CHAPTER I

INTRODUCTION
INTRODUCTION

This thesis incorporates the results of the studies on the effect of one of the cardiotoxic glycosides of the kernel of the seed of Cerbera Odollam Gaertn, on some aspects of the metabolism of the heart. A brief review of the chemistry of the cardiac glycosides of this plant and their pharmacological effects is attempted along with a comparison of digitalis and allied cardiac glycosides. Eventhough digitalis and other cardiac glycosides are extensively used in medicine and their cardiotoxic effects in higher doses are known, it is surprising that little or no work has been carried out on the effect of these substances on metabolism in the heart.

Botanical Characteristics\(^1\)-\(^9\)

Cerbera Odollam (Botanical name - Cerbera Odollam Gaertn - Syn: Cerbera Manghas Linn) is a small poisonous plant or shrub that has wide geographical distribution. It grows in the salt-swamps of the sea-coast and along the backwaters and rivers throughout
India, Sri Lanka, Malaya, China, Australia, Islands of the Pacific, Africa and along Madagaskar sea-coast.

The plant grows to the height of 5-10 metres, with a diameter of 15-30 cms and bears flowers and fruits throughout the year. The leaves are long, dark green and shiny, measuring 30 cms in length and 4-6 cms in breadth. The flowers are white with a light agreeable odour. The fruit has a fibrous mesocarp and the seed inside the endocarp is ovoid, occasionally two, measuring 2 x 1.5 cm, consisting of two kernels. When fresh, the kernel is white, but on exposure, it turns violet and dark brown.

The plant belongs to N.O. Apocynaceae family and is a small tree or a large shrub with an acrid milky poisonous juice, glabrous branchlets, whorled, stout, marked with leaf scars. Leaves-alternate, closely set at the ends of branches, black when dry, coriaceous, lanceolate, oblanceolate or oblangobovate, suddenly acuminate, much tapering to the base. Flowers-white, large, odourous with a yellow throat which is nearly closed by 5 pubescent projecting wing-like ribs. Calyx-glabrous, segments 2-2.5 cm long, oblong, linear oblong, very acute, recurved. Fruit—a drupe, 5-10 cms long, sub-globose, smooth, green with a seed of a dicotylidenous kernel, occasionally two.
LEGENDS TO THE PHOTOGRAPHS

1. Cerbera Odollam - an adult tree

2. Branches showing inflorescence and a fruit
   (Trunk of an aged plant on the background)

3. Fruits of the plant

4. Sections of a fruit showing the epicarp,
   fibrous mesocarp and endocarp with the seed.

5. A. A fresh seed
   B. An exposed kernel
   C. A mature seed
   D. Kernel with the endocarp
   E. Two halves of the seed kernel
   F. A view of two kernels from the same fruit,
      which is seen only occasionally.

6. Enlarged view of D and E
Therapeutic and other applications\textsuperscript{10-16}

The plant has very little economic value. The dried and powdered leaves are used in eczema, rheumatic and bubonic swelling and elephantiasis. Leaves boiled in water are applied externally in paralysis. The extract of the bark is used as a remedy for ring-worm. In Jawa, the leaves are reported to be used as purgative. The kernel mixed with Datura seeds is given as a medication in hydrophobia. It is also used as a fish poison, animal poison and as arrow poison in South-East Africa.

The kernel of the seed is consumed to commit suicide in Kerala and in some places in Tamil Nadu and sometimes for homicidal purpose also. In Kerala, Cerbera Odollam poisoning contributes to more than 75\% of the total vegetable poisoning cases. The incidence of poisoning due to Odollam is next to that of insecticidal poisoning cases in Kerala. About 25\% of all the poisoning cases in females and about 11\% among the males are due to Cerbera Odollam.\textsuperscript{17}

Chemistry of the cardiac glycosides of Cerbera Odollam

The kernel of the seeds of Cerbera Odollam contains a number of cardiototoxic glycosides, some of which have been isolated and characterised.\textsuperscript{19-24}
De-Vry in 1881 isolated 'Cerberin' from the kernels.\textsuperscript{25} Greshoff in 1890 isolated 'Odollin' from the seeds.\textsuperscript{26} Plugge in 1893 confirmed that Cerberin is a glycoside with a phenolic body and on hydrolysis he obtained a sugar.\textsuperscript{27} Matsubera in 1937 observed that the sugar obtained by the hydrolysis of Cerberin was methyl pentose, an isomer of Rhamnose which he named 'Cerberose'.\textsuperscript{28} Chen and Steldt in 1942 isolated 'Cerberoside' from the fresh kernel, but they could not isolate any Cerberin from the fresh kernel.\textsuperscript{29} It was suggested that Cerberoside by the action of an enzyme 'Diastase' is converted to 'Cerberin'\textsuperscript{30} and this causes the blackening of the kernel.\textsuperscript{31} Frerejacque in 1948 also isolated Cerberoside from the seed kernels and stated that the sugar, cerberose is not a methyl pentose as suggested earlier, but a methyl methoxy pentose identical with Thevetose and also that cerberin is, therefore, 'monoacetylneriifolin'.\textsuperscript{32} Poti in 1948 isolated 'Odollotoxin' from the alcoholic extract of the kernel and suggested that other glycosides may also be present. He stated that Cerberin, Odollin and Odollotoxin are cardiac glycosides.\textsuperscript{12}

Venkata Rao reviewed the cardiac glycosides of Thevetia Peruviana and Cerbera Odollam.\textsuperscript{33} Both these plants belong to the family of N.O. Apocynaceae and the
seed kernels of both the plants have long been known to be highly poisonous. He had summed up the present knowledge of the chemistry of the cardiac glycosides of both these plants, which have much in common. The cardiac glycosides isolated from these two plants are of the 'cardenolide type'. The cardenolides can be broadly classified into - (1) Polar glycosides (Triosides) and (2) Secondary glycosides (Monosides). All these glycosides give positive Kedde reaction, negative Keller-Kili

Thevetin, the first cardioactive glycoside reported to be present in the seeds of Thevetia Peruviana, was later shown to contain two glycosides, Thevetin A and Thevetin-B. Rangaswamy and Rao found that Thevetin isolated from the seed kernel of Cerbera odollam, contained only Thevetin-B. Thevetin-B was found to be identical with 'Cerberoside', obtained earlier from Cerbera odollam and has the following characteristics.

\[
\begin{align*}
\text{Molecular formula} & \quad C_{42}H_{66}O_{18} \\
\text{Molecular weight} & \quad 858.95 \\
\text{Chemical name} & \quad \beta-D-\text{Glucopyranosyl} - (1 \rightarrow 6) \beta-D-\text{Glucopyranosyl} (1 \rightarrow 4) 6\text{deoxy} - 3-O\text{Methyl} \alpha-L\text{Glucopyranosyl}) \text{Oxyl-14-hydroxy Card-20(22) enolide.}
\end{align*}
\]

\[45\]
Melting Point 197-201°C

\[(\alpha)_D^{24} -61.4^\circ \pm 1.5^\circ (\text{MeOH})\], With Pilzamylase or Cerberase, it is hydrolysed to the monoside - Neriifolin and two glucose units.\(^3\)

'Cerberoside' is believed to be the main primary glycoside present in Cerbera odorallam, and the secondary glycosides are formed only during isolation by enzyme action.\(^4\) But it was subsequently reported that the secondary glycosides could also be isolated from the seed kernels of Cerbera odorallam even when the enzyme action was prevented during extraction.\(^5\) It was further suggested that the secondary glycosides are formed in the seed itself by the action of the enzyme 'Cerberase' present in the seed kernel of Cerbera Odollam and the blackening of the seed kernels on exposure indicates this enzyme action.\(^6\) Rangaswamy and Rao observed that the unblackened kernels of Cerbera Odollam yielded secondary glycoside (monoside), whose yield could be increased with the blackening of the kernels (enzyme action).\(^7\) The secondary glycosides common to both Thevetia peruviana and Cerbera Odollam are Neriifolin\(^8\) and Cerberin\(^9\) (monoacetyl-neriifolin) with the following properties.
NERIIFOLIN

Molecular formula \(-\) \(C_{30}H_{46}O_8\)
Melting Point \(-\) \(226 - 230^\circ C\)
\((\alpha)^{24}D\) \(-\) \(-49.7^\circ C (\text{MeOH})\)

Hydrolysis of Neriifolin yields \(\alpha\) and \(\beta\) anhydro digitoxigenin and L-thevetose.\(^{37}\)

CERBERIN (Monoacetyl Neriifolin)

Molecular formula \(-\) \(C_{32}H_{48}O_9\)
Melting point \(-\) \(212 - 216^\circ C\)
\((\alpha)^{24}D\) \(-\) \(-84.2^\circ (\text{Me OH})\)

In addition, Voigtlander \(\textit{et al}\)\(^{49}\) and also Venkata Rao \(\textit{et al}\)\(^{50}\) isolated 'Acetyl thevetin-B' (Monoacetyl Cerberoside) from the unfermented seed kernels of Cerbera Odollam. Further Venkata Rao and Appa Rao could also isolate 'Diacetyl neriifolin' from the ethanolic extract of Cerbera Odollam seed kernels.\(^{51}\) The structures of Cerberoside,\(^{52,53}\) Neriifolin\(^{46}\) and Cerberin\(^{32}\) are given in figure 1.
<table>
<thead>
<tr>
<th></th>
<th>Aglycone Structure</th>
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<tbody>
<tr>
<td>1. Cerberoside</td>
<td>$R_1$ CH$_3$, $R_2$ H, Gentiobiosyl</td>
</tr>
<tr>
<td>2. Neriifolin</td>
<td>$R_1$ CH$_3$, $R_2$ H, H</td>
</tr>
<tr>
<td>3. Cerberin</td>
<td>$R_1$ CH$_3$, COCH$_3$, H</td>
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The basic structure of the aglycone (genin) is a Cyclopentano-perhydro-phenanthrene nucleus to which is attached an unsaturated lactone ring at C-17. Digitalis and allied cardiac glycosides also contain the same nucleus. The different glycosides differ in the specific positions of the methyl, hydroxy and aldehydic groups in the nucleus. All the naturally occurring aglycones carry a hydroxyl group at C-14 and have an additional hydroxyl group at position 3 where the sugar moieties are usually attached. The unsaturated lactone ring attached at C-17 possesses an $\Delta^\alpha,\beta$ structure and may be 5 or 6 membered. The structural formulae of Digoxigenin and Digitoxigenin along with the aglycone of the odollam glycosides are given in Figure 2.
Figure 2

1. Digoxigenin

2. Digitoxigenin
3. Aglycone of Odollam Glycosides

Thus the glycosides of Cerbera odolam and the digitalis glycosides have very similar aglycones. The pharmacological action of these cardiac glycosides resides in the aglycone and the particular sugar moieties attached to position 3 of the aglycone modify their water and lipid solubility and also the potency of the resulting glycoside. Major contributions in this field have been made by Chen and Henderson (1954), Fieser and Fieser (1959), Tamm (1963), Marshall (1970) and Brown et al (1986). Saturation of the lactone ring reduces the action by ten fold or more and increases the speed of development in the cardiac action. Opening of the ring completely abolishes toxic activity. Digoxin and Digitoxin are the only cardiac glycosides used frequently and consists of the corresponding aglycone with three molecules of 'Digitoxose', a 2, 6-dideoxy hexose, joined in glycosidic linkage and
attached to position 3. The sugars attached to the aglycone in the cardiac glycosides of Odollam are:

- Thevetose in Noriifolin \(^{37,61}\)
- Acetyl thevetose in Cerberin \(^{48}\)
- Thevetose-gentiobiose in Cerberoside \(^{44}\)

The structures of these sugars are given in Figure 3.

**Figure 3**

1. Thevetose

![Thevetose structure]

2. Acetyl thevetose

![Acetyl thevetose structure]
3. Threitolose-gentiobiose
Pharmacological action

Initially, the pharmacological action of Thevetin was studied by Chopra and Mukherjee, Chen and Chen, Arnold et al, and subsequently by a number of other investigators. Since Thevetin was shown to be a mixture of Thevetin-B with varying quantities of Thevetin-A, this early work might not be of much significance. Chen and Steldt studies the pharmacology of pure 'Cerberoside'. These workers also studied the pharmacology of 'Cerberin'. Rangaswamy and Rao observed that what the above workers were considering as Cerberin was probably Neriifolin. Huang et al also studied the pharmacology of Neriifolin.

Detailed pharmacokinetic studies have been carried out mostly with peruvoside, present in the seed of Thevetia peruviana, a plant very closely related to Cerbera Odollam. Peruvoside resembles cerberin very closely, the only difference being R₁ is CHO and R₂
is H in the structure given in figure 1 whereas cerberin has CH₃ and CO.CH₃ in the corresponding positions.

Primary pharmacological studies carried out by Kohli and Vohra indicated that Peruvoside has an overall pattern of action which resamble Ouabain. It however differs in one important respect, that is the absorbability from the oral route. Arora et al from their pharmacological and toxicological evaluation studies, as well as limited clinical trials reported that peruvoside is an orally administrable drug and as potent as Ouabain. They reported peruvoside to be a promising drug for congestive heart failure. Several groups of pharmacologists and clinicians studied the pharmacology and clinical properties of peruvoside (Encordin (R)). These studies indicated that this glycoside has a markedly positive ionotropic effect on the heart of anaesthetised cats and dogs with experimental heart damage induced by barbiturates and phenylbutazone. The effect of cardiac contraction equalled approximately that of Ouabain. In animal experiments, peruvoside showed a wider margin between doses with positive ionotropic effect and those inducing arrhythmias.
Experiments with ouabain. Tritium-labelled peruvoside confirmed the good enteral absorption of peruvoside, as well as more rapid elimination in comparison to digitoxin. The major part is excreted via the bile and intestine, whereas kidney plays only a minor part in the excretion of peruvoside. The most important metabolite of peruvoside was found to be peruvoside carboxylic acid. Comparison of the kinetic properties of peruvoside with those of ouabain, digitoxin and digoxin showed a greater similarity towards digoxin than towards the other two glycosides.79

Kazutake et al (1986)80 showed that cardiac steroids having a doubly linked sugar and their related compounds have a higher inhibitory action on Na\(^+\)K\(^+\)ATPase and also that their activity is dependent on the presence of ring carbon substituents.

Compared to the work on peruvoside, relatively less pharmacological data is available on cerberin. Not much work has been carried out on the pharmacological effects of cerberoside, the primary glycoside of Cerbera Odollam, except for the report that it resembles cerberin, showing much weaker action. In lower doses, cerberin produces a positive ionotropic effect, while in higher doses it is cardiotoxic.81 In this respect, the effects of Cerbera Odollam glycosides resemble those produced by digitalis.
The main pharmacodynamic property of digitalis is its ability to increase the force of myocardial contraction. The beneficial effects of this drug in patients with heart-failure are increased cardiac outputs, decreased heart-size, venous pressure and blood-volume, diuresis and relief of edema - all explained on the basis of increased contractile force and positive ionotropic action.82

The second important action of digitalis is to slow the ventricular rate of atrial fibrillation or flutter.82 It appears that the major component to the positive ionotropic effects of digitalis is direct inhibition of the membrane-bound sodium-potassium activated Adenosine triphosphatase (Na⁺-K⁺-ATPase) which leads to an increase in intra-cellular calcium ion concentration and associated increase in a slow inward current during the action potential.83 This current is the result of the movement of Ca²⁺ ions into the cell. Digitalis glycosides bind specifically to Na⁺-K⁺ ATPase, inhibits its enzymatic activity and impair the active transport of these two monovalent cations.84-88 As a result, there is a gradual increase in intra-cellular Na⁺ concentration and a gradual, small decrease in intracellular K⁺ concentration.
It is the increase in the concentration of Na\(^+\) that is at present considered to be crucially related to the positive ionotrophic effect of digitalis.\(^89-92\) When the intra-cellular Na\(^+\) is increased as a result of the inhibition of Na\(^+\)-K\(^+\)-ATPase by digitalis, the exchange of extra-cellular Na\(^+\) for intra-cellular Ca\(^{++}\) is diminished and hence intra-cellular Ca\(^{++}\) concentration increases. The probable consequence of this is an increased store of Ca\(^{++}\) in the sarcoplasmic reticulam and with each action potential, a greater release of Ca\(^{++}\) to activate the contractile apparatus.\(^93-94\) The mechanism proposed thus assumes that Na\(^+\)-K\(^+\)-ATPase is the pharmacological receptor for digitalis and when digitalis binds to this enzyme, it induces a conformational change that decreases the active transport of Na\(^+\).\(^95\) Many studies have provided evidence that digitalis binds to the ATPase in a specific and saturable manner,\(^96\) that the binding results in a conformational change in the enzyme,\(^97\) that the rate of binding is increased by Sodium ion concentration and decreased by Potassium ion concentration\(^98\) and that the binding site is probably the external surface of the membrane.\(^99\) Furthermore, the magnitude of the ionotrophic effect of digitalis has been reported to be proportional to the degree of inhibition of the enzyme.\(^100\) In spite of the evidence that the positive ionotropy results
from inhibition of Na\(^+\)-K\(^+\) ATPase and the resultant elevation of intracellular concentration of Ca\(^{++}\), some data suggest other possible mechanism of action of digitalis. A number of studies have shown that very low concentrations of the glycoside cause stimulation of Na\(^+\)-K\(^+\) ATPase and a concomitant positive ionotropic effect.\(^{101-104}\) Other studies attributed the effect of digitalis to an alteration in the Ca\(^{++}\) binding by sarcolemal phospholipids.\(^{105}\) The question of alternate mode of action of digitalis has been discussed by several workers such as Wiengarten and Marban and Tsein.\(^{92}\) It does seem clear that a positive ionotropic effect is not prominent until there has been some inhibition of the active transport of Na\(^+\) and K\(^+\).

Toxic effect of high dose of digitalis can be severe or lethal.\(^{106-107}\) The most important toxic effects are those that involve the heart. Digitalis can cause marked sinus bradycardia and a complete SA block.\(^{108}\) Toxicity can also be manifested as disturbances of atrial rhythm.\(^{109}\) High levels of AV-block and appearance of accelerated AV junctional rhythms are manifestations of digitalis toxicity.\(^{108}\) The most typical disturbance appears as escape of beats or as a norperoxicimal AV junctional tachycardia.\(^{110}\) The disturbances of ventricular rhythm frequently caused by digitalis are premature depolarisation that appears as coupled beats.\(^{111}\) Digitalis toxicity can
also cause ventricular tachycardia and ventricular fibrillation. 112-113

Most of the available reports on the pharmacological studies of Odollam glycosides relate to Cerberin, which is considered to be the most physiologically active constituent of seed kernel of Odollam. Very little work has been carried out on the mechanism of action of Odollam glycosides, except for the report that the positive ionotropic effect in this case may also be due to inhibition of Na$^+$-K$^+$ ATPase. 114

Guruswamy et al observed that Cerbera Odollam glycosides possess action similar to that of digitalis. 81 But the beneficial cardiotonic effect was better with Cerbera Odollam as was evidenced by the results obtained when equal strength of these two drugs used under identical conditions. In normal as well as failing hearts of frogs, perfusion with a crude extract of Cerbera Odollam produced initial progressive stimulation of amplitude of contraction of the heart. On continuing the perfusion, the amplitude gradually decreased, rhythm became irregular and heart stopped in systole.

In anaesthetised cats and dogs, I.V. administration of Cerbera Odollam extract at the rate of 100 mgs,
every 5 minutes produced an initial rise of blood pressure (20-30) mm) with slowing of heart followed by sudden fall of blood pressure and death. Respiration was not seriously affected. ECG showed myocardial improvement in the initial stages followed by irregularities.

Chopra et al. observed that cerberin at a concentration of $10^{-6}$ increased the amplitude of verticular contraction in dogs, the rhythm remaining unchanged (cardiotonic effect i.e. positive ionotropism). In rabbits, cerberin at a concentration of 1 in 40,000 doubled the amplitude of contraction (positive ionotropism). At a concentration of 1 in 20,000, the amplitude remained unchanged, but the heart rate was diminished (negative chronotropism) and finally disturbance of excitability was noted (negative bathmotropism). Cerberin at a concentration of $10^{-6}$ to $5 \times 10^{-5}$ stimulated the intestinal musculature of isolated guts of cats and guinea pigs. It increased both the tonus and peristaltic movements. These actions were antagonised by atropine, but potentiated by Pilocarpine. In isolated dog-intestine at a concentration of $5 \times 10^{-5}$, the tonus was slightly increased and the amplitude of peristaltic movement was stimulated. Cerberin behaves as a parasympathomemetic. 1-2 mgs of cerberin given intravenously in cats, stimulated and then
paralysed the sympathetic nerve endings, produced increase in blood pressure, relaxation of bronchi, followed by marked constriction, increased tone and movement of intestine, uterus and urinary bladder, and finally paralysis. Neurovascular preparation of frog was tetanysed and fatigued sooner than the normal and recovery was delayed. Chopra reported that subcutaneous injection of cerberin in experimental animals caused vomiting, urging and syncope. Vijayaraghavan et al observed that in pithed frog, there was significant increase in the heart rate, proportional to the concentration of cerberin in alcoholic solution. They also observed that in perfused frog's heart, amplitude of contraction increased, but the diastolic relaxation was incomplete. There was bradycardia and terminally the arrest of heart in systole. In dogs, cerberin at a dosage of 100-350 µg/kg, produced a sustained elevation of blood pressure with no change in rhythm. Above 350 µg/kg, 60% of the dogs developed AV-dissociation. At 650 µg/kg, 80% of the dogs developed AV-dissociation and showed a change in mean frontal axis of QRS Complex in all cases, bidirectional rhythm in 6 cases, ST-depression and T wave inversion in 3 cases prior to arrhythmia, while in others they developed along with arrhythmia. Ventricular rate increased and tachycardia or fibrillation was observed. In cats, administration of 400-500 µg/kg of cerberin,
produced T wave flattening followed by inversion. At or above 600 µg/kg, all the cats developed prolongation of PR interval and then atrial flutter in 5-8 minutes. This was associated with change in mean frontal axis of QRS complex, lasted for 10-12 minutes and was followed by atrial fibrillation and then ventricular fibrillation in all the cats without further increase in dosage.

Cerberoside is reported to be weaker but to resemble cerberin in action. As detailed above, cerberin produces negative chronotropic, dromotropic and bathmotropic effect in the heart. Ionotropic effect varies with the dose, as also the blood pressure. It stimulates intestinal musculature and acts as cathartic. Lethal dose (subcutaneous) of cerberin reported by Dymock et al is 1.8 mgs/kg for dog, 3.1 mgs/kg for rats and 50 mgs/kg for rabbits. Guruswamy et al reported the lethal dose of the crude extract as 100 mgs/kg in anaesthetised dogs and rats, while Narendranathan reported the kernel of one fruit as lethal in man.

Clinical Aspects

There do not seem to be many reports on the clinical aspects of Odollam poisoning. The available
reports in this connection show that the clinical symptoms and findings with Odollam poisoning are more or less similar to those reported for the lethal dose of digitalis.\textsuperscript{86,102,108-111, 119,120}

Werdan \textit{et al.}\textsuperscript{121} reviewed the development of tolerance to cardiac glycoside (digitalis) in beating heart muscle in guineo pigs and also during long term digitalis therapy in patients with congestive heart failure.

Kini and Pai\textsuperscript{122} were the first to report on the clinical aspects of Odollam poisoning. Nausea, vomiting and syncopical attacks were the main presenting symptoms. A variety of ECG findings resembling very closely those produced by toxic dose of digitalis were noted by them - like sinus bradycardia, sinus arrhythmia with second degree of AV block, sinus arrhythmia with second degree of SA and AV block, AV dissociation, atrial fibrillation and atrial tachycardia, ventricular fibrillation, widening of QRS complex and sagging of ST segment with inversion of T waves.

Kunhali \textit{et al.}\textsuperscript{123} reported nausea, retching and vomiting in Odollam poisoning cases and also observed presence of extra systoles.
Narendranathan et al\textsuperscript{114} reported nausea, vomiting, retching, epigastric pain, diarrhoea, colicky abdominal pain, blurring of vision, general weakness, drowsiness, unconsciousness, bradycardia, cardiac arrhythmias, conduction defects, hyporeflexia, hypotonia, non-specific slowing of EEG and hyperkalemia. They have also reported ECG changes in a patient even after 2 years suggesting a permanent effect. The cardiac actions were mainly negative chronotropic, dromotropic and bathmotropic.\textsuperscript{118}

Vijayaraghavan et al\textsuperscript{116} reported vomiting and epigastric pain. According to them, the glycosides in Cerbera Odollam have inhibitory action on the SA node, AV junction as well as sino-atrial and atrioventricular conduction.

Vasudeva Iyer and Narendranathan\textsuperscript{124} reported neurological findings in some cases of Odollam poisoning which consisted of hypotonia, hyporeflexia (in 50\%) and non-specific slowing in the EEG in the theta range (in 50\%). There was no correlation between the severity of cardiac changes and the EEG abnormalities. The neurological findings were, however, trivial and they suggested that it is possible that the active principles either may not have any direct action on the nervous system or they may not penetrate the blood-brain barrier in sufficient amounts.
The hypotonia and hyporeflexia noted in 50% cases may suggest a depressive action on the peripheral nerves or at spinal synapses.

Mohandas reported the results of the studies of 20 autopsy cases of poisoning with Cerbera Odollam. The following conclusions were drawn by him. The cardiac arrhythmia and conduction defects are due to structural changes caused by the toxic principles of Cerbera Odollam. The neurological signs elicited in the clinical cases can be explained by the toxic principles having crossed the blood-brain barrier. The mucosal changes in the stomach reveal that the poison is irritant in nature.

A method of isolation and identification of Odollam glycosides in biological fluids by Thin Layer chromatography has also been reported.

Biochemical Aspects

Eventhough the pharmacological effects of the cardiac glycosides on the heart have been studied in detail and some work on the mechanism of this action in the case of digitalis has been carried out, very little work seems to have been done on how these glycosides affect the cardiac metabolism. Most of the available
reports in this respect relate to their inhibition of Na\(^+\), K\(^+\) ATPase\(^{86,97,100,126-129}\) and their binding site on cardiac muscle.\(^{98,130-133}\) Influence of derivatisation of cardiac glycosides (26 digitalis and 6 strophanthus comprising of aglycone; mono, bis, tris sugar; alkylated, acylated etc.) on lipophilicity and inhibitory potencies on the myocardial Na\(^+\), K\(^+\)-ATPase has also been studied by Dzimri et al.\(^{134}\)

The other reports in this connection include inhibition of phosphodiesterase activity,\(^{135}\) stimulation of phospholipase-C activity\(^{136}\) in rat pineolocytes, interaction with biomembrane lipids, enhancing conformational motility of lipids causing additional binding of Ca\(^{++}\) with the membrane\(^{137}\) and the effect of digitalis on renal Na\(^+\) reabsorption\(^{138}\) etc.

Gervias et al\(^{105}\) studied the effect of cardiac glycosides on alternation in Ca\(^{++}\) binding by sarcolemal phospholipids. Kolomiets and Merzon\(^{139}\) investigated the effect of cardiac glycosides (such as digoxin, strophanthin and corglycon) and Ca\(^{++}\) antagonists (such as verapamil and norepinephrine) on the serum Ca\(^{++}\) concentration in patients.

Practically no work seems to have been carried out on the effect of Odollam glycosides on cardiac metabolism. Narendranath et al. suggested inhibition of Na\(^+\)-K\(^+\) ATPase
to be responsible for their action. They also suggested that Cerbera Odollam induces its effect through biochemical alterations and not by any pathological change in the conducting tissue.

Very little systematic work seems to have been carried out on how the metabolism of lipids, carbohydrates, amino acids and proteins as well as that of the macromolecular components of the intercellular matrix viz. glycosaminoglycans and glycoproteins, is affected by the cardiac glycosides in general. In view of this it was considered necessary to investigate in detail some of these aspects using Odollam glycosides. Cerberoside was chosen for this purpose mainly because it is the primary glycoside present in Cerbera Odollam kernel and because it is far less toxic than the other well studied glycoside of Cerbera Odollam – viz. cerberin. Investigations carried out on the effect of cerebroside in this respect using rats as experimental animals include:

A. Toxicity of cerberoside

B. Effect on the metabolism of carbohydrates in the heart and liver.
   1. Concentration of blood glucose
   2. Concentration of hepatic glycogen.
   3. Activity of some glycolytic enzymes in the heart.
   4. Activity of some enzymes involved in glycogen metabolism in the heart.
5. Activity of some enzymes involved in gluconeogenesis, pentose phosphate pathway and citric acid cycle in the heart.

C. Changes in the metabolism of lipids in the heart.
   1. Concentration of cholesterol, phospholipids, triglycerides and free fatty acids.
   2. Concentration of lipids in the serum lipoproteins.
   3. Activity of HMGCoA reductase and incorporation of labelled acetate into the lipids in the heart.
   4. Concentration of hepatic bile acids and fecal excretion of neutral sterols and bile acids.
   5. Activity of some lipogenic enzymes in the heart.

D. Changes in the metabolism of glycosaminoglycans in the heart.
   1. Concentration of total and individual glycosaminoglycans.
   2. Activity of some enzymes involved in the biosynthesis of precursors of glycosaminoglycans.
   3. Activity of some enzymes involved in the degradation of glycosaminoglycans.
   4. Sulphate metabolism.

E. Changes in the metabolism of glycoproteins in the heart.
   1. Concentration of carbohydrate components of glycoproteins.
2. Activity of glycohydrolases

F. Changes in the DNA, RNA, amino acids and proteins in the heart.

G. The effect of sub-lethal dose of cerberoside on the severity of myocardial infarction induced by isoproterenol.
   1. Activity of serum CPK
   2. Activity of LDH in the serum and heart.
   3. Changes in lipids in the serum and heart.

H. Effect on some aspects of brain metabolism.
   1. Changes in lipids
   2. Activity of acetyl cholinesterase
   3. Metabolism of glycoaminoglycans
   4. Metabolism of glycoproteins

The results of these investigations are discussed in this thesis.
CHAPTER 2

MATERIAL AND METHODS