جامعة الزهراء للبنات
Al-Zahraa University for Women

FACULTY OF PHARMACY

Pharmacology and Toxicology Department

Fourth Year

General Toxicology

Laboratory Course

Semester 2

Course Organisers

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Lab. No1

General introduction to practical toxicology.

Toxicology:- is the study of toxic effects (adverse effects) of chemicals on living organisms.

It is seeks to characterized the potentially adverse effects of foreign chemicals and their dose response relationships to protect public health.

Toxicity:- any harmful effect of a chemical or drug on target organ.
Note :- Any mishandling of cosmetics, pesticides, building materials and food additives may lead to hazard, so caution must
taken in handling these agents.

Note:- All chemicals are toxic , but the proper use of these agents determine whether these become toxic or safe.

Toxicologist:- Is a specialist trained to examine the nature of the adverse effects of chemicals or drugs and to assess probability of their occurrence.

Note:- All substances are poisonous , the right dose
differentiates a poison and remedy .
Poison:- Any agent capable of producing a deleterious response in biological system seriously injury function or producing death.
Poison**,** any substance that produces disease conditions, tissue injury, or otherwise interrupts natural life processes when in contact with or absorbed into the body. Most poisons taken in sufficient quantity are lethal. A poisonous substance may originate as a mineral, vegetable, or an animal, and it may assume the form of a solid, liquid, or gas. A poison, depending on the type, may attack the surface of the body or, more seriously, internal organs or the central nervous system.

II. KINDS OF POISON

Poisons in humans are usually classified according to their effects as corrosives, irritants, or narcotics; the last named are also known as systemic or nerve poisons.

1- Corrosives include strong acids or alkalis that cause local tissue destruction, externally or internally; that is, they "burn" the skin or the lining of the stomach. Vomiting occurs immediately, and the vomitus is intermixed with blood. Common or so-called household corrosive poisons include hydrochloric acid, carbolic acid, bichloride of mercury, and ammonia.
2- Irritants such as arsenic, mercury, iodine, and laxatives act directly on the mucous membrane, causing gastrointestinal irritation or inflammation accompanied by pain and vomiting; diluted corrosive poisons also have these effects. Irritants include cumulative poisons, those substances that can be absorbed gradually without apparent harm until they suddenly take effect.
3- Narcotic poisons act upon the central nervous system or upon important organs such as the heart, liver, lungs, or kidneys until they affect the respiratory and circulatory systems. These poisons can cause coma, convulsions, or delirium.

Narcotic poisons include alcohol, opium and its derivatives, belladonna, turpentine, potassium cyanide, chloroform, and strychnine, also include one of the most dangerous poisons known, botulin toxin, a potent bacterial toxin that is the cause of acute food poisoning .

Blood poisoning, bacterial in nature, is a condition that occurs when virulent microorganisms invade the bloodstream through a wound or an infection. Symptoms include chills, fever, prostration, and often infections or secondary abscesses in various organs . Most poison gases also affect the bloodstream. Because these gases restrict the body's ability to absorb oxygen, they are often considered in a separate category called asphyxiants, to which group ordinary carbon monoxide belongs. Gas poisons, however, may also be corrosives or irritants .

About 50 percent of all human poisoning cases in the U.S. involve commonly used drugs or household products such as aspirin, barbiturates, insecticides, and cosmetics. Because barbiturates are easily available, toxic effects resulting from their misuse are not infrequent. Acute poisoning may result from overdosage or interaction with other drugs, especially alcohol. The victim of acute barbiturate poisoning may become agitated and nauseated, or may pass into a deep sleep marked by increasingly shallow respiration. Coma and heart failure may follow. Chronic barbiturate poisoning, caused by prolonged use of the drugs, is usually marked by gastrointestinal irritation, loss of appetite, and anemia. In advanced stages of chronic barbiturate poisoning the victim may show mental confusion

.**Common target tissues** :- any tissue or organ within the body can potentially be affected by a chemical toxin , and most chemicals adversely affect more than one tissue.

- non-selective action:-exposure to corrosive compounds leads to a local irritation and / or caustic effects that are non-selective in nature and occur at any site of application or exposure is located.

Selective actions:- many chemicals produce their toxic effects by interfering with the functions of specific biochemical pathways
and/or macromolecules within a tissue.

- **immediate and delayed actions**:- many compounds have toxic actions that will quickly lead to symptoms following exposure. Another chemicals exert effects that have latency periods for as long as several decades.

Risk and safety:-

Risk:- the probability that a substance will produce
harmful effect under specified condition.

Safety:- the probability that harmful effect will not
occur under specified conditions.

* high risk may be acceptable in the use of life saving drugs but would be unacceptable for food additives , So we have what called (risk against benefit or safety).

*** Some factors must be considered in**
determining an acceptable risk:-

1- benefit gained from the use of a substance.
2- adequacy and availability of an alternative.

3- economic consideration.
4- effect on environmental quality

Characteristic of exposure:-

- toxic effect of chemicals in biological system are not produced unless the chemical or its metabolites reach appropriate sites in the body at a concentration and for length of time sufficient to produce the toxicity.

Whether a toxic response occurs or not depend on :-

1- physico-chemical properties of the agent.

2- exposure situation route, duration ,frequency (of administration).

3- susceptibility of the biological system

Duration of exposure:-

1- Acute:- exposure for < 24 hours(as single or repeated within 24 hours.
2- subacute:- exposure for ≤ 1 month.
3- subchronic:- exposure for 1 or 3 months.
4- chronic:- exposure for > 3 months.

* for many agents , the toxic effects following acute exposure are quiet different from those produced by repeated exposure.

Example :- benzene Acute – CNs depression

chronic -- leukemia

Frequency of exposure

- fractionation of the dose reduces the effect , this allow the biotransformation or excretion to minimize the toxic concentration of the agent.

-chronic toxic effect occur , therefore if the chemical accumulates in the biological system.(i.e. absorption > metabolism or excretion).

III. TREATMENT

Various treatments may counteract the effect of a poison. In most cases the use of

1- dilution is advisable, that is, the ingestion of large quantities of water or milk.
2- emetic, a substance that induces vomiting and rids the stomach of certain poisons. An emetic may act locally, as on the gastric nerves, or systematically on the part of the brain that causes the vomiting. Household emetics, which act locally, include a tablespoon of salt dissolved in warm water or two tablespoons of mustard dissolved in a pint of water. Emetics must not be given to a person who has swallowed a corrosive poison.

3-An antidote, unlike an emetic, is a remedy that counteracts the effects of a poison chemically, although it may result indirectly in vomiting. An antidote may work against a poison by neutralizing it, rendering it insoluble, absorbing it, isolating it, or producing an opposite physiological effect generally. In any instance of poisoning, it is imperative that remedial treatment be started immediately.

Lab. No. 2

Acute toxicity study, determination of LD50.

Ld50 is defined as the dose which has proved to be lethal to 50% of the test animals. Is usually an initial step in the assessment and evaluation of the toxic characteristics of a substance .It's an initial assessment of toxic manifestations (provides information in health hazards likely to arise from short term exposure to drugs)and is one of the initial screening experiments per formed with all compounds .

There are various methods to calculate LD50 values; like the graphical method, arithmetical method and statistical approach.

- Arithmetical method of Karber method

The sum of the product was divided by the number of animals in a group and the resulting
quotient was subtracted from the least lethal dose in order to obtain LD50 value.

LD50 = the apparent least dose lethal to all in a group $-\sum(a*b)/N$
Where
N = number of animals in each group,
a = dose difference
b = mean mortality.

GROUP	DOSE mg/kg	Dose difference (a)	No. of animals dead	Mean mortality (b)	Product (a*b)
1					
2					
3					
4					
5					
6					

Aim :
To determine the LD50 of a drug in a given species.
Procedure:
1.devide 48 rats into 6 groups
2.each group get different dose of Phenobarbital the 1st is 330 mg/kg the 2nd is 300 the 3rd is 280 ,4th is 250 , 5th is 220 and 6th is 120
3.weigh the animals and mark them.
4.calculate the doses of the drug that that will be given to the animals
5. inject group with the indicated dose intraperitoneally in the center of the lower left quadrate of the abdomen of the animal
6.one hour after the injection , record the number of death and survivals .
7. record your results as indicated in the sample sheet.

Lab. No. 3

Drug toxicity on liver.

Liver is the primary site for any toxic substance .The liver plays a central role in transforming and clearing chemicals and is susceptible to toxicity from these agents .certain medicinal agents when taken in overdoses and sometimes even when introduced within therapeutic ranges ,may injure the organ .other chemical agents, such as those used in laboratories and industries ,natural chemicals and herbal remedies can also induce hepatotoxicity .chemicals that cause liver injury are called hepatotoxins.

Paracetamol (N-acetyl –p- aminophenol, acetaminophen) is a slightly bitter ,white ,odorless , crystalline powder, non –opiate, non –salicylate analgesic and antipyretic which is remarkably safe when taken in therapeutic doses and is associated with significant hepatotoxicity when taken in overdose .

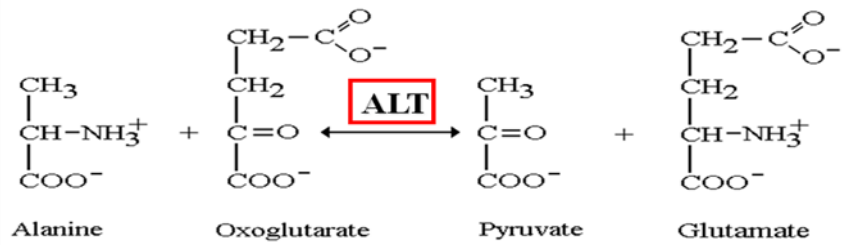
Generally safe for human w doses (1000 mg per single dose and up to 4000 mg per day)for adults,

The hepatic glutathione conjugates the NAPQI to produce *N*-acetyl-p-aminophenol (APAP) mercapturate and APAP-cysteine which are both nontoxic metabolites

Acetaminophen exposure becomes toxic when glucuronidation and sulfation pathways become saturated and cellular glutathione stores are depleted

In such cases, NAPQI binds to cellular proteins and membranes, causes disruption of protein function and damage to cell membranes, and leads to cell injury and death, causing centrilobular hepatic necrosis

- Alanine transaminase or ALT is a transaminase enzyme .It is also called serum glutamic-pyruvic transaminase (SGPT)



- **ALT or sGPT (serum glutamate pyruvate transaminase)**

GPT (ALT) catalyses the transfer of amino-groups from alanine to 2-oxoglutarate and thus the formation of glutamate and pyruvate.

Source & biological action

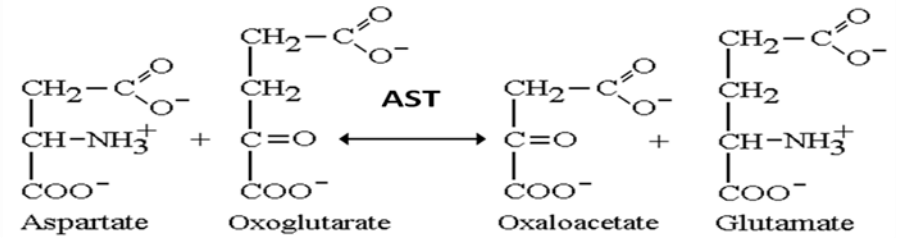
GPT (ALT) is widely distributed in cells throughout the body. GPT (ALT) is found predominantly in the cytoplasm of hepatic parenchymal cells and is widely considered to be specifically for the liver. In addition, it is also active in the heart, skeletal muscle, pancreas, and the kidney.

GPT (ALT) activity in the liver is about 3,000 times higher than its activity in the serum. Only if cells are damaged GPT (ALT) will be excreted into the blood. In the plasma, GPT (ALT) has no biological function because the necessary substrates and co-substrates are lacking there.

when the liver is injured, ALT is released into the bloodstream.

Elevated levels of ALT may indicate :

- alcoholic liver disease ,cancer of the liver ,cholestasis or congestion of the bile ducts ,cirrhosis or scarring of the liver with loss of function , death of liver tissue ,Hepatitis or inflammation of the liver ,noncancerous tumor of the liver ,use of medicines or drugs toxic to the liver



Aspartate aminotransferase (AST)

AST or sGOT (serum glutamate oxaloacetate transaminase

- AST also reflects damage to the hepatic cells and is less specific for liver disease. It can also be released with heart, muscle and brain disorders. Therefore, this test may be ordered to help diagnose various heart, muscle or brain disorders, such as a myocardial infarct (heart attack).

Elevated levels of AST may indicate :

acute hemolytic anemia, acute pancreatitis or inflammation of the pancreas ,acute renal failure or loss of kidney function.., cirrhosis of the liver ,Hepatitis ,recent surgery ,severe burns ,muscle injury, *heart attack*

AST and ALT is in the different distribution of the hepatocytes. ALT exists primarily in the cytoplasm of liver cell. if there is a slight liver cell damage, ALT firstly leak into the bloodstream, so that the serum ALT increased. The AST mainly in the "mitochondria“ of liver cells, the mitochondria are "bubble" in the liver cell cytoplasm. if there is a slight liver cell damage, AST don`t leak into the bloodstream.

Procedure:

1. In this experiment two groups of male rats each contain 3 rats .
2. Weigh the rats and mark them.
3. The 1st group rats were treated with 500mg /kg daily orally for 7 days.
4. Paracetamol should be suspended with GW.
5. Control group were drenched with same amount GW.
6. After 7 days we take blood sample from heart for both groups.
7. Blood samples centrifuged to get serum .
8. AST and ALT are measured according to the method of Reitman et al by using readymade kit.
9. Before leaving the lab. Give the instructor a copy of data collected by your group , each student should submit results.

Lab. No.4

Nicotine toxicity.

Exposure to nicotine occur during processing or extraction of tobacco during mixing, storage or application or extraction of tobacco.
Also during the mixing, storage or application of insecticides containing nicotine or during smoking of cigarettes. The fatal dose of pure nicotine alkaloid from the leaves in about 10mg. nicotine first stimulates, then depressed and paralyze the cells of peripheral autonomic ganglia, brain ,spinal cord and skeletal muscles including the diaphragm.

Procedure:-

1- Toxic effects of pure nicotine alkaloid.

Administer orally one drop of pure nicotine alkaloid to a rat using the tip of a glass rod. Record the time of the onset of effects. Notice the excitement, salivation , hyperpnea prostration, clonic and tonic convulsions and piloerection are usually followed by reparatory cardiac arrest. After death expose the intestines and observe the motility stimulate the phrenic nerve and note wether or whether not the the corresponding half of the diaphragm contracts.

Record your results:-

2- Nicotine and the frog

Inject 0.1 ml of 0.1% nicotine sulfate solution into a lymph sac of frog. Note the rapidly appearing peculiar fibrillary twitching the muscles and characteristic position of the hindlege.

3- Cigarette smoke and the frog,

Fill a bell jar or a beaker with cigarette smoke. Place a frog in the smoke and notice the signs of poisoning. Compare the effects with those seen in 2.

4- Aqueous extract of cigarette and the frog.

Crush five cigarette of one of the common brands in 100 ml beaker , add 20 ml of distilled water and allow to macerate for 15 minutes. Press (squeeze out) and filter the liquid and inject 1 ml of the extract into the anterior lymph sac of frog.
Observe the animal for signs of nicotine poisoning, compare with those observed in 2.

5- Nicotine and picrotoxin.

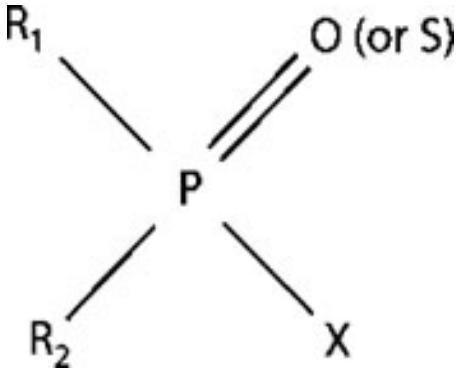
Inject intraperitoneally 0.1 ml , of 3% picrotoxin solution to frog . after that inject the same frog with 0.1ml, of 0.1% nicotine sulfate solution.

Observe the effects.

Lab. No. 5

Pesticide toxicity.

Organophosphorous compounds toxicity:
Although a number of organic phosphorus (OP) compounds were synthesized in the 1800s, their development as insecticides only occurred in the late 1930s and early 1940s. The German chemist Gerhard Schrader is credited for the discovery of the general chemical structure of anticholinesterase OP compounds, and for the synthesis of the first commercialized OP insecticide [Bladan, containing TEPP (tetraethyl pyrophosphate) as the active ingredient], and for one of the most known, parathion, in 1944. Since then, hundreds of OP compounds have been made and commercialized worldwide in a variety of formulations. More than half of the insecticides used are OPs, and some OPs are among the most extensively used pesticides .The general structure of OP insecticides can be represented by



Where X is the so-called "leaving group," that is displaced when the OP phosphorylates acetylcholinesterase (AChE), and is the most sensitive to hydrolysis;R1 andR2 are most commonly alkoxy groups (i.e., OCH3 or OC2H5), though other chemical substitutes are also possible; either an oxygen or a sulfur (in this case the compound should be defined as a phosphorothioate) are also attached to the phosphorus with a double bond. Based on chemical differences, OPs can be divided into several subclasses, which include phosphates, phosphorothioates, phosphoramidates, phosphonates, and others . The problem with organophosphates is that they affect an important neurotransmitter common to both insects and mammals. This neurotransmitter, acetylcholine, is essential for nerve cells to be able to communicate with each other. Acetylcholine released by one nerve cell initiates communication with another nerve cell, but that stimulation must eventually be stopped. To stop the communication, acetylcholine is removed from the area around the nerve cells, and an enzyme, acetylcholinesterase, breaks down the acetylcholine. Organophosphates block the enzyme and disrupt the proper functioning of the nerve cells. Hence, these insecticides are called acetylcholinesterase inhibitors.

The principle pharmacological action of toxic phosphate esters is the inhibition of cholinesterase consequently the effects of a toxic dose of an organophosphates is due to the resultant accumulation of acetylcholine. This provides the practitioner with a rational means of therapy since it is known that atropine will competitively antagonize

the muscarinic effects of excess acetylcholine. A second group of compound employed of organophoshate intoxication are hydroxylamine derivatives , the most important on being pyridine 2-aldoxime methiodide (2-PAM) . these compounds act by displacing the organophosphate from the active site of cholinesterase and therefore permit to hydrolyze acetylcholine .

caution:
malathion is highly toxic substance that is readily absorbed through intact skin .

procedure:

1. Administer intraperitoneally malathion 96% solution in adose of 400 mg/kg to 3 group mice.
2. When established signs of toxicity are observed from the appearance of the animals, antidote will be administered .
3. Group 1 will be administered atropine sulphate 5mg/kg I.P. group 2 will be administered pralidoxime (2-PAM)25 mg/kg I.P. , Group 3 will be administered atropine sulphate 5mg/kg+(2-PAM)25 mg/kg I.P.
4. If the condition of the animals continues to deteriorate after the administration of antidotes ,a second dose of atropine may be administered after 5 -15 minutes.

Lab. No. 6

Metal toxicity

Toxicity of Heavy Metals and Antidotal Therapy (Mercury and BAL):
The increasing threat of contamination of the environment and the wide spread mercury and its compounds in industry and agriculture, and the potential hazard of high intake of toxic forms of the mercury by large group of the population have focused agreat deal of attention on the fate of mercury in the environment.
Exposure to mercury and its compounds may result in toxic effects to man. The toxicity is mainly due to the inhibition of some enzyme systems by reaction with their sulfhydryl groups (-sh) .there for as a therapy would be of great importance to remove as much as the the toxic compound before it gets absorbed and bound to the tissue protein . poisoning by mercury specifically combated by British Antilewisite (BAL) .
BAL when administered to man , will chalet with mercury and remove from the sites to which it is combined .this experiment is designed to demonstrate the use of BAL as an antidote for inorganic poisoning .

Procedure:
One group of rats will be given mercuric chloride alone , second and third groups will be treated with BAL and EDTA respectively , following the administration of mercuric chloride procedure .
All of the animals were started for 24 hours .

1. Weight the animals and mark them .
2. All of the animals will be received an intraperitoneal injection of mercuric chloride 60mg/kg B.W
3. After the onset of the toxic symptoms of mercury ,(about 30 minutes) administer, intraperitonealy ,animals No. II and III in 30 minutes intervals 18 mg /kg BAL for total dosage of 54mg/kg ,and 25m/kg EDTA for a total dosage of 75 mg /kg respectively.
4. Throughout the experiment ,observe the appearance of the toxic manifestions of mercury alone and after administration of BAL and EDTA.
   Report your results.

Lab. No.7

The sedative and toxic effects of Alcohols

Aim:

To measure the sedative and toxic effects of methyl and ethyl alcohols.

Principle:

Both methanol and ethanol are competing for the same enzyme for their hepatic metabolism that is alcohol dehydrogenase.
This fact can be useful in the management of methanol poisoning.

Methanol:-

Also known as **methyl alcohol, wood alcohol**, **wood naphtha** or **wood spirits**, is a chemical with the formula CH_3OH (often abbreviated MeOH). Methanol acquired the name "wood alcohol" because it was once produced chiefly as a byproduct of the destructive distillation of wood. Modern methanol is produced in a catalytic industrial process directly from carbon monoxide, carbon dioxide, andhydrogen.

Methanol is the simplest alcohol, and is a light, volatile, colorless, flammable liquid with a distinctive odor very similar to, but slightly sweeter than, that of ethanol (drinking alcohol). At room temperature, it is a polar liquid, and is used as an antifreeze, solvent, fuel, and as a denaturant for ethanol.

Methanol is produced naturally in the anaerobic metabolism of many varieties of bacteria, and is commonly present in small amounts in the environment

Methanol burns in oxygen including open air, forming carbon dioxide and water:

$$2\ CH_3OH + 3\ O_2 \rightarrow 2\ CO_2 + 4\ H_2O$$

Methanol ingested in large quantities is metabolized to formic acid or formate salts, which is poisonous to the central nervous system, and may cause blindness, coma, and death. Because of these toxic properties, methanol is frequently used as a denaturant additive for ethanol manufactured for industrial uses. This addition of methanol exempts industrial ethanol (commonly known as "denatured alcohol" or "methylated spirit") .

Toxicity:-

Methanol has a high toxicity in humans. If as little as 10 mL of pure methanol is ingested, for example, it can break down into formic acid, which can cause permanent blindness by destruction of the optic nerve, and 30 mL is potentially fatal, although the median lethal dose is typically 100 mL (4 fl oz) (i.e. 1–2 mL/kg body weight of pure methanol.Toxic effects take hours to start, and effective antidotes can often prevent permanent damage. Because of its similarities in both appearance and odor to ethanol (the alcohol in beverages), it is difficult to differentiate between the two (such is also the case with denatured alcohol).

Methanol is toxic by two mechanisms. First, methanol (whether it enters the body by ingestion, inhalation, or absorption through the skin) can be fatal due to its CNS depressant properties in the same manner as ethanol poisoning. Second, in a process of toxication, it is metabolized to formic acid (which is present as the formate ion) via formaldehyde in a process initiated by the enzyme alcohol dehydrogenase in the liver. Methanol is converted to formaldehyde via alcohol dehydrogenase (ADH) and formaldehyde is converted to formic acid (formate) via aldehyde

dehydrogenase (ALDH). The conversion to formate via ALDH proceeds completely, with no detectable formaldehyde remaining. Formate is toxic because it inhibits mitochondrial cytochrome c oxidase, causing the symptoms of hypoxia at the cellular level, and also causing metabolic acidosis, among a variety of other metabolic disturbances.

Treatement:-

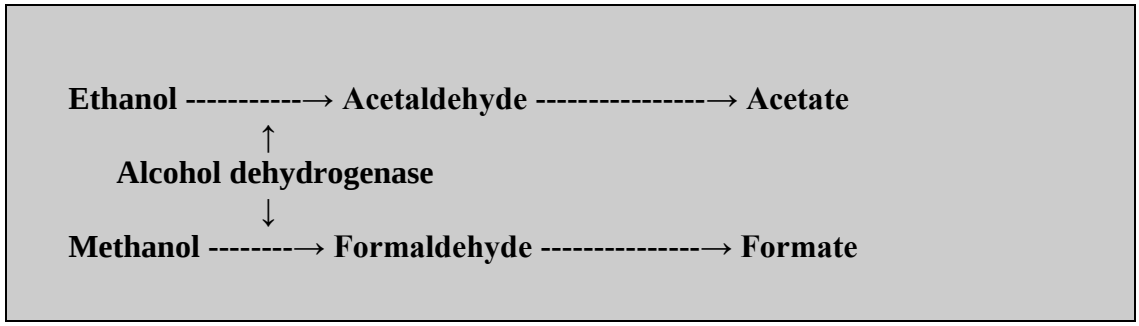
Methanol poisoning can be treated with the antidotes ethanol or fomepizole. Both drugs act to reduce the action of alcohol dehydrogenase on methanol by means of competitive inhibition, so it is excreted by the kidneys rather than being transformed into toxic metabolites. Further treatment may include giving sodium bicarbonate for metabolic acidosis, and hemodialysis or hemodiafiltration can be used to remove methanol and formate from the blood. Folinic acid or folic acid is also administered to enhance the metabolism of formate.

Symptoms of toxicity:-

The initial symptoms of methanol intoxication include central nervous system depression, headache, dizziness, nausea, lack of coordination, and confusion. Sufficiently large doses can cause unconsciousness and death.

Ethanol:-

Ethanol also called ethyl alcohol , pure alcohol, beverage alcohol, or drinking alcohol, is a volatile, flammable, colorless liquid with the structural formula CH3CH2OH, often abbreviated as C2H5OH or C2H6O. Ethanol is a psychoactive drug and is one of the oldest recreational drugs still used by humans. Ethanol can cause alcohol intoxication when consumed. Best known as the type ofalcohol found in alcoholic beverages, it is also used in thermometers, as a solvent, and as a fuel. In common usage, it is often referred to simply as alcohol or spirits.



As ethanol has higher affinity for alcohol dehydrogenase enzyme than methanol, so it is life saving in the emergency treatment after accidental administration of methanol. Methanol causes blindness, acidosis, respiratory depression and death.

Methods:

1- measure the weight of two rats and check the following parameters:
Gait, pain reflex, respiratory rate, onset of anesthesia and death if occurs.
2- Inject one rat with methanol in a dose of 3mg/kg I.P and the other rat with ethanol in the same dose.
3- Record the time of administration and recheck the above parameters every 5 minutes to see the effects of both alcohols.

Experimental needs: Measuring balance, forceps, syringes, needles, methanol, and ethanol.

Lab. No. 8

Drug-induced toxicity-Acute toxicity

Acute toxicity:- describes the adverse effects of a substance that result either from a single exposure or from multiple exposures in a short space of time (usually less than 24 hours). To be described as acute toxicity, the adverse effects should occur within 14 days of the administration of the substance.

Acute toxicity is distinguished from chronic toxicity, which describes the adverse health effects from repeated exposures, often at lower levels, to a substance over a longer time period (months or years).

It is widely considered unethical to use humans as test subjects for acute (or chronic) toxicity research. However, some information can be gained from investigating accidental human exposures (e.g., factory accidents). Otherwise, most acute toxicity data comes from animal testing or, more recently, in vitro testing methods and inference from data on similar substances.

Benzodiazepines

The commercial use of benzodiazepines began with the introduction of chlordiazepoxide for anxiety in 1961 and shortly thereafter of diazepam for seizures in 1963.

Benzodiazepines are used principally as sedatives. Temazepam and triazolam are exceptions; they are used as hypnotics to produce sleep. Clonazepam is the only benzodiazepine approved for use as a chronic anticonvulsant agent.

The benzodiazepines are organic bases with a benzene structure and a 7-member diazepine moiety.

Similar to barbiturates, various side chains at R1, R2, R2', R3, R4, R5, and R7 influence potency, duration of action, metabolites, and rate of elimination.

Toxicokinetics:

Benzodiazepines tend to be highly protein bound and lipophilic. They passively diffuse into the CNS, their main site of action. Because of their lipophilic nature, benzodiazepines are extensively metabolized via oxidation and conjugation in the liver prior to their renal elimination.

Benzodiazepine receptors:

Benzodiazepines associated with GABA receptors at specific areas in the CNS. Two structurally different [central] benzodiazepine receptors are found in the brain: type I and type II .Type I receptors tends to be located throughout the brain and contain the $GABA_A$ subunit. They are hypothesized to affect anxiety, sleep, and amnesia. Type II receptors are concentrated predominantly in the hippocampus, striatum, and the spinal cord. They are hypothesized to affect muscle relaxation and dependence.

Peripheral benzodiazepine receptors:

Benzodiazepines are also active at certain types of benzodiazepine receptors that are not associated with the GABA receptor. These receptors differ structurally, pharmacologically, and physiologically from GABA-associated benzodiazepine receptors. The function and structure of these receptors are not well defined

Several types of endogenous benzodiazepinelike substances, endozepines, are proposed to bind to these receptors.

Peripheral benzodiazepine receptors may play significant role in modulating pathologic conditions such as hepatic encephalopathy, anxiety disorders, and abnormal immune function.

Tolerance and Dependence:

Tolerance to the sedative effects of the benzodiazepines occurs more rapidly than does tolerance to the antianxiety effects.

Abrupt discontinuation following long-term use of benzodiazepines may precipitate benzodiazepine withdrawal, which is characterized by autonomic instability, changes in perception, paresthesias, headaches, tremors, and seizures.

Alprazolam and lorazepam are associated with more severe withdrawal symptoms and more frequent recurrent symptoms compared with chlordiazepoxide and diazepam,drugs that may protect the user because of the effects of their active metabolites.

Overdose:

A unique property of the benzodiazepines is their relative safety even after substantial ingestion, which probably results from their GABA receptor properties.

Unlike many other sedative-hypnotics, benzodiazepines do not open GABA channels independently at high concentrations. Benzodiazepines are not known to cause any specific systemic injury, and their long-term use is not associated with specific organ toxicity.

Deaths resulting from benzodiazepine ingestions alone are extremely rare. Most often deaths are secondary to a combination of alcohol or other sedative-hypnotics.Supportive care is the mainstay of treatment.

Flumazenil

Flumazenil is a water-soluble benzodiazepine analog with a molecular weight of 303 daltons. It is a competitive antagonist at the benzodiazepine receptor, with very weak agonist properties in animal models and in humans.

The benzodiazepine receptor modulates the effect of GABA on the $GABA_A$ receptor by increasing the frequency of Cl^- channel opening, leading to hyperpolarization. Agonists such as diazepam stimulate the benzodiazepine receptor to produce anxiolytic, anticonvulsant, sedative, amnestic, and muscle-relaxant effects at low doses and hypnosis at high doses.

Inverse agonists bind the benzodiazepine receptor and result in the opposite effects of anxiety, agitation, and seizures.

Antagonists, such as flumazenil, competitively occupy the benzodiazepine receptor without causing any functional change and without allowing an agonist or inverse agonist access to the receptor.

Investigations reveal that 1.5 mg flumazenil leads to an initial receptor occupancy of 55%, whereas 15 mg causes almost total blockade of benzodiazepine receptor sites.

Physicochemical and Pharmacologic Properties of Flumazenil

pK_A	Weak base
Volume of distribution	1.06 L/kg
Distribution half-life	5 minutes
Metabolism	Hepatic: three inactive metabolites High clearance
Elimination	First order
Protein binding	54-64%
Elimination half-life	53 minutes
Onset of action	2 minutes
Duration of action	Dependent on dose and elimination of benzodiazepine, time interval, dose of flumazenil, and hepatic function

The reversal effects of Flumazenil:

Volunteer studies demonstrate the ability of flumazenil to reverse the effect of benzodiazepines.Reversal is dose dependent and begins within minutes. Peak effects occur within 6-10 minutes. Most individuals achieve complete reversal of benzodiazepine effect with a total IV dose of 1 mg.

Use in Benzodiazepine Overdose:
Flumazenil has no role in cases of unknown overdose because seizures and dysrhythmias may occur when the effects of a benzodiazepine are reversed in a mixed overdose.

Flumazenil appears safe and effective for reversal of sedation and partial reversal of amnesia and cognitive impairment.

Flumazenil does not reliably reverse the respiratory depression induced by intravenous benzodiazepines but does reverse central nervous system (CNS) depression.

Flumazenil also has the potential to induce benzodiazepine withdrawal symptoms, including seizures in patients who are benzodiazepine dependent.

Contraindications to Flumazenil Use

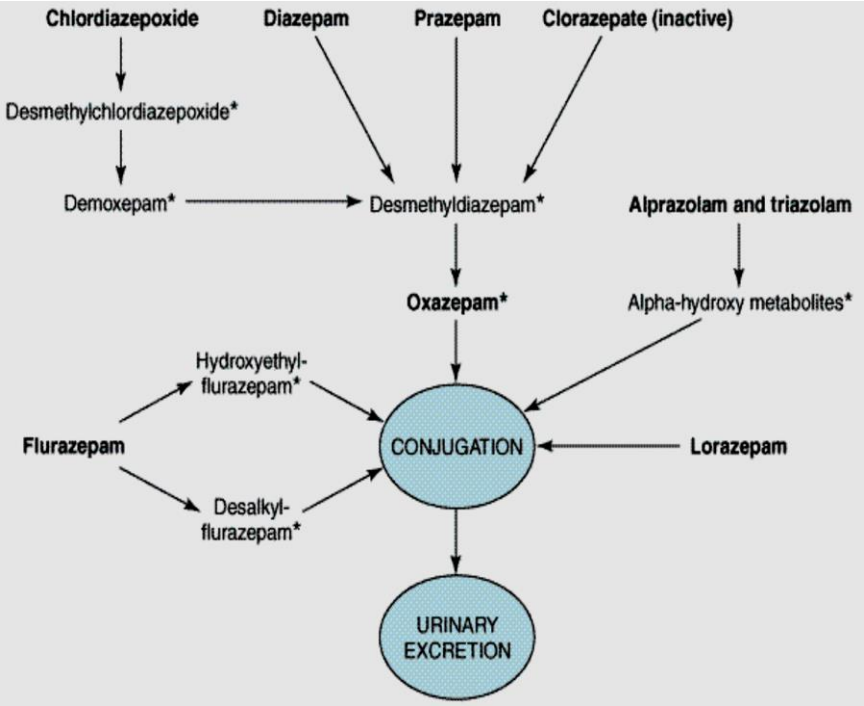
- Prior seizure history or current treatment of seizures
- History of ingestion of a xenobiotic capable of provoking seizures or cardiac dysrhythmias

- Long-term use of benzodiazepines

Procedure:-

- IP injection of 0.2 mg Diazepam in mice 1
- IP injection of 0.4 mg Diazepam in mice 2
- Observe the effects for 6-10 min.
- IP injection of 20 µg Flumazenil in mice 1
- IP injection of 40 µg Flumazenil in mice 2
- Observe the effects for 3-5 min.

Discussion :
What are the expected effects in mice 1 ??
What are the expected effects in mice 2 ??



Appendix

I.Laboratory safety instructions

1. Personal Protective Equipment (PPE)
-Wear Lab Coats: Always wear a lab coat to protect your clothing and skin.
- Safety Goggles: Use safety goggles to protect your eyes from chemical splashes or flying debris.
- Gloves: Wear appropriate gloves for handling chemicals or biological materials.
- Closed-Toe Shoes: Wear closed-toe shoes to protect your feet from spills or dropped objects.

2. Know the Emergency Procedures
- Emergency Exits: Familiarize yourself with the location of all emergency exits.
- Emergency Equipment: Know the locations of fire extinguishers, safety showers, eyewash stations, and first aid kits.
- Fire Evacuation Plan: Understand the fire evacuation plan and practice it regularly.

3. Chemical Safety
- Labeling: Ensure that all chemicals are properly labeled with hazard information.
- Material Safety Data Sheets (MSDS): Review MSDS for chemicals you will be using to understand hazards and first aid measures.
- Proper Storage: Store chemicals according to their compatibility and hazard class.
- No Eating or Drinking: Never eat or drink in the laboratory to avoid contamination.

4. Safe Handling of Equipment
- Proper Use: Use equipment only for its intended purpose and follow operational guidelines.
- Inspect Equipment: Check equipment for damage before use. Don’t use damaged equipment.
- Report Issues: Immediately report any malfunctions or accidents to a supervisor.

5. Waste Disposal
- Segregate Waste: Dispose of hazardous waste in designated containers.
- Follow Protocols: Adhere to your institution's waste disposal protocols.

6. General Laboratory Conduct.
- Keep Work Areas Clean: Clean up spills immediately and keep work areas organized.
- Limit Personal Items:** Keep personal items (bags, phones) out of the work area.

7. Training and Awareness
- Mandatory Training: Attend all required safety training sessions.
- Stay Informed: Keep updated on safety protocols and procedures.

8. Handling Biological Materials
- Biosafety- Biosafety Guidelines: Follow biosafety levels appropriate for the materials you are working with and adhere to all related protocols.
- Decontamination Procedures: Always decontaminate surfaces and equipment after working with biological materials.
- Sharps Disposal: Use designated sharps containers for needles and other sharp objects.

9. Electrical Safety
- Avoid Overloading Circuits: Do not overload electrical outlets or use damaged cords.
- Unplug Equipment: Disconnect equipment when not in use and before performing maintenance.
- Use Ground Circuit Interrupters (GFCIs): Ensure that electrical equipment in wet areas is equipped with GFCIs.

II. How to write a laboratory report

Each student should write his report by himself using his own words to improve his language.

DO NOT COPY. The report should be brief, precise and including the following items:

1- The name of the student and the experiment.

2- The principle of the experiment.

3- The aim of the experiment.

4- Materials and methods used.

5- Results, including the measurements and observations during the experiment that sometimes should be arranged in a form of table.

6- Discussion and conclusion, which is the **most important** item in the report as it shows the ability of the student to discuss his findings and compare them with those mentioned in the textbook.

Pharmacology 4th Stage
PRACTICAL LAB REPORT

Student name: **Date and group:**

Name of the experiment:

Aim:

Equipment:

Materials:

Results:

Discussion

References

1- Casarett_Doulls_Essentials_of_Toxicology_3E_medibos.blogspot.com
2- Goldfrank's Toxicologic Emergencies 9th Ed [PDF]
3-laboratory manual of toxicology by Assistant Professor Mazin Hamid Ouda
4https://www.academia.edu/11411581/Basic_Toxicology_course_1801545_Laboratory_Manual