CHAPTER 2

REVIEW OF LITERATURE
CHAPTER 2

REVIEW OF LITERATURE:

Historical review of drugs, poisons and their analysis in Toxicology:

Toxicology has played a relevant part in the history of man. The earliest medical record, the Ebers papyrus contains information of many recognized poisons like hemlock, aconite, opium, lead, copper and antimony and other plant substances like belladonna and digitalis. The Vedas (900 B.C) also mention many poisons. Hippocrates (400 B.C), the founder of Greek medicine added a number of poisons and wrote about their treatment in therapy and overdose. Ancient Greek literature contains many references to poisons and their use. Theophrastus (370-280 B.C.), a student of Aristotle included many references to poisonous plants in De Historia Plantarum. Dioscorides, a physician in the court of Nero attempted the classification of poisons with descriptions and drawings. He also separated plant, animal and mineral poisons and also offered therapy in poisoning cases.

In ancient Greece, poisoning with plant and animal poisons was common. Socrates (470-399 B.C.) was made to drink hemlock. Demosthenes took poison hidden in his pen. The Romans have made much political use of poisons. King Mithridates VI of Pontus protected himself from poisoning by enemies, by regularly ingesting a mixture of 36 ingredients. When he was finally captured by enemies, he could not kill himself with poison because of his successful concoction and had to use his sword. Nero became the Emperor of Rome by killing Claudius with arsenic. Later Nero also poisoned Claudius’s son Britannicus using arsenic. Catherine de Medici of Rome must be credited as the earliest toxicologist. Under the guise of delivering provender, she experimented with various poisons using the sick and poor as subjects. The Antidotarium of Nicholaus - 17th century was a commission appointed by the Pope of Matthioulus to search for specific antidotes. In the 4th century B.C. there was a conspiracy of women to remove those, from whose death they might profit, by poisoning. In 82 B.C. the Lex Cornelia - 1st law against poisoning was issued. In Italy, ample political use of poisons was made by professional poisoners. A lady named Hieronyma Spara used poisoning to attain marital and monetary objectives, forming a notorious club of wealthy married women who killed their husbands.

Maimonides (A.D- 1135-1209) wrote a volume entitled “Poisons and their Antidotes” a first aid guide to the treatment of accidental and intentional poisonings. Paracelsus (1493-1541) formulated many revolutionary views which still remain an integral part of the present structure of toxicology, like degree of specificity and dosage. This view presaged Paul Ehrlich’s therapeutic index, or Dose response relationship.
Orfila (1787-1853) is cited as the founder of Toxicology. He attempted a correlation between the chemical and biological information of the then known poisons. He also was the first to point out the necessity of chemical analysis for legal proof of lethal intoxication and devised methods for detecting poisons. He introduced the use of autopsy material for detecting accidental and intentional poisonings. In other words, he introduced the speciality area of Forensic Toxicology.

Later, many analytical developments came through for detection of poisons like the development of a test for arsenic by Marsh in 1836. Magendie (1783-1855) studied the mechanism of action of Emetine, Strychnine and arrow poisons. His student Bernard (1813-1878) identified the site of action of curare and noted the formation of a carbon monoxide haemoglobin complex. Louis Levin (1854-1929) studied the toxicology of methyl-, ethyl- and higher alcohols and opiates and Chloroform. He published a text on toxicology.

In the 20th century, toxicology developed further. British Anti Lewisite (Dimercaptol) an antidote to Arsenic containing war gases was developed. Carl Voegtlin (1923) studied the mechanism of action of organic arsenicals. Dichloro Diphenyl Trichloroethane (DDT) and organophosphorous pesticides were discovered with the increase in the discovery of new toxins and drugs. Toxicology and with it forensic toxicology emerged as a major discipline.

At present, in forensic toxicology, the poisons and drugs are analysed from blood, viscera and various tissues using traditional and modern analytical methods to detect their presence. Recently, high performance liquid chromatography and derivative ultraviolet spectroscopy are analytical methods found to be very useful in the detection and differentiation of poisons and drugs.
LITERATURE REVIEW OF INDIVIDUAL DRUGS

IMIPRAMINE

Since the introduction of Imipramine, there have been numerous reports about the ingestion of massive quantities of this drug. Some were fatal whereas other patients survived without sequelae (91,106). Desipramine, a metabolite of Imipramine has a rapid action that makes it the drug of choice when a prompt antidepressant response is desirable (157). Imipramine is the original dibenzazepine derivative (91) and belongs to the class of drugs known as tricyclic antidepressants. Although they are effective in the management of psychiatric depression, the use of these potent and dangerous drugs in enuresis has been questionable (66). As a class, the tricyclics have a bewildering array of additional pharmacological effects including sedative actions, alpha-adrenergic blockade, ganglionic blockade and anti-cholinergic activity (66). The danger in children of accidental ingestion of these drugs has become increasingly recognised in recent years (152) but there is an impression that their effects are not so dramatic or dangerous in adults as in children (117). However, reports indicated that severe symptoms and death occurred in adults in very high doses (500 to 750 mg/kg) than in children (20 mg/kg) (68). Cardiac arrhythmias and conduction defects are usually associated with severe poisoning.

Structure:-

![Chemical structure of Imipramine]

Molecular weight :- 280.4

Chemical formula :- C19 H24 N2.

Solubility :- Imipramine is a yellow powder practically insoluble in water, soluble in Ethanol, Chloroform and Ether.

Disposition :- Imipramine is readily absorbed after oral administration and is widely distributed throughout the tissues. (32) It appears to be distributed into many regions of the brain (30) and also appears in breast milk (149). Bio-availability is about 50 percent but there is considerable inter-subject variation.
Volume of distribution: 0-20 L/kg (61)
Protein binding: 89-94% (20)
Tmax in humans: 30-60 minutes

Metabolism and Elimination:

Imipramine and Amitriptyline are absorbed quickly from the intestinal tract and are largely bound to plasma proteins. They accumulate in tissues rapidly and their serum concentration can never be high. After medium toxic doses, the tissue concentration is 10-30 times that of serum concentrations. The metabolism is very fast and no drug can be found in tissues in 24 hours after a toxic dose. Only small and important amounts of free drug can be found in the urine after administration of higher doses (166). Imipramine undergoes considerable first pass metabolism, mainly by N-demethylation to the primary active metabolite Desipramine. Other major metabolic reactions include hydroxylation at the 2 or 10 position followed by conjugation. The extent of 2 or 10 hydroxylation seems to be genetically determined (166). The half-life of Imipramine is 8-16 hours and that of Desipramine is 10-26 hours. Less than 10% of a dose is excreted in urine unchanged. A large number of metabolites have been identified in the urine. A total of about 40.0% of a dose is excreted in the urine in 24 hours and about 70.0% in 72 hours. The proportion of present drugs and metabolites found in the urine are 1-4% for Imipramine and Desipramine, 40-60% for conjugated metabolites, 15-35% for non-conjugated metabolites and the remaining percentage for non-extractable polar materials (30, 61). Following a single oral dose of 50 mg to three subjects, peak plasma concentrations of 0.010 to 0.083 mg/ml (mean 0.03) of Imipramine and 0.004 to 0.014 mg/ml of Desipramine (mean 0.08) were attained in 3-4 hours and 4-8 hours respectively. After daily oral dose of 50-300 mg to 24 subjects, the steady state plasma concentrations reported were:

- Imipramine :- 0.01 to 0.11 mg/ml (mean 0.05)
- Desipramine :- 0.02 to 0.33 mg/ml (mean 0.09)
- 2-OH-Imipramine :- 0-0.02 mg/ml (mean 0.01)
- 2-OH-Desipramine :- 0-0.06 mg/ml (mean 0.007)

Toxicity:

Tricyclic antidepressants have produced many cases of serious and fatal intoxication. Toxic effects have been reported both in adults and children and consists of circulatory, cardiac, central nervous system and haematological disturbances (167). The toxicity symptoms are:

(A) Headache, nausea, vomiting.
(B) Drowsiness, ataxia and stupor, that may progress to coma, which is usually short lasting, rarely respiratory depression, absence of light reflex.
(C) Restlessness, hyperactivity, involuntary movements of limbs.
(D) Abnormal ECG, widening of QRS, prolonged QT interval, tachycardia and almost every possible arrhythmia and conduction defects that may progress to complete heart block, ventricular fibrillation, and cardiac arrest.

(E) Abnormal ECG patterns, hallucinations, acute psychosis and irreversible cerebral damage.

(F) Hypothermia and hyperpyrexia.

(G) Oliguria and aspiration pneumonitis (66)

Severe poisoning is characterised by the development of convulsions and coma, in addition to respiratory depression, cardiac arrhythmias, ECG changes and profound hypotension. (152)

Cases of poisoning reported:

Poisoning of 60 children with Amitriptyline hydrochloride and Imipramine hydrochloride is reported. The minimum lethal dose for both the tricyclic antidepressants is probably 30 mg/kg body weight (65). The accidental poisoning of a healthy boy of 5 years and 4 months with 50-60 tablets of Amitriptyline (55 mg) is reported. The presence of Amitriptyline in gastric aspirate was established by extraction at alkaline pH and ultraviolet spectroscopy. The recovery was only 30 mgs. The gastric washings represent the most suitable clinical material to make identification of an ingested drug, since metabolic changes are minimal and actual concentration may be higher than any available body fluids, particularly if aspiration is carried out within a short time of ingestion. Some quantitative assessment of the toxicity of Imipramine and Amitriptyline was also made by studying reported cases of such poisonings. The smallest fatal case in a child was 32 mg/kg and the largest amount taken by one who survived was 112 mg/kg. In adults too, this variation in susceptibility was seen. Deaths had been recorded after ingestion of only 625 mg (9mg/kg) but survival was seen even after 105 5 gms (80 mg/kg) (152). A case of an acute intoxication with Desipramine was reported where a 59 year old woman undergoing treatment for maniac depressive psychosis ingested 2500 mg of Desipramine. She had grand seizures and became comatose, remaining so for 5 hours. Blood and urine samples contained Desipramine (157).

These antidepressants are generally used in the treatment of endogenous depression. These drugs have a sedative effect in addition to antidepressant action. While sedation occurs at once, the antidepressant effects begin to appear only after a week or more. Acute poisoning using these drugs is common and its incidence is increasing. Inevitably these drugs are prescribed to the very people who are most likely to indulge in self poisoning or to attempt suicide and the risk of after dosage is high as reported in above cases and the fatal dose varies from 1 to 2 grams (124). These poisoning cases are frequently referred to the Forensic Toxicologist for detection of poison and hence stability studies for Imipramine is assessed in this study.
CHLOROQUINE

Chloroquine is used in the treatment of malaria. It is readily available over the counter. Although it is relatively less toxic, doses as low as 1 gram have caused death in children and fatalities have occurred in adults after the ingestion of 3 - 44 gms of the drug. Quite a few cases of fatal Chloroquine poisoning have been reported (37, 19, 40) and in such cases it is necessary to determine the drug in the acquired biological tissues.

Molecular weight: 318.9

Chemical formula: C18H22ClN3

Structure:

\[
\text{NH} \cdot \text{CH} \cdot \{\text{CH}_2\}_3 \cdot \text{N(C}_2\text{H}_5\}_2
\]

Distribution and disposition in the body: Chloroquine is rapidly absorbed after oral administration. Bio-availability is 80-90%. Half life of Chloroquine is 25 - 60 days.

Distribution in Blood:

Plasma/Whole Blood Ratio: 0.3

Protein binding in plasma is 50-70%

It undergoes N-dealkylation and deamination followed by conjugation, possibly with glucuronic acid. Metabolites of Chloroquine include Mono-desethyl and Didesethyl Chloroquine. Chloroquine is excreted slowly and may persist in tissues for prolonged periods. About 55% of a dose is excreted in urine and 310 mgs taken daily for 14 days is eliminated in the faeces in 96 days. Of the material excreted in urine, 70% is unchanged, 23% is as Mono-desethyl Chloroquine, 1 - 2% as Didesethyl Chloroquine and an unidentified metabolite and 1-2% as a conjugated Carboxylic acid metabolite.

Toxicity and reported cases of Chloroquine poisoning:

Plasma concentrations greater than 0.6 μg/ml may produce toxic effects and concentrations greater than 3 μg/ml may be fatal. In a review of nine fatal cases of overdose, the tissue
concentrations reported were

Brain = 2.8 - 50 μg/gm. (mean 16)
Kidney = 110 - 640 μg/gm (mean 303)
Liver = 200 - 750 μg/gm (mean 410) (87)

In 2 cases, the blood concentrations were reported to be 30 mg/ml. (136). In 2 fatal cases of overdosage, in which about 3 gms and 10 gms had been ingested, the post-mortem concentrations were

Blood - 16 and 124 μg/ml.
Kidney - 70 and 300 μg/gm.
Liver - 175 and 344 μg/gm.
Liver blood - 10 and 44 μg/ml.
Lung - 38 and 98 μg.
Urine - 20 and 68.4 μg

A 26 year old woman died after ingesting an unknown quantity of Chloroquine, possibly to induce abortion, post-mortem concentrations were.

Blood - 4.2 μg/ml.
Brain - 3.8 μg/gm.
Kidney - 32.9 μg/gm.
Liver - 71 μg/gm. (118)

Kemmenoe reports the death of a two and half year old male child following ingestion of an amazing 60, 200 mg tablets of Hydroxy-chloroquine. It was noted that the large quantity was absorbed from the gut very quickly and a substantial portion of the drug remained unmetabolized (81).

Due to the increasing number of cases of Chloroquine poisoning, intentional, accidental and sometimes as a result of medical mishaps, Forensic Science laboratories are expected to give reports regarding the nature of the drug ingested and whether the concentrations in tissues or blood are toxic or therapeutic. Stability studies of Chloroquine in tissues are therefore essential and have been made in the present study.
STRYCHNINE

Strychnine is an alkaloid obtained from the seeds of Strychnos Nuxvomica and other species of Strychnos plant which grow in India. The ripe fruits of the plant contain seeds which are poisonous. The seeds are hard and flat, about two centimetres in diameter and half centimeter in thickness and slightly convex on one side and concave on the other. They have intensively bitter taste and contain the active principles Strychnine, Brucine and Logain. The bark, wood and leaves of this tree contain Brucine but no Strychnine. Strychnine is a spinal poison, acts mainly on the spinal cord, the cerebral symptoms being either slight or absent. The action may be a stimulating one resulting in the production of spasms (113).

Structure :

\[
\text{\[
\begin{array}{c}
\text{N} \\
\text{H} \\
\text{H} \\
\text{H} \\
\text{H} \\
\text{O} \\
\end{array}
\]
}
\]

Molecular weight :- 334.40
Chemical formula:- C21 H22 N2 O2
Physical properties :-
Strychnine appears to be a white crystalline powder with melting point of 268-290 C and boiling point 270-280 C. Solubility of Strychnine in water at room temperature is 143 mg per litre. It is slightly soluble in Benzene and Chloroform, very slightly soluble in Ether and petroleum ether and insoluble in Ethanol and Diethyl ether. The salts are more soluble in water, for example the sulphate is 3.2% soluble in water at 25 C. (71)

Uses :-
Strychnine was used traditionally in clinical medicine as a gastric and respiratory stimulant and tonic in the form of alkaloid salts, various elixirs and syrups (69). Nuxvomica tincture, Nuxvomica fluid extract, and dried powder extract containing Strychnine concentrations ranging from 0.12% - 7.7% are extensively used in Homeopathic medicine. Strychnine increases the tone of skeletal muscles and is used as a tonic in connection with fatigue, general weakness, incontinence, impotence, and collapse of varied origin. Strychnine is also used as a rodenticide and it forms the chief ingredient of several vermin killers (113).
Absorption :-

Strychnine is promptly absorbed orally and parenterally mainly from the intestines and its absorption from the stomach is a question. Strychnine is taken up to some extent by R.B.C. Although Strychnine acts principally on the spinal cord, it is not concentrated there. In poisoning cases of Strychnine, the highest concentration were found in the blood, liver and kidneys.

Metabolism :-

Strychnine is largely detoxified in the liver by microsomal oxidation (129). The rate of metabolism of Strychnine can be accelerated in animals by pretreatment with Phenobarbital or other inducers of microsomal enzyme activity (76). At least four metabolites occur after incubation of Strychnine with rabbit liver slices. One of these- 2 Hydroxy strychnine, is about 100 fold less toxic than the parent compound (163). Deacetylation also results in loss of biological activity. The half life of Strychnine in human plasma is 10 hrs. About 11-20% of the therapeutic dose of 4 mg whether given by ingestion or intramuscular injection, is excreted unchanged in the urine. The percentage of excretion decreases with increasing dose. Of the portion excreted by the kidneys, about 70% appears in the first six hours and nearly 90% in the first twenty four hours. Strychnine may be detected in the urine in 0.5-3 hours in humans. Traces may persist for four days or even five days. In animals excretion of Strychnine is increased slightly by diuresis (167). In rats, Strychnine is metabolised by the microsomal enzymes of the liver. It is reported that the rate of transformation produced by microsomal fraction was 144 and 64 µg/g of liver (84).

Biochemical changes :-

Strychnine at a concentration of 1 x 10 M or less inhibits Carbonic anhydrase and increases the activity of Cytochrome oxidase (162). It was shown that Strychnine reduces Choline esterase activity to 81% of normal at a concentration of 37.5 ppm and to 55% and 39% of the normal at concentrations of 75 and 150 ppm respectively. It was thus concluded that intoxication by Strychnine could be explained by its ability to inhibit Cholinesterase. About twice as much Acetylcholine was reported to be present on the brain of a frog intoxicated with Strychnine than on the brain of a normal frog. However it is to be said that if inhibition of Cholinesterase is involved, then it must be critically influenced by the distribution of Strychnine. But interestingly, the far more powerful organophosphorous inhibitors of Cholinesterase activity do not have a Strychnine like effect (71). A recent report stated that the characteristic symptoms of Strychnine poisoning cannot be explained by the degree of Cholinesterase inhibition it produces (2). Strychnine also increases rapidly the activity of Na-K activated ATPase of brain microsomes, but has no significant effect on magnesium activated ATPase activity (123). However it is not known whether the above mentioned changes in enzymes activity causes the characteristic symptoms of Strychnine poisoning.

Almost all the actions of Strychnine are attributable to its antagonism of the inhibitory neurotransmitter- Glycine. (11) Glycine is the major inhibitory transmitter in the spinal cord and
brain stem and it is the disturbance of the normal balance between excitation and inhibition produced by blockade of Glycine receptors in these areas that causes the powerful motor symptoms seen in Strychnine poisoning (73). Strychnine has a high degree of affinity and specificity for Glycine receptors. At high doses, Strychnine has more widespread action, which includes inhibition of Sodium, Potassium and Chloride conductance and antagonism of GABA and noradrenaline (11).

Toxicity:

Nuxvomica seeds swallowed as a whole are non-poisonous on account of the hard pericap, which cannot be dissolved by the digestive juices. When broken seeds are taken or the seeds chewed, there is an intensively bitter taste in the mouth. Within 15 minutes to an hour, symptoms of poisoning appear. Strychnine stimulates anterior horn cells of spinal cord causing greatly increased reflex excitability. This results in a loss of normal inhibition of spread of motor cell stimulation so that any stimulus such as noise, light, or air breeze causes a violent reflex action producing general contraction of muscles and as a result the poisoned animals go tetanic. Strychnine apparently acts simultaneously on all the portions of central and peripheral nervous system to increase excitability. Effects on other organ systems appear to be entirely secondary to these actions. The toxic symptoms that are seen are an immediate choking sensation in the throat, stiffness and twitching of the muscles of the face and the neck. Within 15-45 minutes, the patient gets generalised convulsions, tonic at first and then clonic. The complete seizure cycle involves a prodromal period, immediately preceding the convulsions. The prodromal symptoms include cramps in the legs and tightness in the chest. The seizure begins with an expulsion of air from the chest followed by violent contractions of muscles. The chest and diaphragm are fixed, stopping respiration and producing Cyanosis. The muscles are hard. The contractions are sometimes violent enough to cause compression fractures of the vertebrae. The back is arched with the victim sometimes resting on his heels and back of his head. This condition is known as Opisthotonos. During the paroxysms the face becomes cyanosed and wears an anxious apprehensive look of impending death. The eyes are staring and the eyeballs are prominent. The features are drawn into a grin (the risus sardonicus) and the mouth is covered with froth, frequently stained with blood. The legs are adducted and extended with the feet curved inward and arches bowed. The arms may be flexed over the chest and extended rigidly with the fists clenched. The jaws are fixed and the pulse may be difficult to detect. The first seizure usually lasts one or few minutes. In severe poisonings, each convulsion tends to last longer than the one before and the intervals between the convulsions tend to grow shorter. As many as 10 convulsions separated by an interval of 10-15 minutes may be experienced, but death commonly occurs between the second and fifth paroxysm. The victim may tolerate being lifted and moved provided the action is expected and gentle, but a sudden unexpected light touch may throw him into tonic convulsions (113).

Death may occur at any time. Rarely, with very high doses, it may follow sudden collapse before convulsions develop but is usually due to brain damage, secondary to apnea from uncontrolled
seizures or to cardiac arrest a medullary paralysis. If recovery occurs, it is remarkably prompt and complete in spite of the violence of the illness. The soreness and tightness of muscles may persist for a day or two. The body temperature may be normal, but it may sometimes rise and be abnormally high for a day or more, long after the convulsions have stopped (119).

**Lethal doses of Strychnine reported in literature:**

<table>
<thead>
<tr>
<th>Type</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDLo oral Human</td>
<td>30 mg/kg</td>
</tr>
<tr>
<td>LDLo unknown Human</td>
<td>1103 µg/kg</td>
</tr>
<tr>
<td>LD50 oral rat</td>
<td>16 mg/kg</td>
</tr>
<tr>
<td>Intraperitoneal rat LD50</td>
<td>2500 µg/kg</td>
</tr>
<tr>
<td>Scutaneous rat LD50</td>
<td>1200 µg/kg</td>
</tr>
<tr>
<td>Intravenous rat LD50</td>
<td>960 µg/kg</td>
</tr>
</tbody>
</table>

**Reported cases of Strychnine poisonings:**

In a review of nine fatalities due to Strychnine, the following post-mortem tissue concentrations of Strychnine were reported.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Concentration (µg/g)</th>
<th>Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>0-61 µg/g (mean 25, 5 cases)</td>
<td></td>
</tr>
<tr>
<td>Brain</td>
<td>0.47, 4.2 and 5 µg/g (3 cases)</td>
<td></td>
</tr>
<tr>
<td>Kidney</td>
<td>0.07-90 µg/g (mean 36, 6 cases)</td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>0-209 µg/gm (mean 95.5, 8 cases)</td>
<td></td>
</tr>
<tr>
<td>Spinal Cord</td>
<td>0.1, 1.8, 1.9 µg/g (3 cases)</td>
<td></td>
</tr>
<tr>
<td>Urine</td>
<td>1, 2.5, 7.7 µg/g (3 cases)</td>
<td></td>
</tr>
</tbody>
</table>

A typical case of suicidal Strychnine poisoning by a rodenticide is presented. The deceased, a 56 year old male was found within a locked apartment, in pain. He confessed to having ingested half a can of mole poison with suicidal intent. The can contained green pellets with an original capacity of 140 gms with 0.35% Strychnine sulfate. The patient died soon. The autopsy was performed 3 hours after death and the post-mortem findings included the presence of 61 gms of thick green, mortar-like material in the stomach similar to the contents of the can. The light microscopic study of the spinal cord indicated haemorrhages around capillaries. Some neurons showed chromatolysis. Toxicological analysis of blood, bile, vitreous fluid, urine as well as various body tissues performed revealed the presence of Strychnine. The concentrations of Strychnine in the tissues were 175.0 mg/L in stomach contents, 149 mg/L in stomach, 9.2 mg/L in liver, 3.3 mg/L in blood to 1.4 mg/L in the urine. (127)

A case of acute Strychnine poisoning is reported where an enormous dose of Strychnine caused a rapid death with little evidence of the classical symptoms of Strychnine poisoning. The deceased was a pharmacist. At 10 o'clock one morning, he was in his shop when he sent all but one
of his assistants on various errands. Eighteen minutes later his assistant found him collapsed in his room. Five minutes after death the doctor attending him predicted his death at 10.18 a.m. itself. There is good evidence that death occurred within 15 minutes of swallowing the poison, and probably even less. There was no evidence of convulsions and no trace of muscular rigidity. A bottle partly filled with Strychnine hydrochloride was found beside the man and crystals of Strychnine hydrochloride was found in his mouth and down the front of his pullover and on the floor surrounding the body. Chemical analysis of the viscera and body fluids showed the presence of Strychnine. The stomach and its contents contained 653 mgs, liver 125 mgs, blood 4 mgs/100ml, kidneys 30 mgs, urine trace amounts and bowel 4,608 mgs of Strychnine. Assuming a circulation volume of 5 litres, the total amount of Strychnine in the blood would be 200 mgs. There was a total recovery of 5.6 gms of Strychnine from the main viscera and it was estimated that the deceased had consumed 340 ml of Strychnine hydrochloride solution containing 3.4 gms of Strychnine hydrochloride but the amount of crystalline Strychnine hydrochloride taken was not known. (102).

Eight cases of unusual Strychnine poisoning and their treatment have been reported, where 8 young adults sniffed quantities of Strychnine in the mistaken belief that it was Cocaine. Apart from one patient in whom the poisoning was fatal and the most minimally affected, who recovered without specific treatment, the remaining 6 patients were comparable in terms of intoxication levels and concentrations of Strychnine in urine (not detected after 36 hours). The urine analysis of the patient who had ingested the largest amount of Strychnine showed large amounts of Strychnine (69).

The number of fatal accidents caused by Strychnine intended for use as a rodenticide, from 1939 to 1959 decreased, but then levelled off (72). However, cases of accidental poisoning and suicide are still seen. In 1978 and 1981 there were 13 deaths from Strychnine poisoning in the U.S.A. (129) and in 1983 there were 30 hospital admissions (41). A disturbing trend is that several recent cases have involved adulteration or replacement of illicit drugs with Strychnine, like inhaled Cocaine (22) or intravenous Heroin (39).

In India, many cases of homicidal, suicidal and accidental poisonings due to Strychnine are recorded in the Textbook of Medical Jurisprudence and Toxicology by N.J. Modi (113).

The case of a 36 year old woman is described who was prescribed a medicinal dose of 1/2 ounce of liquor Strychnine. When the contents of the bottle had been emptied, she added some water and drank off the contents. An hour afterwards she suffered from Strychnine poisoning and died within two and a half hours. In another case an Anglo Indian lady took a teaspoonful of Strychnine with suicidal intent and died an hour later. A thirty two year old male suicide took Strychnine and died within 15 minutes. This case too like the one described by Lloyd showed the absence of classical symptoms of Strychnine poisoning like spasms and convulsions (113).
Homicidal poisonings by Strychnine have occurred in India. A case was reported in which a man in Sioni was poisoned when he took some betel offered to him at a singing party by 2 persons who were not on good terms with him. Strychnine was detected in the stomach wash and the soil in which the man had spat (113). In another case the adopted son of a Hyderabad millionaire was killed by administration of pills containing Strychnine while in another case, a person administered Strychnine in a cup of wine to another who died within 3 hours (113). A case was recorded by the chemical examiner of Uttar Pradesh in his annual report for 1948. A person in Moradabad was given some wine with Strychnine. He threw it out of his mouth but his son drank the remaining wine in the cup and died within half an hour. Strychnine was detected from portions of his viscera and vomit.

Nuxvomica seeds, which contain Strychnine and Brucine have been used in India for suicides and homicides. A case was recorded by the chemical examiner of Bombay in 1927 where three brothers of Malvar committed suicide after consuming milk in which Nuxvomica seeds were boiled. Strychnine and Brucine could be detected from their viscera. In another case recorded by the Chemical Examiner of Bombay, a 22 year old girl died of drinking a decoction in which particles of Nuxvomica leaves were later detected. Brucine and Strychnine were detected from the stomach and intestines (113). The chemical examiner of Bombay cites two cases of accidental poisoning. In one case, two female children aged three and five years respectively were given some powder as a quack remedy for worms and both died of convulsions within half an hour, while in the other case a woman was given some stuff which turned out to be Nuxvomica and she died. Examination of viscera revealed the presence of Strychnine and Brucine.

All these cases indicate that Nuxvomica is used for poisoning in India and such cases are referred to the Chemical Examiner for analysis of Strychnine and Brucine. Therefore stability studies of Strychnine are conducted to provide information in the diagnosis of poisoning cases.
ACETYL SALICYLIC ACID.

Acetyl Salicylic acid (Aspirin) is an analgesic drug which relieves pain without inducing sleep or narcosis and an antipyretic drug which lowers the temperature in case of pyrexia. It has also got antithrombotic and anti-inflammatory properties. It is one of the household remedies for pains, aches and pyrexia (124).

Salicylates were one of the oldest compounds discovered from Willow Bark (133). Further in Germany, Koth managed to synthesise Salicylic acid. It was found by Hofmann that Salicylic acid caused irritation of the gastric area on consistent ingestion and in a search for better medication, Acetyl salicylic acid was synthesised. Today more Salicylates have been synthesised and are one of the least expensive and most widely used drugs (110). The massive consumption of Aspirin is alarming as it is a dangerous drug causing problems of overdosage, especially in children.

Structure :-

```
 COOH
```

Molecular Formula :- \( C_9 H_8 O_4 \).

Molecular Weight :- 180.2.

Solubility :- Soluble 1 in 300 of water, 1 in 6 of Ethanol, 1 in 17 of Chloroform and 1 in 20 of Ether.

Absorption, Fate and Excretion :-

Acetyl salicylic acid is usually administered orally. Its rate of absorption is dependent upon the amount of available non-ionised and non-protein bound drug and surface area of the absorptive membrane (100). It is rapidly absorbed orally. Oral bio-availability is 80-100%. \( t_{\text{max}} \) and \( c_{\text{max}} \) (75, 99) vary with preparation with buffered Aspirin performing better than unbuffered preparation or enteric coated tablets. Aspirin administered rectally is absorbed slowly (98). Large, potentially lethal doses of Aspirin taken accidentally or with suicidal intent may be absorbed much more slowly than therapeutic doses due to the inhibitory effect of Aspirin on gastric emptying and the impaired dispersion of the drug in GI fluids. Plasma concentrations of the drug in acutely overdosed patients may rise continually for as long as 24 hours and the appearance of toxic effects may be delayed. Gastric
lavage or induced vomiting can remove ingested Aspirin from the stomach but not from the intestine. Activated charcoal is an effective inhibitor of Aspirin absorption from the stomach and intestinal tract. The t_max of Aspirin is 1-2 hours (102). Once absorbed, salicylates are converted to Salicylic acid within a few minutes.

**Distribution :-**

The distribution of Aspirin is reflected by the Volume of Distribution (Vd) of the drug. Vd ranges from 150-200 ml/kg at therapeutic concentrations. Newborns may have a higher Vd due to the binding of Salicylic acid with plasma albumin. The pharmacological effect of Salicylic acid is produced by the unbound drug and not by those bound to plasma or tissue proteins. (98)

**Metabolism and Elimination :-**

Aspirin is rapidly hydrolysed to Salicylic acid, which is an active ingredient. Salicylic acid further undergoes metabolism by conjugation with Glycine and Glucoronic acid. The Glycine conjugate, Salicyl uric acid is the major metabolite. The glucoronide conjugates, are Salicyl acyl glucoronide and Salicyl phenolic glucoronide. These on further hydroxylation yield Gentisic acid and Gentisuric acid (98). The formation of Salicyl uric acid and Salicyl phenolic glucoronide are the two quantitatively most important biotransformation pathways. Due to the limited capacity of two of the elimination routes, the quantitative composition of the urinary metabolites of Salicylic acid is dose-dependent with small doses being excreted mainly as Salicyl uric acid and Salicyl phenolic glucoronide, the rest as Salicyl acyl glucuronide, Gentisic acid and Salicylic acid. On increasing the dose, the proportion of the latter compounds increase. (98) t 1/2 for Acetyl salicylic acid is 15-20 minutes. (140).

The excretion of Salicylic acid and its metabolites in the urine is pH dependent with 80% of the dose appearing unchanged in urine at pH 8 and 10% at pH 4 (99).

**Pharmacological actions:**

Aspirin inhibits by acetylation, cyclooxygenases which convert Arachidonic acid to cyclic endoperoxides, the first step in the synthesis of thromboxanes and prostacyclins (25, 138). Inhibition of thromboxanes synthesis underlines the antithrombotic effect while inhibition of prostaglandin synthesis may explain the anti-inflammatory properties. The action of Aspirin in reducing temperature and pain was postulated to be due to its effects on the hypothalamic nuclei (103). Aspirin treatment in patients of hyperpyrexia and pain reduces fever and offers relief from mild to moderate pain such as might occur with headache, arthritis, myalgia and dysmenorrhoea. It is used as an anti-inflammatory agent in rheumatic fever and radiation colitis. Regular Aspirin intake is associated with a decreased incidence of myocardial infarction (21) and with a decreased incidence of thrombosis in patients with hemodialysis. Where inhibition of platelet function and thrombosis are required, low dose Aspirin seems preferable to higher doses since thromboxane synthesis may be inhibited preferentially to the...
production of Prostacyclin which has antiplatelet aggregating properties (8, 25). Inhibition of Prostaglandin synthesis may also produce side effects such as peptic ulceration and bronchospasm (154).

Toxicity:

Poisoning from Aspirin results from idiosyncrasy or excessive dose. The symptoms include giddiness, buzzing in the ears, oedema of the face and eyelids, cyanosis and breathlessness. The patient is seldom drowsy or unconscious and these signs are of serious significance when present. Aspirin in doses larger than 150 mg/kg irritates the mucous membrane of the stomach and produces nausea and vomiting. Serious toxicity can be seen with ingestion of greater than 400 mg/kg. The principal effects of Aspirin toxicity are severe vomiting, hyperventilation due to stimulation of the respiratory centre of the brain leading to hyperpnea and respiratory alkalosis. It also uncouples oxidative phosphorylation leading to increased oxygen utilization and glucose demand, which further leads to increased gluconeogenesis and increased heat production. The Krebs cycle enzymes are also inhibited leading to decreased glucose availability, an increase in organic acids and alterations in lipid metabolism and amino acid metabolism enhancing metabolic acidosis (16). Clinical conditions may progress to pulmonary oedema and acute renal failure or death (5). Aspirin causes severe acidosis due to reduction in alkaline reserve. Ketosis, albuminuria and glycosuria are not uncommon.

Reported cases of Acetyl salicylic acid poisoning:

Salicylate intoxication is most commonly due to the ingestion of Acetyl salicylic acid. In a study of 73 adults hospitalized with Salicylate intoxication, 53 patients volunteered that they consumed salicylates in large quantities and were young in age. The remaining had been unintentionally poisoned and were older. They had been using salicylates for medical problems (5). Four cases of Aspirin poisoning treated without dialysis were reported (58).

(A) One man aged 18 consumed 32 grains of Aspirin and after being admitted to the hospital a stomach wash was performed and he survived. However salicylate levels in serum were not reported.

(B) A 47 year old man, in a state of anxiety had taken 32 gms of Aspirin at night and was admitted to hospital where a stomach wash was carried out the next afternoon. The plasma salicylate level reported was 66 mg/100 ml.

(C) A chronically depressed unemployed man aged 46 years took 42 gms of Aspirin and on hospital admission, stomach wash was carried out. Plasma salicylate was found to be 72 mg/100 ml and the patient died suddenly.

(D) A 28 year old man ingested 32 gms of Aspirin and stomach wash was done. Blood salicylate acid level was not reported.

Another report describes the treatment of 460 patients over 12 years of age admitted to the Regional Poisoning Treatment Centre, Royal Infirmary, Edinburgh U.K. over a period of 3 years for Salicylate poisoning and the highest measured plasma salicylate concentrations ranged from 14 mg/100 ml to 106 mg/100 ml (130).
A case of a 44 year old man admitted to Georgetown University Hospital was reported. He had been found unconscious in his room and was suspected to have consumed 210 gms of Aspirin. Blood salicylate levels were 91 mg/100ml after 7 hours past admission. He had been taking 5 tablets per day for headache and had gradually increased the dose to 50 tablets per day. On the day before admission to hospital he admitted to have consumed the usual amount, but had combined it with Coca-Cola which had produced synergistic effects. (146)

Another case of a 19 year old woman is recorded. She claimed to have dissolved 83 gms (1 bottle) of Aspirin in a glass of water and drunk half of it. The blood salicylate level was 90 mg/cc. Serum salicylate slowly declined and disappeared on the 6th day. The recovery from urine was 3.4 gms (146).

Another report describes the successful treatment of 10 adults suffering from salicylate overdosage with Sodium bicarbonate and Acetazolamide. Their initial serum salicylate concentrations ranged from 51 mg/100ml to 88 mg/100ml. After treatment for a time ranging from 6-7 hours there was a recovery of salicylate ranging from 2.2 gms to 9.8 gms. The salicylate half life in these patients ranged from 3.2 to 9.8 hours.

In the Regional Poisoning Treatment Centre Edinburgh U.K., 208 patients were hospitalised after taking overdose of salicylates. Out of them three patients died. Nine patients selected for haemodialysis had an estimated intake of 53, 100, 53, 23 and 60 gms Aspirin and showed serum salicylate levels of 58, 67, 79, 100 and 115 mg/100ml. Another patient showed a serum salicylate level of 75 mg/100ml which on admission 3 hours later rose to 115 mg/100ml. He died before haemodialysis could be undertaken. (16)

A multidrug poisoning case was reported about a 35 year old Caucasian male found dead in the kitchen floor. He was a known user of abused drugs and had been taking Aspirin alone or in combination with Phenacetin and Caffeine for relief of joint pain. The toxic substances present in the blood and in urine respectively were Salicylate (185 & 2750 mg/L), Caffeine (16 & 37 mg/L) and Phenacetin (9.6 and 20 mg/L). Acetaminophen was also present in plasma and urine (28).

All these above cases indicate that Aspirin is widely used in poisoning. As there is an increasing number of Aspirin poisoning cases in India and as very often, samples of Aspirin poisoning are received by Forensic Science Laboratories for chemical analysis, stability studies of Aspirin are essential and hence were taken up in the present study.
PARACETAMOL (ACETAMINOPHEN).

Paracetamol, a metabolite of Phenacetin, is now becoming a more common cause of overdosage because of its widespread use in place of Aspirin (124). It has analgesic and antipyretic properties comparable to Aspirin, but devoid of anti-inflammatory action (124). Paracetamol was introduced in medical use in 1893, however it did not gain popularity until the 1950's (150).

Structure:

\[
\begin{align*}
\text{OH} \\
\text{NH} \cdot \text{CO} \cdot \text{CH}_3
\end{align*}
\]

Molecular formula: \( C_8 H_9 N_02 \)

Molecular weight: \( 151.2 \)

Melting point: \( 168 \) to \( 172 \degree \) C.

Synonyms: Acetaminophen, N-acetyl p-aminophenol

It is in the form of white crystals or crystalline powder, soluble, 1 in 70 of water, 1 in 10 of Ethanol, slightly soluble in Chloroform and practically insoluble in ether (32).

Distribution and Disposition:

Small doses are readily absorbed, but the absorption of larger doses is influenced by gastric emptying rates, the presence of food and the time of day. Oral bio-availability of Paracetamol is 100%. It has a tmax of 1/2 to 1 hour depending upon the preparation (13). Paracetamol has a volume of distribution of 0.8 to 1.0 L/kg (148). It is widely distributed in most of the body fluids and is present in the saliva at concentrations parallel to that of plasma. Therapeutic concentrations in the plasma range from 10-20 \( \mu g/ml \). Following a single oral dose of 1.5 gms to 14 subjects peak plasma concentrations of 7.4 to 3.7 (mean 24) \( \mu g/ml \) could be attained in 0.5 to 8 (mean 1.4) hours (\( \). Following daily oral doses of 1.8-3.6 gms to 8 subjects, peak plasma concentrations obtained one to two hours after a dose ranged from 9.9 to 43.3 (mean 23.7) \( \mu g/ml \) (\( ). About 25% of an absorbed dose of Paracetamol is bound to protein with highest concentration in liver.
Metabolism:

Paracetamol undergoes first pass metabolism and is metabolised mainly by conjugation to glucuronic acid, and ethereal sulphates. Following conjugation, 3-hydroxylation or O-methylation of the hydroxy group takes place. Oxidation to a reactive metabolite, thought to be acetyl aminophenol, occurs to a smaller extent but becomes more significant after larger doses. This metabolite and a minor deacetylation product and intermediate N-hydroxyl derivatives are relevant to toxicity. About 90% of a therapeutic dose is excreted in urine within 24 hours. Of the excreted material, 1-4% remains unchanged, 20-30% is conjugated with sulphates and 40-60% with glucuronic acid. About 5-10% consists of 3-hydroxy 3 sulphate, 3-methoxy glucuronide and 3 methoxy 3 sulphate metabolites and about 5-10% consists of Mercapturic acid and cysteine conjugates. Larger amounts of Mercapturic acid and cysteine conjugates are excreted in overdose. There is a slight diurnal variation in metabolism (148). The t 1/2 of Paracetamol is 2-4 hours. Although t 1/2 is similar throughout life, the infant and young child produce more sulphate than glucuronide (111).

Pharmacological Actions:

Although equivalent to Aspirin as an effective analgesic and antipyretic agent, Acetaminophen differs by its lack of anti-inflammatory properties. It does not affect uric acid levels and lacks platelet-inhibiting properties. This drug is useful in mild to moderate pain such as headache, myalgia, postpartum pain and other circumstances. It is preferable to prescribe Aspirin in patients with haemophilia or a history of peptic ulcer and in those in whom Bronchospasm is precipitated by Aspirin (85). Acetaminophen has become popular because it is not known to cause gastrointestinal bleeding or to affect blood clotting (112).

Toxicity symptoms:

In acute poisoning, Paracetamol does not behave like Aspirin. Vomiting which usually occurs a few hours after the tablets have been taken is seldom severe or accompanied by other symptoms and the patient remains fully conscious. This initial absence of gross disturbances not infrequently leads to a false sense of security. There may however be anorexia, nausea and epigastric pain. Case histories of acute Paracetamol poisoning include liver damage in many instances, gastro-intestinal haemorrhage, cerebral oedema and renal tubular necrosis. The fatal dose has individual variation but usually results from ingestion of more than 20 tablets containing 500 mgs of Paracetamol (124). Paracetamol when taken in overdosage may produce acute centrilobular hepatic necrosis. There are no specific early symptoms or signs of Paracetamol poisoning and consciousness is not impaired (88).

The minimum lethal dose of Paracetamol is about 10 gms/kg body weight (32). Symptoms of hepatic damage do not occur for at least 12 hours after overdose, but may not appear until 4-6 days later. Liver necrosis is possible at concentrations of about 120-300 ugs/ml in blood. Although
recovery of liver function in a small minority of severely poisoned patients has been reported, it is often fatal. Acute renal failure after Paracetamol intoxication was observed (143). Toxic symptoms of Paracetamol reported in humans are oral LDLo's varying from 143 mg/kg to 357 mg/kg and oral TDLo's varying from 77 mg/kg to 490 mg/kg. (137)

Post-mortem tissue concentrations reported in fatalities are as follows:

- Bile: 180-900 μgs/ml.
- Liver: 385 μgs/gm.
- Liver blood: 200-475 μgs/ml.
- Urine: 180, 620 μgs/ml. (159)

Reported cases of Paracetamol poisoning

The use and abuse of Paracetamol in Dade County, Florida over a ten year period was reported (128). Ninety five cases of Acetaminophen were detected. In four of these cases Acetaminophen was the cause of death and blood drug concentrations were 348 mg/l, 30 mg/l, 77 mg/l and 130.8 mg/l. Suicides and accidental deaths comprised 2/3rd of the total 95 cases. Paracetamol overdose deaths in England and Wales numbered 35 in 1972 and 190 in 1978 (128). Acetaminophen as a suicide tool is a poor choice as the lethal hepatic necrosis takes several days to develop. However, it is consumed as a suicidal agent mainly because of its easy availability. Few of the suicides knew that Acetaminophen causes hepatic necrosis. However, even with this knowledge, they expected to become rapidly unconscious. It appears that advertising and easy availability of Acetaminophen over the counter are responsible for the increased use and concomitant abuse of the drug (128). In India too, recently the Forensic Laboratories often receive samples of Paracetamol poisoning for chemical analysis.

Following are some of the cases of Paracetamol poisoning reported in literature:

1. A 65 year old female was found stuporous, with a history of suicidal ingestion of 75 Tylenol. She survived for 3 1/2 days. Blood Paracetamol concentration on admission was 348 mg/l. (159)
2. A 31 year old female epileptic with past episodes of drug overdose and mental illness ingested approximately 35 Tylenol capsules. Subsequently she vomited. She survived for five days. On admission blood Paracetamol concentration was 30 mg/L. (159)
3. A 49 year old female was found unconscious following a past history of mental illness and drug overdose. She survived for two days. Admission blood drug concentration of Paracetamol was 77 mg/l (159)
4. A 72 year old female ingested Tylenol and died 4 days later. She was a constant user of
Analgesics for hip joint pain. She also suffered from acute myocardial infarction. Blood Paracetamol concentration on admission was 130.8 mg/l. (159)

(5) The case history of a 26 year old emaciated woman who developed liver necrosis and acute renal failure after ingestion of thirty, 500 mg tablets is described. Under the influence of enzyme systems, reactive metabolites may be produced in the liver causing hepatic necrosis. Similar metabolic activation may occur in the renal tubules as well, but under the influence of different enzyme systems, malnutrition may potentiate the hepatotoxic and nephrotoxic action of Paracetamol as observed.

These observations of Paracetamol poisoning cases show that people started using Paracetamol for self-poisoning. Such drugs need stability studies for accurate prediction of dose used and time of poison. Therefore Paracetamol has been included in the present stability studies of poisonous substances.
Benzodiazepines are one of the modern sedative-hypnotic drugs introduced in the treatment of psychosomatic disorders such as anxiety and insomnia. They are also used in the management of alcohol withdrawal and epilepsy. While Meprobamate offered questionable advantages over Phenobarbital, the Benzodiazepines exemplified by Chlorodiazepoxide and Diazepam were improvements. They were not necessarily more efficacious, although prevailing opinion is that they were far safer in regard to physical dependence and suicidal overdose (17).

Now Benzodiazepines and related compounds are a rapidly expanding group of drugs. For some years, Diazepam has been the most widely prescribed drug in most of the countries, with its close relative Chlorodiazepoxide, also retaining higher degrees of use (92). Success has brought competition first with Oxazepam and Chlordiazepoxide and more recently with Lorazepam, Prazepam and others. Flurazepam is the only Benzodiazepine promoted as a hypnotic, but it may soon have competition from a chemically close relative - Triazolam (77). Benzodiazepines have been taken by more persons and this extensive medical use provided innumerable opportunities for misuse by every conceivable, stable and unstable person. A number of clinical reports of spontaneous dependence on Benzodiazepines have appeared in literature (107, 3). The Benzodiazepines that are commonly prescribed are listed below.

(a) Anxiolytic and muscle relaxants:
   Long Acting: - Diazepam, Chlorodiazepoxide, Nordiazepam.
   Short Acting: - Oxazepam, Lorazepam.

(b) Hypnotics:
   Long Acting: - Flurazepam, Nitrazepam.
   Short Acting: - Midizolam, Triazolam

(c) Anticonvulsants: - Clonazepam.
   Among the Benzodiazepines, Diazepam is described in detail below.

DIAZEPAM
Structure:
Chemical formula: C16H13ClN2O

Molecular Weight: 284.78

Solubility: Slightly soluble in water, soluble 1 in 25 of Ethanol, 1 in 2 of Chloroform and 1 in 39 of Ether.

Absorption: Diazepam is rapidly and completely absorbed after oral administration. The oral bio-availability of Diazepam is 75% and the oral tmax is 1 hour after administration (89). Intra muscular bioavailability is erratic and intra muscular tmax is 35-75% (104).

Distribution: Volume of distribution of Diazepam is 1.1 L/kg. Protein binding in plasma is 98-99%. Diazepam and its metabolite, N-desmethyldiazepam appear in fetal circulation and in breast milk (34). 1-4% of Diazepam and 3-4% of the metabolite plasma concentrations appear in cerebrospinal fluid and these equal the unbound fraction in plasma.

Metabolism: Diazepam is partially metabolized by demethylation to the active metabolite N-desmethyldiazepam and by hydroxylation to the active metabolite Oxazepam and temazepam. N-desmethyldiazepam is metabolized to Oxazepam which is the major metabolite in urine.

Excretion: Half life of Diazepam is 20-100 hours in the plasma and it appears to increase in neonates and the elderly. Most Benzodiazepines including Diazepam are characterized by a long elimination half life and are biotransformed into pharmacologically active metabolites. Thus it is unlikely that the rapid elimination of active compounds will account for recovery. Only small traces of unchanged Diazepam are excreted in urine and the relative amounts of metabolites are variable and appear to be dose dependant. About 70% of a dose is excreted in the urine, mainly as Oxazepam glucuronide and conjugated Desmethyl diazepam together with smaller amounts of conjugated Temazepam. About 10% of a dose may be eliminated in the faeces (32).

Pharmacological Actions: The major effects of the Benzodiazepines is to depress the central nervous system at all segmental levels. This accounts for most of their clinical uses which range from sedation to anaesthesia (70). The sedatives, antianxiety and hypnotic drugs cannot be clearly separated pharmacologically because drugs that are classified in one category may also fit into another category if the dose is appropriately adjusted. These Benzodiazepines produce muscle relaxant effect, may be central (via) an interneuronal process that directly affects electrical stimulation of facilitatory and inhibitory interneurons in the brainstem reticular system. It restores behavior that has been suppressed by punishment and reduces behavior that has been motivated by punishment.
Reduction in Brain Dopamine, may biochemically explain some of its effects. However more recently, the actions of Benzodiazepines on gamma-aminobutyric acid mediated synapses have been emphasized. Areas of their action include the amygdalo-hippocampus systems and the non-adrenergic projections from the Locus ceruleus to the cerebral cortex. It has been demonstrated that specific Benzodiapine receptors exist in the human brain and are concentrated in the cerebral cortex.

**Toxic Symptoms:**
Paradoxical excitement, hostility or rage reactions are adverse effects that "Behavioral-Toxicity" infrequently manifests. Three cases of Rage reactions were described during the early clinical study of Chlorodiazepoxide. Two additional patients in this study showed irritability and hyper activity. Oral Clonazepam is used as an anticonvulsant and during such use it has been reported to produce irritability, irrational social behavior and outbursts of aggressiveness. "Strange" behavior has been observed with Oxazepam. One patient reported unusual body sensations, another became unusually argumentive and a third was arrested for disrobing in public. Recent experiences of drug surveillance programmes indicated that of 2086 patients treated with chlorodiazepoxide only 6 (0.3%) had central nervous system excitation (Insomnia, agitations, hallucinations etc.). Of 2623 patients treated with Diazepam only 4 cases of such excitation were observed. Some anxious patients treated with Benzodiazepines seem to become depressed. Nothing in the known Pharmacological actions of these drugs would suggest that they are "depressogenic" Anticholinergic symptoms such as dry mouth, tachycardia, dilated pupils and absence of bowel sounds may be seen.

The individual toxicity of drugs ie. Diazepam, Oxazepam and Chlorodiazepoxide are described below.

**Diazepam**
Diazepam is a tranquilizing agent with anticonvulsant and muscle relaxant properties used therapeutically in doses of 2 to 10 mg, three to four times a day. Maximal single dose in man (suicidal attempt) is reported to be 300 to 400 mg. This patient was drowsy and ataxic for 8 hours. Common side effects include fatigue, dizziness and ataxia. Diazepam may potentiate the respiratory depressant effects of opiates and barbiturate-like sedatives.

**Oxazepam**: It is one of the Benzodiazepine group of drugs used in the management of anxiety-tension states. A poisoned infant (unknown dose) exhibited lethargy, ataxia, paradoxical excitation, depressed reflexes and facial oedema. These signs persisted several days after detectable blood levels of the drug much longer than the usual sedation seen in adults. One adult (unknown dose) was in a deep coma for 3 days.
**Chlorodiazepoxide Hydrochloride:**

Chlorodiazepoxide is a tranquilizer with a chemical structure related to Diazepam and Oxazepam. It can be described as a useful ataractic, a potent muscle relaxant, an effective anticonvulsant and a weak analgesic. In high doses it produces ataxia, emesis, and diarrhoea. With usual doses (up to 40 mg daily) side effects are not common. Drowsiness, ataxia and emesis may be seen with doses up to 100 mg daily. Occasionally, small doses result in sleep or coma (172, 147).

**Reported cases of poisoning:**

Benzodiazepine overdoses have been reported between 1962 and 1975. 773 patients with acute overdosage of drugs were admitted to Massachusetts General Hospital. Benzodiazepines were involved in 99 cases (13.0%) with a gradually increasing proportion over the years (62). An extensive survey of 27 medical examiners' or coroners' offices in United States and Canada was conducted during the later part of 1976. The combined jurisdictional population of these states was 79.2 million people. Diazepam was found to be present on toxicological analysis in 1239 cases of death (50). Three case histories of Triazolam deaths have been described. All cases were suicides and Triazolam levels present in these cases were above therapeutic levels (10). All these reveal that Benzodiazepines are widely consumed as poisons and such cases are also referred to Forensic Science Laboratories in India for chemical detection of this poison. Considering this, stability studies for Benzodiazepines are included in the present investigation.
LITERATURE REVIEW ON THE STABILITY OF DRUGS IN BIOLOGICAL TISSUE.

Analysis of biological tissue like blood, serum, urine and post-mortem viscera for drugs are performed for various reasons. Some of the reasons are:

- to indicate exposure to a drug,
- to correlate physiological or behavioral effects with a drug,
- to treat cases of drug overdose and
- to predict involvement of drug in fatalities.

Both ante-mortem and post-mortem samples may be available to the toxicologist. Ideally they must be analysed immediately in order to minimise any decomposition of drugs or metabolites. This is not always practically possible. Toxicological analyses, are frequently performed some time after the acquisition of the sample. This interval may vary from one to two weeks and may even be three to four months in laboratories overloaded with work. As the result of these tests are frequently involved in criminal or civil litigation it is essential that the results accurately reflect the drug value present at the time of sample acquisition.

Various factors which affect drug stability in biological samples are:

1. RBC to plasma distribution:-
   Haemolysis increases the plasma level of a drug strongly bound to RBC. Chloroquine is one such drug. RBC can stabilise some solutions and may absorb drugs displaced from protein binding sites.

2. Temperature:-
   Reducing the temperature effects the RBC to plasma ratio, reduces the rate of decomposition and decreases the activity of enzymes like esterases. Errors may however arise due to stratification of drugs near the surface making them more prone to oxidation and loss due to thawing.

3. Specific inhibitors of enzyme activity:-
   They help increase drug stability, for instance Physostigmine prevents the deactylation of Acetyl salicylic acid.

4. Presence of anticoagulants, preservatives and exposure to daylight (Appendix 3):-
   Some of the drugs, whose stability in biological tissues have been studied are Barbiturates, Benzodiazepines, Cannabinoids, Ethanol, Cocaine, L.S.D and Phencyclidine. Other drugs on whom stability studies have recently been performed are Chlorpromazine and Diltiazem in plasma, Diazepam, Phenobarbitone, Phentoin and Desipramine in formalin fixed tissues and formalin blood solutions and stability of Diazepam in injections. Numerous researchers have studied the stability of
the barbiturates in blood. In one study (36) a dog was dosed Pentobarbital. Its blood containing the drug was then stored at 4°C and 25°C and assayed at set intervals up to a period of two months. No significant changes in drug concentration were observed in the samples stored at 4°C. However, a significant reduction in concentration was observed in the samples stored at 25°C. This was attributed to the evaporation of blood and oxidation of barbiturates in the putrefactive process. In another study by Garriot et al. (56), in postmortem blood samples containing one or more barbiturates, minimal changes (less than 30%) in the blood concentrations of barbiturates (Amobarbital, Phenobarbital, Butabarbital and Pentobarbital) were observed after a period of two weeks. Levine (94) studied the stability of six barbiturates in blood over a three month period, stored at 4°C and 25°C. Greater than 78% of the original blood concentrations were present at the end of three months in both the cases indicating the barbiturates to be stable in blood. Phenobarbital in serum is stable at 4°C for twelve weeks (145) and at room temperature for six months (168).

In tissue, a 100% increase in the concentration of Pentobarbital is observed after a liver obtained from a Pentobarbital death was analysed after storage for 90 days at room temperature (1). Levine studied the stability of barbiturates in 10 gm portions of tissue up to a period of two months stored at room temperature and at 4°C. Only minimal changes in drug concentration were observed (94). Recent studies thus indicate the barbiturates to be quite stable in biological tissue.

The benzodiazepines have also been studied. Levine studied the stability of Chlorodiazepoxide and Norchlorodiazepoxide (97). Chlorodiazepoxide was found to be very unstable and a 5 mg/ml concentration stored at room temperature could not be detected after 8 days. Norchlorodiazepoxide was unstable, but could still be detected after two months. Sodium fluoride and oxalate related degradation of Chlorodiazepoxide, Norflurazepam and Demoxepam, two breakdown products, accounted for some but not all of the lost drug. A post-mortem blood sample containing 26 mg/L of Chlorodiazepoxide showed a 27% decrease within 7 days of storage at room temperature (56). Serum concentrations of Chlorodiazepoxide remained unchanged for nineteen days while blood concentrations decreased significantly. Chlorodiazepoxide in liver at a concentration of 77 mg/kg decreased below the limit of detection within three days (153).

Benzodiazepines with a nitro group, Nitrazepam and Clonazepam showed a significant decrease in concentration within a three week span at room temperature but remained stable at -20°C and 4°C over a one week period. Storage in the presence of light, increased decomposition. Sodium metabisulphite and antireductases like Silver nitrate slowed down but did not prevent the decomposition of Nitrazepam in plasma stored either in light or dark. In liver tissue Nitrazepam showed 70% losses after 3 days. The instability of Nitrazepam and Clonazepam may be due to reduction of nitro group to amino group. Aminonitrazepam, however could not be detected. Other benzodiazepines studied like Diazepam, Flurazepam and N-Desalkylflurazepam were stable at room temperature up to a period of five months.
Another group of drugs on whom stability studies have been carried out are the cannabinoids. The stability of cannabinoids and their metabolites depended upon the pH of the solution, the amount of oxygen and the presence of free radical compounds. (54, 55, 109, 133, 164). The rate of oxidation of cannabinoids depended upon the storage medium. Tetrahydrocannabinol (THC) in dry form, in Chloroform, Carbontetrachloride and Hexane solutions deteriorated about 10% each month. THC in Ethanol solutions was stable for greater than 75 days at room temperature and for greater than one year at 0-5°C. THC in blood and plasma is stable at room temperature up to two months, but shows a loss of 90% in 6 months. However at -10°C and 4°C it remained stable for up to 6 months (82). Atmospheric conditions, freezing and thawing and elevated temperatures for one day had no effect (170). THC is a lipophilic molecule and binds to hydrophobic surfaces causing losses. Studies confirming absorption of THC to rubber, plastic containers and unsilanized glass have been reported in literature. (55)

Cocaine is susceptible to chemical and enzymatic hydrolysis. Benzyl ecgonine and Ecgonine methyl ester are the hydrolysis products of cocaine. Cocaine in blood preserved with fluoride or organophosphates at physiological pH is hydrolysed to Benzoyl ecgonine only (78). Cocaine is most stable in blood when preserved with fluorides or organophosphates at pH 5. The stability of Cocaine in urine is dependent on pH and temperature (12, 57, 33) and in saliva is a function of temperature, container and preservative.

There is a large amount of data in literature indicating that under certain storage conditions, Ethanol can be produced in blood in vitro. These include high temperature, contamination with certain microorganisms and the absence of chemical preservatives. Ethanol concentration remain essentially unchanged for short periods of time regardless of storage temperature in the presence of fluoride. Long term storage of fluoridated blood samples causes decrease in ethanol concentrations (27). In urine specimens ethanol is less likely to be produced except in rare instances of high glucose concentrations and the presence of certain microorganisms (9). Preserving with sodium fluoride and storing at as low temperature as possible minimises changes in ethanol concentration.

L.S.D (Lysergic Acid Diethylamide) is much more stable in serum and urine than in blood at room temperature after a period of three days. (132) L.S.D. can however still be detected after six weeks in urine and blood at both room temperature and under frozen conditions. (126) In urine stored for 6 months at room temperature with or without fluoride, a decrease was observed. L.S.D concentrations were within 10% of control after four weeks of storage in polyethylene bottles protected or unprotected from light (52). L.S.D was stable in refrigerated urine.

Phencyclidine is stable in stored blood at room temperature up to 18 months with less than 30% differences from the original concentration. The regression line correlating the original and reanalysed blood concentration of Phencyclidine had a slope of 1. (30)
The stability of Diltiazem, a calcium antagonist widely used in the treatment of angina and related disorders, has been studied in plasma samples obtained from volunteers receiving Diltiazem (171). The major metabolites of Diltiazem are N-Desmethyl diltiazem, Deacetyl diltiazem and Deacetyl desmethyl diltiazem. No deterioration of Diltiazem occurred up to 8 weeks. But after twelve weeks, deterioration to N-Desmethyl-diltiazem and Deacetyl diltiazem occurred on storage at -20°C and -70°C. However in plasma samples spiked with Diltiazem, deterioration occurred after 4-6 weeks of storage with no concomitant increase in the two metabolites N-Desmethyl diltiazem and Deacetyl diltiazem. Thus decomposition of Diltiazem and N-Desmethyl diltiazem was effected by the plasma material. These compounds also appear to be more stable at -70°C than at -20°C.

The stability of Chlorpromazine and its metabolites in plasma has been studied by a few researchers. The effect of storage on the plasma concentration of Chlorpromazine in patients' plasma samples stored frozen, suggested that the plasma should be assayed on the same day. The concentration of Chlorpromazine and six of its metabolites in plasma samples of patients stored at -20°C for 24 hours and at -20°C for 1 week and -70°C for 4 weeks were compared. Chlorpromazine and 6 of its metabolites spiked onto human plasma and stored at -70°C up to 12 months were also studied. In all the above cases no significant differences were seen in the concentration. Thus Chlorpromazine and its metabolites can be stored up to one week in a freezer and upto 12 months in a biofreezer before analysis (29).

In order to assess the shelf life of pharmaceutical preparation, their stability has to be studied. Fyllinger et al (53) studied the long term stability of Diazepam injections. The hydrolysis of Diazepam in solutions is well documented. The two main degradation products of Diazepam are 2 Methyl Amino -5-Chlorobenzophenon (MACB), 3-Amino-6 Chloro-1 Methyl-4 Phenyl carbostyril (ACMP) and N-Desmethyl diazepam. Diazepam and its main degradation product (MACB) were quantitatively determined by H.P.L.C. and Spectrophotometry in 1 to 10 year old Diazepam injections. Not greater than 1.5% degradation of Diazepam was found after 10 years.

Stability of compounds in formalin-fixed tissues and blood solutions were studied (169). Buffered formalin solutions were added to spiked blood samples containing Diazepam, Phenytoin, Carbon monoxide and Cyanide to give formalin whole blood solutions of 5% and 8%. Drug losses were monitored daily for upto 30 days. At least 41% and 33% losses of Diazepam and Phenytoin were found over the 30 day period. Cyanide could not be detected immediately after addition of formalin and Carbon monoxide could not be detected after one week. Liver samples positive for Desipramine, and Phenobarbital when preserved in 5% and 8% formalin showed greater than 60% losses while Phenytoin under the same conditions showed little change. Therefore drugs at toxic concentrations can be detected with variable recoveries upto thirty days after fixation in formalin.
All the above studies have been made on temperature and storage conditions different from those encountered in India. In India, during the summer months, temperatures rise up to 43°C and in most laboratories due to the overload of work, storage and preservation facilities are inadequate. Thus the biological specimen and the poison and drugs present in them undergo degradative changes. This leads to instability of the drug and may even lead to degradation to a point when it is no longer detected. It is therefore very essential to study the stability of poisons and drugs in the temperature and storage conditions that prevail in the country and compare the results with earlier reports and suggest improvements and early analysis wherever necessary. Only then would the forensic report be justified.
REVIEW OF LITERATURE ON EXTRACTION AND QUANTIFICATION OF DRUGS FROM BIOLOGICAL TISSUE.

Extraction:-
Before a drug quantification can be made, it has to be removed from the biological matrix for analysis. For such extractions, disruption techniques like homogenisation, ultrasonication, peristaltic bag device, enzymatic digestion and chemical stabilization with detergents are used. Protein precipitation techniques have been used widely as a first step in separating a drug from its matrix. Precipitating agents such as Aluminium chloride, Tungstate, heat and strong acids have been used (74, 80, 79).

Once a biological matrix has been disrupted, liquid-liquid partitioning with a solvent chosen for its easy volatility and property for dissolving the drug to be extracted is done. In homogeneous solvent extractions, solvents are added to the sample which are miscible with it. Afterwards a salt or another liquid is added which effects a phase separation. Simultaneous extraction of acidic, basic and neutral drugs in gastric contents, urine and plasma with Ammonium sulphate added to separate the phase have been reported. pH plays a very important role in solvent extractions where the drugs are separated into various classes by selective partitioning between an organic and an aqueous phase at various pH values using a knowledge of the pKa values of the drugs concerned (80, 79, 156, 44) Other extraction processes include liquid/solid extractions where the drugs are first adsorbed on to columns (silica, ODS etc.) and are then eluted out with solvents such as Acetone, Diethyl ether, Chloroform or Methanol. Recoveries were similar to direct solvent extractions (26).

Quantification:-
Drug quantification methods include, thin layer chromatography, gas liquid chromatography which includes capillary GLC, GC-MS, GC-HS and pyrolysis gas liquid chromatography, high performance liquid chromatography and spectrometry. The two techniques of high performance liquid chromatography and derivative ultraviolet spectroscopy have been reviewed in detail.

Derivative ultraviolet spectroscopy:-
Ultraviolet spectroscopy may be in principle used to analyse solutions containing any member of light absorbing solute components. The spectra of 100 drugs in 0.05 M Sulphuric acid have been examined and reported (135). The ultraviolet spectra of several drugs are recorded in several texts (135, 175). Derivative ultraviolet spectroscopy is used to increase the selectivity and sensitivity of ultraviolet spectroscopy. Using second derivative ultraviolet spectroscopy, Paraquat could be determined in serum to 0.1 mg/l (81) Second and fourth derivative spectra decreased interference from Diquat in the analysis of Paraquat. (49) Second derivative spectra has also been used in the
This analytical technique was introduced in 1955 (67) and used in resolving two spectral lines of nearly equal wavelength range. This technique has proved particularly useful in eliminating interferences from spectral overlapping of structurally related compounds. The theoretical aspects of derivative spectroscopy have been reviewed in detail (67, 120). Many reports on the analytical aspects of derivative spectroscopy have appeared in literature in the past few years, which includes their application in Forensic toxicology (59), Pharmacology (115, 38), Biomedical application (48, 125) and in illicit drug analysis (95, 94, 93, 6 and 7).

Despite the advantages of derivative spectroscopy in eliminating interferences in zero order ultraviolet spectroscopy, few applications of derivative spectroscopy in forensic toxicology have been published.

This work includes the derivative ultraviolet spectroscopic studies of poisons and drugs and its application in their analysis in the presence of each other and from biological tissue.

**High Performance Liquid Chromatography (H.P.L.C):**

The advantages of H.P.L.C. to the toxicologist are that

1. It offers a complete measure of a drug's metabolic profile, from non-polar drugs to their polar metabolites.
2. It separates structurally similar compounds.
3. Recovery of the intact drug is possible because of its mild operating conditions.
4. It is possible to increase the specificity and sensitivity of the analysis by choice or by combination of detector systems.
5. High molecular weight compounds and those that are lightly hydrophilic can be chromatographed (112).

H.P.L.C. system for the analysis of 166 drugs of toxicological interest have been tabulated (121). Methods for the detection of toxic drugs in serum are described by Kabra et al (83). Numerous publications also give the relative elution orders of drugs under a variety of conditions (51). The reverse phase H.P.L.C. analysis of benzodiazepines have been described by several authors in various publications, some of which are

The determination of Chlorodiazepoxide and its N-Demethyl metabolite in blood samples by H.P.L.C. (64).


The H.P.L.C. separation of benzodiazepines and their metabolites (60).

Report on the H.P.L.C. analysis of antidepressants include a report by Dong and Dilesare (42), where a silica column was used along with a mobile phase of Acetonitrile: Methanol: Ammonia using ultraviolet and fluorescent detection for the analysis of Imipramine and Desipramine. Twitchett et. al (165), also report a method for the detection of antidepressants using Methanol, Ammonia and Ammonium nitrate with UV detection at 254 nms.

H.P.L.C. analyses of Paracetamol include Ramsay's report (131) where a Lichrosorb RP 8 column with Methanol water containing 5 gms of Sodium acetate and 5 ml of Acetic acid per litre as mobile phase was used with UV detection at 240 nms.

Quite a few reports on the H.P.L.C. analysis of Chloroquine and its metabolite Monodesethyl chloroquine are published in literature (15, 151). A rapid H.P.L.C. procedure for the simultaneous determination of Chloroquine, Monodesethyl chloroquine, Diazepam and Nordiazepam in blood is reported by Estadieu et. al (47).

Reports on the HPLC analysis of Strychnine include that of Elliot et al. (4), where a Silica column was used along with a mobile phase of Ammonia: Methanol with UV detection at 254 nms.

Reports on the H.P.L.C. analysis of Aspirin include that of Reid (134) where a Lichrosorb RP 18 column is used with a Methanol: water mobile phase at pH 3 with UV detection at 280 nms.