MARRI LAXMAN REDDY INSTITUTE OF PHARMACY
(Approved by AICTE & PCI, New Delhi and Affiliated to JNTUH)
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PHARMACOLOGY – III

LAB MANUAL

B. PHARMACY III-II

About MLRIP

VISION

To be an educational Institute of par excellence and produce competent pharmacy professionals to serve the community through research and the ever-increasing needs of Industry.

MISSION

1. Imparting quality education and innovative research for various career opportunities.
2. Creating conducive academic environment to produce competent pharmacy professionals.
3. Indoctrination of students adorned with high human values and make them aware of their responsibility as health care professionals.

Program Educational Objectives

PEO 1: To produce graduates with sound theoretical knowledge and technical skills required for their career opportunities in various domains.

PEO 2: To incite the students towards research and to address the challenges with their innovative contributions for the benefit of the mankind.

PEO 3: To instill the essence of professionalism, ethical commitment to become a health care professional with sound integrity and adherence to the core human values in the service of the society.

PROGRAM OUTCOMES

1. **Pharmacy Knowledge:** Possess knowledge and comprehension of the core and basic knowledge associated with the profession of pharmacy, including biomedical sciences; pharmaceutical sciences; behavioral, social, and administrative pharmacy sciences; and manufacturing practices.
2. **Planning Abilities:** Demonstrate effective planning abilities including time management, resource management, delegation skills and organizational skills. Develop and implement plans and organize work to meet deadlines.
3. **Problem analysis:** Utilize the principles of scientific enquiry, thinking analytically, clearly and critically, while solving problems and making decisions during daily practice. Find, analyze, evaluate and apply information systematically and shall make defensible decisions.
4. **Modern tool usage:** Learn, select, and apply appropriate methods and procedures, resources, and modern pharmacy-related computing tools with an understanding of the limitations.
5. **Leadership skills:** Understand and consider the human reaction to change, motivation issues, leadership and team-building when planning changes required for fulfillment of practice, professional and societal responsibilities. Assume participatory roles as responsible citizens or leadership roles when appropriate to facilitate improvement in health and well-being.
6. **Professional Identity:** Understand, analyze and communicate the value of their professional roles in society (e.g. health care professionals, promoters of health, educators, managers, employers, employees).
7. **Pharmaceutical Ethics:** Honour personal values and apply ethical principles in professional and social contexts. Demonstrate behavior that recognizes cultural and personal variability in values, communication and lifestyles. Use ethical frameworks; apply ethical principles while making decisions and take responsibility for the outcomes associated with the decisions.
8. **Communication:** Communicate effectively with the pharmacy community and with society at large, such as, being able to comprehend and write effective reports, make effective presentations and documentation, and give and receive clear instructions.
9. **The Pharmacist and society:** Apply reasoning informed by the contextual knowledge to assess societal, health, safety and legal issues and the consequent responsibilities relevant to the professional pharmacy practice.
10. **Environment and sustainability:** Understand the impact of the professional pharmacy solutions in societal and environmental contexts, and demonstrate the knowledge of, and need for sustainable development.
11. **Life-long learning:** Recognize the need for and have the preparation and ability to engage in independent and life-long learning in the broadest context of technological change. Self-assess and use feedback effectively from others to identify learning needs and to satisfy these needs on an ongoing basis.

EXPERIMENT 1

Dosage calculation and stock solution preparation in experimental animals' studies

Dosage calculation and stock solution preparation in preclinical studies, involving the use of experimental animals is important in screening and development of new drugs. Experimental animals have been of very important tools in the history of non-human research models. Dosage calculation and stock solution preparation based on dosage rationale formula are prerequisites to drug administration in experimental animals.

2. Vehicle of choice, drugs dissolution and volume selection rationale

A vehicle is any substance that acts as a medium in which a drug is administered. Vehicle, which is an essential consideration in all animal research should be biologically inert, have no toxic effects on the animals and not also influence the results obtained for the compound under investigation.

Example of suitable vehicles for animal research include; water, normal saline (0.9% sodium chloride), 50% polyethylene glycol, 5 to 10% Tween 80, 0.25% methylcellulose or carboxymethylcellulose.

According to the OECD's (organization of economic corporation and development's) guidelines, dosage of drug (mg) should be constituted in an appropriate volume not usually exceeding 10 ml/kg (1 ml/100g) body weight of experimental animals (mice and rats) for non-aqueous solvent in oral route of administration. However in the case of aqueous solvents, 20 ml/kg (2 ml/100g) body weight can be considered.

Final dilution volume should not exceed 20 ml/kg.

Based on 10 ml/kg volume selection, required dose volume for a 100 g rat can be calculated as follows:

$$\frac{100\ g}{1000\ g} \times 10\ \text{ml} = 1\ \text{ml}$$

NB: 1kg = 1000 g

Based on 20 ml/kg volume selection, required dose volume for a 100 g rat can be calculated as follows;

$$\frac{100\ g}{1000\ g} \times 20\ \text{ml} = 2\ \text{ml}$$

This is well illustrated below (table 1) in the OECD'S guideline on volume selection using animals of different body weights.

Table 1: OECD'S guideline on volume selection.

Standard volume	Animal's body weight (g)	Calculated volume (ml) based on animal's body weight
10 ml/kg (Appropriate volume)	100 g	1.00 ml
	120 g	1.20 ml
	130 g	1.30 ml
	135 g	1.35 ml
	150 g	1.50 ml
20 ml/kg (Maximum volume)	100 g	2.00 ml
	120 g	2.40 ml
	130 g	2.60 ml
	135 g	2.70 ml
	150 g	3.00 ml

3. Dosage calculation and preparation of stock solution of crude plant extract for experimental animals: With reference to table 1 above, stock solutions and doses of a plant extract (With selected doses, 200 mg/kg and 400 mg/kg) for a rat weighing 120 g be calculated as follows;

Step 1: **Dosage calculation**

Body weight of animal =120 g

$$\text{Dosage in mg} = \frac{\textbf{Body weight of animal (g)}}{\textbf{1000 } g} \times \text{dose (mg)}$$

$$\text{Dosage in mg} = \frac{\textbf{120 g}}{\textbf{1000 } g} \times \textbf{200 (mg)} = 24 \text{ mg.}$$

Step 2: **Dissolution of dose in a suitable vehicle for oral administration**

From the OECD's guidelines,

120 g rat requires **24 mg** of the crude plant extract which should be constituted in not more than **1.2 ml** of normal saline (see table 1 above) according to the OECD guildline.

In a nut shell, **120 g ≡ 24 mg ≡ 1.2 ml** of normal saline.

Bulk volume of the stock solution required for large number of animals can be calculated by multiplying both sides by a constant value as follows;

24 mg = 1.2 ml

40 x 24 mg = 40 x 1.2 ml

960 mg of crude plant extract will be dissolved in 48 ml of normal saline = $\frac{\textbf{960 } mg}{\textbf{48 } ml}$ = 20 mg/ml.

1 ml of dissolved plant extract from a given stock solution (960 mg/48 ml = 20 mg/ml) is the required dose (from selected dose of 200 mg/kg) for a rat weighing 100 g. However, 1.2 ml from the same stock solution is the required volume for a rat weighing 120 g (which

is meant to receive 24 mg of the plant extract). Having successfully prepared a stock solution (960 mg/48 ml = 20 mg/ml) for a selected dose of 200 mg/kg, stock solution of the same plant extract with a higher selected dose (400 mg/kg) can be easily be prepared by dissolving 960 mg of plant extract with half the volume (24 ml) used in the previous stock (960 mg/48 ml), thereby yielding a higher concentration (960 mg/24 ml = 40 mg/ml) which is twice the concentration of the formal stock' as shown in table 2 below. In this case animals with similar body weight from two different selected dose categories (200 mg/kg and 400 mg/kg respectively) will receive the same volume, but different concentrations.

Table 2: Showing stock solutions from two selected doses of a crude plant extract.

Selected dose	Stock solution	Animal body weight (g)	Calculated dose (mg)	Equivalent dose in ml
Low dose, 200 mg/kg	960 mg/48 ml (20 mg/ml)	120 g	24 mg	1.20 ml
		130 g	30 mg	1.30 ml
		135 g	26 mg	1.35 ml
		150 g	30 mg	1.50 ml
High dose, 400 mg/kg	960 mg/24 ml (40 mg/ml)	120 g	48 mg	1.20 ml
		130 g	60 mg	1.20 ml
		135 g	52 mg	1.35 ml
		150 g	60 mg	1.50 ml

4. Dosage calculation and preparation of stock solution of a reference drug (example: Sylimarin) for experimental animals. Sylimarin used in animal model of screening for agents with hepatoprotective, nephroprotective and anti-oxidant properties at standard doses ranging between 25 mg/kg to 200 mg/kg body weight.

For example: The required dose of sylimarin (70 mg per tablet) for a rat weighing 130 g at a standard dose 25 mg/kg can be calculated as follows;

Step 1: **Dosage calculation**

$$\text{Required dose for 130 g rat} = \frac{\textbf{Weight of animal (g)}}{\textbf{1000 g}} \times \text{Standard dose (mg)}$$

$$= \frac{\textbf{130 g}}{\textbf{1000 g}} \times 25 \text{ mg} = 3.25 \text{ mg.}$$

Step 2: **Dissolution of sylimarin in a suitable volume of vehicle for oral administration**

From the above calculation, 130 g rat requires 3.25 mg of sylimarin and this dosage (3.25 mg) should be constituted in not more than 1.3 ml of normal saline according to the OECD's guideline (see table 1 above).

In a nut shell, **130 g ≡ 3.25 mg ≡ 1.3 ml** of normal saline. If **3.25 mg** would be constituted in **1.3 ml** of normal saline,

Then, one tablet of sylimarin (**70 mg**) would be constituted in

$\frac{1.3\ ml}{3.25\ mg}$ x 70 mg = **28 ml** of normal saline. That is $\frac{70mg}{28ml}$ = **2.5 mg/ml**

From this stock solution, dosages can be administered to animals of varying body weights based on the OECD's 10 ml/kg standard volume rationale as shown in table 3 below.

Table 3: Showing stock solution preparation for sylimarin tablet and required doses for animals of different body weights.

STANDARD DOSE	STOCK SOLUTION	Animal's body weight (g)	Calculated dose in mg	Equivalent dose in ml
Sylimarin, 25 mg/kg	70 mg/28 ml = (2.5 mg/ml)	130 g	3.25 mg	1.30 ml
		135 g	3.38 mg	1.35 ml
		140 g	3.50 mg	1.40 ml
		150 g	3.75 mg	1.50 ml

Conclusion:

EXPERIMENT -2

ANTI-ALLERGIC ACTIVITY BY MAST CELL STABILIZATION ASSAY

AIM: To screen the anti-allergic activity of the drugs.

PRINCIPLE: In allergic diseases mast cells plays an important role by defending the antigens. IgE antibodies formed in response to antigen antibody complex attaches to the surface receptors of mast cells and rises calcium influx leading to degranulation of mast cells which releases some pro-inflammatory mediators such as histamine and eicosanoids.

REQUIREMENTS:

Animals: Guinea pigs of 400-600 g of either sex, Albino rats of 175-200 g of either sex

Drugs :	Histamine dihydrochloride aerosol (0.2% w/v)
	Chlorpheniramine maleate (2 mg/kg, s.c.)
	Disodium chromoglycate (50 mg/kg. i.p.)
Reagents:	Saline solution (0.9%)
	RPMI 1640 buffer medium (PH 7.2-7.4)
	egg albumin (100 µg/mL)
	toluidine blue solution (1%)
Instruments:	Microscope with 10X magnification lens.

PROCEDURE: Evaluation of bronchoconstriction in guinea pigs by using histamine aerosol. Selected guinea pigs are divided into two groups consisting of 3 animals in each. Animals have to be fasted overnight. Group-1 receives normal saline. Group-2 animals receive Chlorpheniramine maleate (2 mg/kg, S.C.). Before the drug treatment animals should be exposed to histamine aerosol (0.2%) in histamine chamber. Then determine the end point i.e. pre-convulsion dyspnea (PCD) is the time of time of exposure of histamine aerosol to onset of dyspnea that leads to convulsion. As early as PCD is observed the animals should be removed from chamber and placed in fresh air and time of onset of PCD is to be noted on day zero. Then animals have to treat with drug after 24 hours. After 1 hr of drug administration once again animals are exposed to histamine aerosol and PCD is determined. Percentage (%) of protection offered by the drug can be calculated by the below formula.

Percentage (%) protection= (1-T1/T2) ×100

Where T1= mean value of PCD before drug administration, T2= mean value of PCD after drug administration.

OBSERVATIONS

Group	Percentage protection
Group-1 (saline)	
Group-2 (Chlorpheniramine maleate (2 mg/kg, S.C.)	

Mast cell stabilization activity

Albino rats of either sex are divided into two groups consisting of 3 animals in each. Group-1 receives normal saline, and Group-2 receives Disodium chromoglycate (50 mg/kg. i.p.) for 3 days. Inject 10 ml/kg of 0.9% saline into peritoneal cavity on 4th day to each animal. Massage the peritoneal region of the animal gently for 5 min, then collect the peritoneal fluid and transfer to the test tube which is carrying 7-10 ml of RPMI buffer. Centrifuge the fluid for 400-500 RPM. Discard the supernatant and wash the pellets of mast cells twice with same buffer by centrifugation. Add egg albumin to the above cell suspension and incubate at 37oC for 10 min. Later the suspension has to stain with 1% toluidine blue solution and observe the slide under microscope for calculating number of granulated and degranulated mast cells in each group (total 100 cells are having to be counted from different visual areas).

OBSERVATIONS

Group	Total number of cells (n=100)	
	Granulated	Degranulated
Group-1 (saline)		
Group-2 (Disodium chromoglycate (50 mg/kg. I.P.)		

CONCLUSION

Pre-treatment of animals with standard drugs stabilizes mast cell membrane and generates nitric oxide as defensive mechanism that inhibits the release of chemokines, which are responsible for vasoconstriction.

EXPERIMENT -3

STUDY OF ANTI-ULCER ACTIVITY OF A DRUG USING PYLORUS LIGAND (SHAY) RAT MODEL

AIM:

To study the anti-ulcer activity of a drug using pyloric ligand (SHAY) rat model.

PRINCIPLE:

Peptic ulcer is one of the most prevalent gastrointestinal disorders. The aim of the present study is to demonstrate the antiulcer activity of drugs using pylorus ligand (SHAY) rat model. This was first demonstrated by Shay in 1945. Ligation of rat pylorus results gastric acid accumulation in the fore-stomach leads to acute gastric ulcers. This procedure is used to screen the drugs for their anti-secretary and antiulcer activity.

REQUIREMENTS

Animals	:	Albino Wistar rats of 150-200 g are selected for the study.
Drugs	:	Ether (anesthetic), Ranitidine 20 mg/kg, p.o, 0.9% normal saline
Reagents	:	0.1 N NaOH, Phenolphthalein, Topfer's reagent,
Instruments	:	Dissecting microscope, Burette, PH meter, Surgical instruments.

PROCEDURE

Animals are to be divided into two groups consisting of 3 animals in each group. Saline is to be administered to control group and Ranitidine (20 mg/kg, P.O.) to other group. Animals have to be fasted for one day with free access to water. 30 min prior to ligation process, the drug (Ranitidine) should be given. Under light ether anesthesia a midline abdominal incision is made and pylorus will be ligated with proper care and the wound is closed. Then rats are to be placed individually in separate cages without food and water during this period and allowed them to recover. Sacrifice the animals by decapitation after 4 hours and open the stomach and collect the stomach contents in a centrifuge tube. Determine the PHPH meter. Open the greater curvature of the stomach and clean the part with saline. Under 10X magnification lens ulcers are to be observed and ulcer index is calculated by using the formula given below.

Ulcer index= $(U1 + U2 + U3) \times 10^{-1}$

U1 = Average of number of ulcers per animal

U2 = Average of severity score

U3 = Percentage (%) of animals with ulcer

Intensity of ulcers with scoring: 0 – normal coloration; 0.5 – red coloration; 1 – spot

ulcer; 1.5 – hemorrhagic stress; 2 – deep ulcer; 3 – perforations.
Ulcer score: 1 mm (exact) = 1; 1-2 mm = 2; >2 mm = 3; >3 mm = 4

Analysis of stomach contents:
Measure total volume of gastric content and centrifuge at 1000 rpm for 10 minutes. Pipette out one ml of supernatant liquid of the centrifuged content and dilute with 10 ml distilled water. Titrate the liquid against 0.01N NaOH using Topfer's reagent as indicator, till the end point (appearance of orange colour). The volume of NaOH used is to be noted to estimate free acidity. Titration has to be continued till the appearance of pink colour and the volume of NaOH run down is to be noted to calculate total acidity.

Calculation of acidity is given below:

OBSERVATIONS

Group	**Volume of gastric contents (in ml)**	**P^H**	**Free acidity (mEq/l)**	**Total acidity (mEq/l)**	**Ulcer index**
Control (saline)					
Standard (Ranitidine 20 mg/kg P.O)					

CONCLUSION

Comparison of ulcer index between study groups estimates the potency of antiulcer activity of test drug. Decrease in volume of gastric contents, free and total acidity determines anti-secretory activity of test drug and rise in PH evaluates acid neutralizing action of test drug

Experiment - 4
EFFECT OF DRUGS ON GASTRO INTESTINAL MOTILITY

AIM : To study the effect of drugs on gastro-intestinal motility.

PRINCIPLE: Intestinal motility is regulated by the enteric nervous system of the gut (Auerbach's and Meissner's plexuses) and the activity of this system can be modified by autonomic nervous system. Hence effect of sympathomimetic and parasympathomimetic drugs on intestinal motility can be studied by using isolated piece of intestine. Parasympathomimetic drugs stimulate enteric neurons to release acetylcholine at neuromuscular junctions and enhance muscle tone and rhythmicity of intestine. Sympathomimetic drugs acts on alpha and beta receptors and releases adrenaline which in turn prevents release of acetylcholine and inhibits muscle tone and rhythmicity.

Guinea pig ileum is advantageous for assay purposes as it produces steady baseline for studying effects of drugs. Rabbit intestine (ileum, deuodenum, jejunum) usually jejunum is used for the effects of pendular movements. In the present study rabbit ileum is selected for estimating the effects of selected drugs on intestinal motility.

REQUIREMENTS:

Animals: Medium sized rabbit

Drugs: Adrenaline/Acetylcholine- 10 ug/ml,
Atropine sulphate- 100 ug/ml,
isoproterenol/isoprenaline 10 ug/ml,
Propranolol- 1 mg/ml, Phenylephrine- 10 ug/ml,
phentolamine- 0.1 ug/ml.

Solutions: Tyrode solution

Apparatus used: Kymograph, Dissecting board, Dissecting instruments, scissors, petriplates, Syringe, Frontal writing lever, water bath with temperature controlling unit, organ bath with aeration tube.

PROCEDURE

The procedure adopted for the study is the modified Finkleman method developed by Walker and Scott. Select a medium sized rabbit for the study. Fast the animal for 24 hours prior to experiment as food in gut results in messy dissection and flushing of gut contents may damage the intestine. Before sacrificing the rabbit, prepare Tyrodes Ringer solution and place about 250 ml of this solution in an ice cold flask. Sacrifice the animal by cervical decapitation without use of anesthetic as it may affect the gut motility. Shave the abdomen of the animal and vacuum the surface to remove adhered fur. Make a midline incision

through the skin and abdominal muscles. Locate ileum and a part of ileum was taken 10 cm away from ileocaecal valve. An optimal length of tissue (5-6 cms) is cut carefully and tie the thread to antimesenteric border on both sides and place them in the Tyrode solution.

Record the rhythmic activity of the ileum by using frontal writing lever and kymograph. Suspend the tissue in organ bath of Tyrode solution (100 ml) at 37^0c with adequate oxygen supply (mixture of 95% O2 and 5% of CO_2). Tie one end of the thread of the tissue to fixed point inside the organ bath and the other end to the lever for recording contractions on the kymograph. Stabilise the tissue in the solution to the conditions for about 30 minutes. Ensure the lever should be placed horizontally and record the normal contractions followed by effects of drugs on muscles.

After recording normal contractions inject the drugs one by one and observe for force of contraction and tone, frequency of contractions before and after drug administration. Inject 0.1 ml of drugs in the succession order in the organ bath and the responses are recorded. After noting the effect of every drug, drain the muscle bath and refill with fresh warm Tyrode solution (100 ml). Take the control reading before and after each drug response. Maintain wash out period for 15-20 minutes for change of every drug and check the next drug response only the when the tone and amplitude returned to original value approximately. The drug and dose name should be mentioned in the recording after taking response of each drug.

Order of adding drugs.

1. 0.1 ml of Ach, 1.0 ml of Ach, 0.1 ml of NE, 1.0 ml of NE (Ach increases contraction and NE relaxes the tissue)
2. 1.0 ml of phenylephrine, 1.0 ml of isoproterenol (phenylephrine causes contraction by inhibiting adenylate cyclase- alpha adrenergic agonists; isoproterenol causes relaxation by showing beta agonist action)
3. 1.0 ml of phenatolamine (alpha adrenergic blocker). Wait for 2 minutes then proceed for adding 1.0 ml of phenylephrine.
5. 1.0 ml of propranolol (beta adrenergic blocker). Wait for 2 minutes then proceed for adding 1.0 ml of isoproterenol
6. 1.0 ml of atropine- wait for 3 minutes- add 1.0 ml of Ach- (to check for parasympatholytic activity of atropine)

CONCLUSION

The effect of drugs on intestinal motility can be easily interpreted by the responses taken on kymograph.

EXPERIMENT 5

Effect of Certain Agonists and Antagonists on Isolated Guinea-pig Ileum

Guinea pig ileum is a smooth muscle receive dual nerve supply from autonomic nervous system of both sympathetic and parasympathetic. Parasympathetic tone is dominant in ileum which **Ach** cause contraction by stimulating **M3-R**

Why Guinea pig ileum is used?

1. No myogenic contraction so any drug induce contraction can be seen.
2. Very sensitive to histamine unlike the rabbit due to the presence of histaminase enzyme.

Types of receptors present in guinea-pig ileum

- Cholinergic receptors.
- Serotonergic or tryptaminergic receptors (5-HT1→5-HT7).
- Histaminergic receptors.
- Adrenergic receptors.

Types of receptors in guinea-pig ileum:

1- Cholinergic receptors:

Receptor	Agonist	Antagonist
Nicotinic neuronal receptor (Nn- R)	Dil. Nicotine	Conc. Nicotine
muscarinic receptor (M3 -R)	Ach	Atropine

2-Serotonergic or tryptaminergic (5-HT) receptors :

- Serotonin = 5-HT(5-hydroxytryptamine).
- **5-HT2A** receptor found in smooth muscle and bronchi .

Receptor	Agonist	Antagonist
5-HT2A (Gq11)	serotonin	-Methysergide & ketanserin (selective) -cyproheptadine (non selective block both 5-HT2A &5-HT receptors)

3- Histaminergic receptors:

- Histamine is an autacoids naturally occurring in the body.

Receptor	distribution	Action	Agonist	Antagonist
H1	Smooth muscles	contraction	Histamine	Mepyramine cyproheptadine
H2	Gastric mucosa	Gastric acid secretion	Histamine -	Cimetidine Ranitidine
H3	Presynaptic	Decrease release of Histamine	Histamine	-

4- Adrenergic receptors:

Receptor	Agonist	Antagonist
α1	Adrenaline	phentolamine
α2	Adrenaline	-

Conclusion:

EXPERIMENT 6

Estimation of serum biochemical parameters by using semi- autoanalyser

BILIRUBIN

• Bilirubin (hematoidin) is the yellow breakdown product of normal heme catabolism. Bilirubin is excreted in bile and urine, and elevated levels may indicate certain diseases. It is responsible for the the background straw-yellow color of urine (via its reduced breakdown product, urobilin – the more obvious but variable bright yellow colour of urine is due to thiochrome, a breakdown product of thiamine), the brown color of feces (via its conversion to stercobilin), and the yellow discoloration in jaundice. • Bilirubin consists of an open chain of four pyrrole-like rings (tetrapyrrole) a porphyrin ring. 3

TYPES OF BILIRUBIN

Direct bilirubin (conjugated bilirubin-BC) -In the liver, bilirubin is conjugated with glucuronic acid by the enzyme glucuronyl transferase, making it soluble in water. Much of it goes into the bile and thus out into the small intestine. However, 95% of the secreted bilirubin is reabsorbed by the intestines (Terminal Ileum) and reaches the liver by portal circulation and then resecreted by the liver into the small intestine. This process is known as enterohepatic circulation. The remaining 5%-> large intestine->urobilinogen-> stercobilin-> feces.

Unconjugated or indirect bilirubin (BU) - Insoluble in water.

Total bilirubin ("TBIL") – It measures both BC and BU.

Total and direct bilirubin levels can be measured from the blood, but indirect bilirubin is calculated from the total and direct bilirubin.

TOTAL BILIRUBIN = INDIRECT BILIRUBIN + DIRECT BILIRUBIN

NORMAL LEVELS

• Direct bilirubin: Less than 0.4 mg/dL or 7 μmol/L

• Total bilirubin: less than 1.5 mg/dL or less than 26 μmol/L 5

MEASUREMENT METHODS Originally the Van den Bergh reaction was used for a qualitative estimate of bilirubin. Total bilirubin is now often measured by the 2,5-dichlorophenyldiazonium (DPD) method.

URINE TESTS Urine bilirubin may also be clinically significant. Bilirubin is not normally detectable in the urine of healthy people. If the blood level of conjugated bilirubin becomes

elevated, e.g. due to liver disease, excess conjugated bilirubin is excreted in the urine, indicating a pathological process.

Unconjugated bilirubin is not water-soluble and so is not excreted in the urine. Testing urine for both bilirubin and urobilinogen can help differentiate obstructive liver disease from other causes of jaundice.

INCREASES Total bilirubin is elevated in obstructive condition of the bile duct, hepatitis, cirrhosis, in hemolytic disorders and several inherited enzyme deficiency.

Indirect bilirubin is elevated by prehepatic causes such as hemolytic disorder or liver diseases resulting in impaired entry, transport or conjugation within the liver. Monitoring of indirect bilirubin in neonates is of special importance as it the indirect (or free) bilirubin bound to be albumin that is able to cross the BBB more easily, increasing the danger of cerebral damage.

HYPERBILIRUBINEMIA

Hyperbilirubinemia results from a higher-than-normal level of bilirubin in the blood.

1. Mild rises in bilirubin may be caused by the following:

• Hemolysis or increased breakdown of red blood cells.

• Gilbert's syndrome – a genetic disorder of bilirubin metabolism that can result in mild jaundice, found in about 5% of the population

• Rotor syndrome- non-itching jaundice, with rise of bilirubin in the patient's serum, mainly of the conjugated type.

2. Moderaterise in bilirubin may be caused by:
   • Pharmaceutical drugs-(especially antipsychotic, some sex hormones, Sulfonamides are contraindicated in infants less than 2 months old as the increase unconjugated bilirubin leading tokernicterus.
   • Hepatitis (levels may be moderate or high) • Chemotherapy • Biliary stricture (benign or malignant)

3.Very high levels of bilirubin may be caused by:

• Neonatal hyperbilirubinaemia- where the newborn's liver is not able to properly process the bilirubin causing jaundice unusually large bile duct obstruction, e.g. stone in common bile duct, tumour obstructing common bile duct etc.

• Severe liver failure with cirrhosis(e.g. primary biliary cirrhosis)

DEMO ESTIMATION - by ERBA kit METHODOLOGY-

PRINCIPLE: Bilirubin reacts with diazotized sulphanilic acid in acidic medium to form pink colored azobilirubin with absorbance directly proportional to bilirubin concentration. Direct bilirubin, being water soluble directly reacts in acidic medium. However indirect or unconjugated bilirubin is solubilized using a surfactant than it reacts similar to direct bilirubin.

SAMPLE: Unhaemolysed serum or plasma. Avoid hemolysis as it causes falsely low results. Sample should be protected from bright light as bilirubin is photo labile. Samples may be stored refrigerated for 3 days or frozen for 1 month. REAGENT COMPOSITION Surfactant 1.00% HCL 100 mmol/L Sulphanilic acid 5 mmol/L REAGENT 2: DIRECT BILIRUBIN REAGENT Sulphanilic acid 10 mmol/L HCL 100 mmol/L REAGENT 3: SODIUM NITRITE REAGENT Sodium nitrite 144 mmol/L REAGENT 1: TOTAL BILIRUBIN REAGENT

Conclusion:

ASSAY PROCEDURE TOTAL BILIRUBIN / DIRECT BILIRUBIN Pipette into test tubes marked Blank Standard Test Working reagent 500 µl 500 µl 500 µl Distilled water 25 µl - - Standard/calibrator - 25 µl - Test - - 25 µl

GLUCOSE

Glucose (C6H12O6, also known as D-glucose, dextrose, or grape sugar) is a simple monosaccharide found in plants. It is one of the three dietary monosaccharides, along with fructose and galactose, that are absorbed directly into the bloodstream during digestion. An important carbohydrate in biology, cells use it as a secondary source of energy and a metabolic intermediate. Glucose is one of the main products of photosynthesis and fuels for cellular respiration. Glucose exists in several different molecular structures, but all of these structures can be divided into two families of mirror-images (stereoisomers). Only one set of these isomers exists in nature, those derived from the "particular chiral form" of glucose, denoted D-glucose. The chemical D-glucose is sometimes referred to as dextrose. Glucose is a major source of energy for most cells of the body; insulin facilitates glucose entry into the cells.

INCREASES Due to diabetes mellitus, in patients receiving glucose containing fluids intravenously, during severe stress and cerebro vascular accidents.

DECREASES On insulin administration, as a result of insulinoma, inborn errors of carbohydrate metabolism or on fasting.

FUNCTION

• ANALYTE IN MEDICAL BLOOD TEST Glucose is a common medical analyte measured in blood samples. Eating or fasting prior to taking a blood sample has an effect on the result. A high fasting glucose blood sugar level may be a sign of prediabetes or diabetes mellitus.

• ENERGY SOURCE Glucose is a ubiquitous fuel in biology. Use of glucose may be by either aerobic respiration, anaerobic respiration, or fermentation. Glucose is the human body's key source of energy, through aerobic respiration, providing approximately 3.75 kilocalories (16 kilojoules) of food energy per gram. Breakdown of carbohydrates (e.g. starch) yields mono- and disaccharides, most of which is glucose. Glucose is a primary source of energy for the brain, and hence its availability influences psychological processes. When glucose is low, psychological processes requiring mental effort (e.g., self-control, effortful decisionmaking) are impaired. Use of glucose as an energy source in cells is via aerobic respiration or anaerobic respiration.

NORMAL VALUES Fasting Value Post Prandial Category of a person Minimum Value Maximum Value 2 hours after consuming glucose Normal 70 100 Less than 140 Early Diabetes 101 126 140 to 200 Established Diabetes More than 126 - More than 200.

ESTIMATION OF GLUCOSE Enzymatic methods for glucose determination are classified into three groups: 1. Methods with glucose oxidase, 2. Methods with hexokinase, 3. Methods with glucose dehydrogenase.

PRINCIPLE Glucose oxidase (GOD) converts glucose to gluconic acid. Hydrogen peroxide formed in this reaction, in presence of peroxidase (POD) oxidatively couples with 4aminoantipyrine and phenol to produce red quinoneimine dye. This dye has absorbance maximum at 505 nm (500- 550 nm). The intensity of the colour complex is directly proportional to the concentration of glucose in sample. Principle: (Trinder’s method) -D-glucose Mutarotase -D-glucose +H2O+O2 -D-glucose Glucose oxidase H2O2+ 4-aminophenazone+phenol The intensity of the color concentration in the sample. formed D-gluconic acid+H2O2 Peroxidase is Quinonemine +4 H2O proportional to the glucose

DEMO ESTIMATION - by ERBA kit ASSAY PROCEDURE Pipette into tubes marked Working reagent Distilled water Standard Test Blank 1000ul 10ul --- Standard 1000ul - 10ul -- Test 1000ul --10ul

SGOT

Aspartate transaminase (AST) also called aspartate aminotransferase is commonly known as SGOT(AspAT/ASAT/AAT) or serum glutamic oxaloacetic transaminase (SGOT), is a pyridoxial phosphate (PLP)dependent transaminase enzyme. AST catalyses the reversible transfer of an α- amino group between aspartate and glutamate and, as such, in an important

enzyme in amino acid metabolism. Transaminase or aminotransferase is an enzyme that catalyses a type of reaction between an amino acid and a α–keto acid. An amino acid contains an amine (NH2) group. A keto acid contains a keto (=O) group. In transamination, the NH2 group on one molecule is exchanged with the =O group on the other molecule. The amino acid becomes a keto acid, and the keto acid becomes an amino acid. AST is found in liver, heart,skeletal muscle, kidney, brain and red blood cells, and it is commonly measured clinically as a marker for liver health.

CLINICAL SIGNIFICANCE SGOT is important in the clinical diagnosis of human disease. AST is associated with liver parenchymal cells, heart, skeletal muscle, kidney, brain, red blood cell are released from cells as a part of cell injury that occurs in myocardial infraction, hepatitis, acute pancreatitis, acute haemolytic anaemia, severe burns, acute renal disease, musculoskeletal disease and trauma. Assay of these enzyme activities in blood serum can be used both in diagnosis and in monitoring the progress of a patient during treatment. AST was defined as biochemical marker for diagnosis of acute myocardial infraction earlier. AST is commonly measured clinically as a part of diagnostic liver function test to determine liver health such as liver cancer, liver cirrhosis.

INCREASES: Increased levels are associated with liver diseases or damage, myocardial infraction, muscular dystrophy.

DECREASES: Decreased levels are observed in patients undergoing renal dialysis and those with B6 deficiency. Monitoring the change in the levels over a period of time is beneficial to the physician evaluating myocardial infraction or following chronic or resolving hepatitis. FUNCTIONS Aspartate transaminase catalyses the interconversion of aspartate and α-ketoglutarate to oxaloacetate and glutamate. Reaction catalysed by aspartate aminotransferase. Aspartate(Asp) + α-ketoglutarate ↔ oxaloacetate + glutamate

REFERENCE VALUES • Female-6-34 IU/L • Male-8-40 IU/L

ESTIMATION OF SGOT LEVEL IN SERUM PRINCIPLE AST (Transaminase enzyme) catalyses the following reaction. L-Aspartate +2- Oxaloglutarate Oxaloacetate+ L-glutamate In this present method salts is used which selectively reacts with oxaloacetate to produce a colour complex that is measured photomertically.

DEMO ESTIMATION - by ERBA kit METHOD AST L-Aspartate + 2-Oxaloglutarte Oxaloacetate + L-Glutamate MDH Oxaloacetate + NADH Malate +NAD LDH Sample pyruvate + NADH L-Lactate +NAD AST : Aspartate aminotransferase MDH : Malate dehydrogenase LDH : Lactate dehydrogenase SAMPLE Unhaemolysed serum or Heparinised plasma. According to the IFCC expert panel on enzymes, AST is stable for 3 days at 4oc.

ASSAY PROCEDURE Allow the working reagent to attain 37oc before performing the test. Pipette Volumes Working reagent 1000ul Test 100ul.

SGPT

Alanine transaminase or ALT is a transaminase enzyme. It is also called serum glutamic-pyruvic transaminase (SGPT), or alanine amino transaminase (ALAT). ALT is found in plasma and in various bodily tissues, but is most commonly associated with the liver. An alanine aminotransferase (ALT) test is often part of an initial screening for liver disease. Normally, ALT is found inside liver cells. But if the liver is inflamed or injured, ALT is released into the bloodstream. In a normally healthy individual, the level of SGPT is measurable in the blood. When there is acute liver damage, the level of SGPT tends to rise dramatically. ALT is present in high concentration in the liver and to a lesser extent in kidney, heart, skeletal muscle, pancreas, spleen and lungs. The next stage of the liver test for SGPT is to understand the underlying cause of the liver damage. The liver could be damaged by an infectious disease such as mononucleosis or hepatitis. This damage is generally temporary and heals after the patient has recovered from the condition. The level of SGPT is also elevated in an individual who is suffering from bile related problems. There are many different medications that are likely to cause an elevation in the level of SGPT in the blood. This elevation tends to be temporary and gets reversed as the patient's body absorbs the medication or passes it out of the system in the urine. When a drug overdose has occurred, the patient may suffer from liver damage which, apart from causing an elevation in the level of ALT also causes other typical symptoms of liver damage. The liver test for SGPT is diagnostically relevant and can be used with other tests such as the ALT or SGOT test. These tests can confirm whether the elevation in the level of SGPT is related to liver damage or related to bile duct problems. The liver test for SGPT is almost never conducted in isolation. Significantly elevated levels of ALT (SGPT) often suggest the existence of other medical problems such as viral hepatitis, diabetes, congestive heart failure, liver damage, bile duct problems, infectious mononucleosis, or myopathy. For this reason, ALT is commonly used as a way of screening for liver problems. Elevated ALT may also be caused by dietary choline deficiency. However, elevated levels of ALT do not automatically mean that medical problems exist. Fluctuation of ALT levels is normal over the course of the day, and ALT levels can also increase in response to strenuous physical exercise.

INCREASES

Increases levels are generally a result of primary liver diseases such as cirrhosis ,carcinoma,viral or toxic hepatitis and obstructive jaundice.

DECREASES

Decreased levels may be observed in renal dialysis patients and those with vitamin B6 deficiency.

REFFERENCE VALUES Female ≤ 34 IU/L Male ≤ 45 IU/L FUNCTION It catalyzes the transfer of an amino group from L-alanine to α-ketoglutarate, the products of this reversible transamination reaction being pyruvate and L-glutamate. L-glutamate + pyruvate ⇌ α-ketoglutarate + L-alanine ALT (and all transaminases) require the coenzyme pyridoxal phosphate, which is converted into pyridoxamine in the first phase of the reaction, when an amino acid is converted into a keto acid. ESTIMATION OF SGPT PRINCIPLE ALT (GPT) catalyze the transfer of amino groups from specific amino acids to ketoglutaric acid yielding glutamic acid and oxaloacetic or pyruvic acid respectively. These ketoacids are then determined colorimetrically after their reaction with 2,4dinitrophenylhydrazine (DNP). L-Alanine + α – Ketoglutarate ↔ Pyruvate + L-Glutamate Pyruvate + NADH + H+ ↔ L – Lactate + NAD+

DEMO ESTIMATION - by ERBA kit METHODS ALT(Alanine aminotransferase) L-Alanine + 2- Oxoglutarate Pyruvate + L-Glutamate LDH(Lactate dehydrogenase) L – Lactate + NAD+ Pyruvate + NADH REAGENT RECONSTITUTION Allow the reagent bottle and Aqua-4 to attain room temperature (15-30oc). Add the amount of Aqua-4 indicated on the label to contents of each vial. Swirl to dissolve ,do not shake vigorously.

SAMPLE Unhemolysed serum or heparinised plasma. Anticoagulant such as Heparin or EDTA are suitable. ALT is stable for 3 days at 2-8oc.

ASSAY PROCEDURE Pipette Volumes Working reagent 1000ul Test 100ul 20

Conclusion:

EXPERIMENT - 7

EFFECT OF SALINE PURGATIVE ON FROG INTESTINE

AIM: To study the effect of saline purgative on frog intestine.

PRINCIPLE: Saline purgatives are the salts comprising of highly charged ions and do not crosses cell membrane freely. They remain inside the lumen and retain water through osmotic forces. They increase the volume of the contents of the bowel, stretch the colon and produces normal stimulus for contraction of the muscle that leads to defecation. The aim of the present study is to examine the effect of saline purgative on frog intestine.

REQUIREMENTS

Animal	:	Frog
Reagents	:	0.9% to 0.45% of saline (hypotonic), 27% Magnesium sulphate (hypertonic), Frogs Ringer solution (isotonic)
Instruments used:		Frog's board, pithing needle, dissecting instruments, needle with thread, tuberculin syringe with needle.

PROCEDURE: Pith the frog and place it on a dissecting board. Expose the abdominal cavity and carefully trace the small intestine. Make the small intestine into three compartments by tying threads of different colours in such a way that no fluid can move from one compartment to the other. Inject 0.2 ml of each hypotonic solution into first compartment, 0.2 ml of hypertonic solution to second compartment and 0.2 ml of isotonic solution into third compartment. Wait for 20 minutes and the observations are to be recorded.

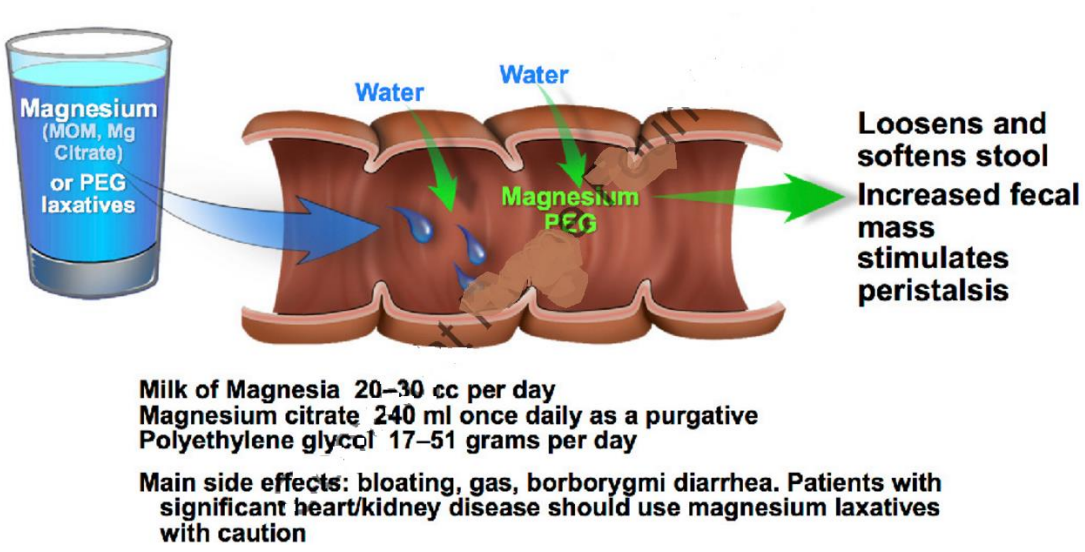

Fig: Representation of Saline purgatives

OBSERVATION

Drug	Compartment	Effect
Hypotonic solution (0.2 ml of 0.9% of saline)	First compartment	Shrunken
Hypertonic solution (0.2 ml of 27% magnesium sulphate)	Second compartment	Swollen
Isotonic solution (0.2 ml of frogs Ringer solution)	Third compartment	No change

CONCLUSION:

Hypotonic solution causes the fluid to move from lumen into circulation by process osmosis thereby shrinks the tissue. Hypertonic solution moves the fluid from cells into the lumen and swells the tissue and isotonic solution did not shows any fluid movement across the intestinal membrane.

EXPERIMENT-8

STUDY THE INSULIN HYPOGLYCEMIC EFFECT IN RABBIT

AIM : To study the insulin hypoglycemic effect in rabbit.

PRINCIPLE: Insulin is a peptide hormone produced by the beta cells of pancreas in response to high glucose levels in the blood. Released insulin acts on the insulin receptors on body cells and activates glucose transporters to absorb more glucose into the cells thereby regulates carbohydrate, protein and fat metabolism in body cells. Reduced blood glucose levels inhibit insulin release and stimulate alpha cells of pancreas to release glucagon to maintain glucose levels in the blood by glycogenolysis and gluconeogenesis. The aim of the present study is to evaluate the effect of insulin in rabbits at different time intervals.

REQUIREMENTS

Animals : Healthy rabbits weighing 1800-3000 gms.
Drugs : 20 units of insulin preparation. One unit contains 0.04082 mg of insulin
Reagents : Normal saline, HCl, 0.5% phenol, 1.4-1.8% glycerin.

PROCEDURE

Select healthy rabbits weighing 1800-3000 gms for the study. They should be maintained in uniform diet for 7 days. Fast the animals for 18 hrs with no access to water before starting the procedure. Select three animals for the study and inject 1 unit/ml of insulin. Prepare drug solution freshly. Weigh 20 units of insulin accurately and dissolve it in normal saline. Acidify the solution by using HCl to pH 2.5. Add 0.5% of phenol as preservative and 1.4-1.8% of glycerin and make the final volume to 20 units/ml of solution. Withdraw 2 ml of blood from marginal ear vein of each rabbit and estimate blood glucose level by using suitable biochemical method and the concentration of glucose can be noted down as initial blood glucose level. Then inject insulin (1 unit/ml) to the animals and check the blood sugar level up to 5 hours at the interval of 1 hour each and the determine blood glucose levels as final blood sugar level and compared both initial and final blood glucose levels.

OBSERVATION

Animals	Initial blood glucose level (in mg/ml)	Final blood glucose level at different time intervals (in mg/ml) in hours				
		1	2	3	4	5
1st animal						
2nd animal						
3rd animal						
Average mean value						

CONCLUSION

Mean percentage decrease of blood glucose levels at different time intervals determines the effect of insulin.

EXPERIMENT 9

Test for pyrogens (rabbit method)

Aim: Learn how to determine the pyrogen in parenteral preparations by injecting the sample in rabbits for pyrogen testing.

Introduction: Pyrogen test is performed to check the presence or absence of pyrogens in all aqueous parenterals. Rabbits are used to perform the test because their body temperature increases when pyrogen is introduced by the parenteral route.

For this test, three healthy rabbits are selected each weighing at least 1.5 kg. No rabbit should be selected if:

1. It has a normal temperature greater than 49.8°C.

2. It was used in a positive test during last two weeks or negative test during last two days.

Method for Pyrogen Test: The pyrogen testing is performed in an air-conditioned room. The food and water is withheld to rabbit overnight. A clinical thermometer is inserted in the rectum of each rabbit to a depth of not less than 7.5 cm. Two readings of the temperature of rabbit in normal conditions should be taken at the interval of half an hour before start the test and mean of the both should be calculated to determine the initial temperature. The equipment, injectors and needles used in the test should be pyrogen-free. These should be washed with water for injection and then heated at 260°C for two hours. The injection is warmed to 38°C before injecting to the rabbits. 0.5 to 1.0 ml per kg dose should be injected through the ear vein. Six reading of temperature is recorded at an interval of half an hour.

Pyrogen Test Results: The response of each rabbit is detected by the difference of initial temperature and the highest temperature recorded. The response of all three rabbits gives the sum of responses and can be concluded as:

i) If the sum of responses does not greater than 1.4°C and any of rabbit shows the response less than 0.6° C, the product passes the test.
ii) I sum of responses is greater than 1.4 °C or any of rabbit shows the response 0.6 or greater, continue the test using 5 rabbits.
iii) If the test is done using 5 rabbits, then if the sum of responses of all 5 rabbits is greater than 3.7°C and the individual response of not more than three rabbits is greater than 0.6°C, the product passes the test.

Conclusions:

EXPERIMENT 10

Determination of acute oral toxicity (LD50) of a drug from a given data:

Median lethal dose, **LD_{50}** is a measure of the lethal dose of a toxin dose required to kill half the members of a tested population after a specified test duration. LD_{50}figures are frequently used as a general indicator of a substance's acute toxicity. A lower LD_{50} is indicative of increased toxicity.

LD_{50} is usually determined by tests on animals such as laboratory mice.

The LD_{50} is usually expressed as the mass of substance administered per unit mass of test subject, nanograms (suitable for botulinum), micrograms, or grams (suitable for paracetamol) per kilogram.

LD_{50} is not the lethal dose for all subjects; some may be killed by much less, while others survive doses far higher than the LD_{50}. Measures such as "LD_1" and "LD_{99}" (dosage required to kill 1% or 99%, respectively, of the test population) are occasionally used for specific purposes.

Lethal dosage often varies depending on the method of administration, LD_{50} figures are often qualified with the mode of administration, e.g., "LD_{50} i.v."

Substance	**Animal, Route**	**LD_{50}**	**LD_{50} : g/kg**
Water	rat, oral	90,000 mg/kg	90
Sucrose (table sugar)	rat, oral	29,700 mg/kg	29.7
Glucose (blood sugar)	rat, oral	25,800 mg/kg	25.8
Vitamin C (ascorbic acid)	rat, oral	11,900 mg/kg	11.9
Glyphosate (isopropylamine salt of)	rat, oral	10,537 mg/kg	10.537
Lactose (milk sugar)	rat, oral	10,000 mg/kg	10
Aspartame	mice, oral	10,000 mg/kg	10
Urea	rat, oral	8,471 mg/kg	8.471
Ethanol (Grain alcohol)	rat, oral	7,060 mg/kg	7.06
Methanol	human, oral	810 mg/kg	0.81
Sodium chloride (table salt)	rat, oral	3,000 mg/kg	3

Substance	**Animal, Route**	**LD_{50}**	**LD_{50} : g/kg**
Paracetamol (acetaminophen)	mouse, oral	338 mg/kg	0.338
Ibuprofen	rat, oral	636 mg/kg	0.636
Psilocybin (from magic mushrooms)	mouse, oral	280 mg/kg	0.280
Ketamine	rat, intraperitoneal	229 mg/kg	0.229
Aspirin (acetylsalicylic acid)	rat, oral	200 mg/kg	0.2
Caffeine	rat, oral	192 mg/kg	0.192

Conclusion:

EXPERIMENT 11

Determination of acute skin irritation / corrosion of a test substance

Aim: To test skin irritation/corrosion by using rabbit

Skin irritation and skin corrosion refer to localized toxic effects resulting from a topical exposure of the skin to a substance.

The Globally Harmonized System of Classification and Labeling of Chemicals (GHS) defines skin irritation as "the production of reversible damage to the skin following the application of a test substance for up to 4 hours" and defines skin corrosion as "the production of irreversible damage to the skin; namely, visible necrosis through the epidermis and into the dermis, following the application of a test substance for up to 4 hours".

Structure of Skin: The skin is the largest human organ, but is more than just a protective covering for the body. The skin is composed of multiple layers and serves many functions important to survival. The outermost layer of the skin is called the epidermis. The epidermis is made up of approximately four layers of epithelial cells called keratinocytes. The epidermal keratinocytes and junctions between these cells form the barrier of the skin, preventing substances from penetrating the skin, and water and electrolytes from leaking out of the body. The deeper layer of the skin is called the dermis. The high content of collagen and elastin in the dermis impart strength and elasticity to the skin. Blood vessels, nerves, sweat glands, and hair roots are also found within the dermis. The innermost layer of the skin, called the subcutaneous layer, is relatively thick and primarily composed of fat cells. It is a source of insulation and physical protection for the body as well as a source of energy for the cells.

Method of testing

A test substance is applied to the shaved bare skin about 6 cm2 of healthy young adult albino rabbits and the area is covered with gauze (OECD Test Guideline (TG) 404; Acute Dermal Irritation/Corrosion). The substance is removed after four hours and the rabbit's skin is observed at specific times for irritant responses for as many as 14 days. One animal is usually tested first. The GHS reports that animal skin irritation and corrosion responses are quite variable, so the document explains a range of responses for classification purposes.

Conclusion:

EXPERIMENT 12

Determination of acute eye irritation / corrosion of a test substance

OBJECTIVES

1. Instill drugs carefully into the rabbit's eye by the pouch method without injuring the cornea.
2. Study the effects of drugs on the rabbit's eye.
3. Record, analyze and interpret the observations obtained during the experiment .

Animals: Rabbits

Apparatus: Droppers, measuring scale, torch, cotton wool, calculator

Drugs & solutions:

1. Saline
2. Eserine salicylate 0.5%
3. Atropine sulphate 1.0%
4. Lignocaine 1.0%

PROCEDURE

Place the rabbit (No.1) on the table. Measure the diameter of both the pupils with the help of a scale. Observe the condition of the conjunctiva (congested or not) and elicit the corneal and light reflexes. Record your findings. In the left eye put one drop of saline and in the right eye one drop of eserine. Use the pouch method for instilling the drops. After adding the drops, the medial canthus should be pressed for 30 seconds. Record the following parameters at one minute, 5minutes and ten minutes after instilling the drug and saline. Parameters to be measured:

1. Diameter of the pupil
2. Light reflex
3. Corneal reflex

Record your observations in a tabular form. Repeat the same procedure for atropine, and lignocaine on separate rabbits (Nos 2, & 3) .

Repeat the experiment in your free time using ExPharm v2 for Windows - A Computer Assisted Learning (CAL) software programme with simulated experiments.

Conclusion:

EXPERIMENT 13

Calculation of pharmacokinetic parameters from a given data

OBJECTIVES : 1. present data in a tabular form.
2. carry out basic statistical analysis of data.
3. interpret the results and draw conclusions.

I. Data Presentation:

An introductory class will be taken on data presentation (30 min) and students will be divided into 4 groups. Each group will be given simulated results (data) of an experiment and asked to devise a table to display the data (30 min). Plenary will be held where each group will present their table.

Task A : Read the following situations and present the data in a tabular form :

1. In an experiment, 2 groups of 6 rats were injected with amphetamine and saline respectively and food intake was measured for 2 hrs. The initial weight of rat food was 10 g for each rat. At the end of 2 hrs, the remaining food was weighed and the following data were obtained:

Group I - Saline - 8.21, 5.3, 6.40, 7.584, 6.120, 7.2 g

Group II - Amphetamine - 9.5, 9.473, 9.24, 8.9, 9.90, 10 g

2. The effects of atropine (1.0 % solution) on the pupil size was studied in 8 rabbits. The pupil size was measured before and after administration of saline in one eye and atropine in the other. The data obtained were as follows.

Right eye	Left eye
Saline(1drop)before 5, 6, 3, 4, 7, 5, 6, 5 mm	Atropine(1 drop)before 6, 4,7,3,5, 6, 5, 6 mm
after 6, 5, 4, 4, 6, 6, 6, 6 mm	after 7, 8, 9, 6, 8, 9, 6, 8 mm

3. The cardiostimulant activity of a plant extract (2 µg) was compared with that of epinephrine (2 µg) Twelve isolated frog heart preparations were used and heart rate was observed after administration:

Preparation no. 1- 6:

Basal heart rate- 58, 62, 54, 45, 75, 80 bpm

Epinephrine - 90, 85, 62, 60, 85, 91 bpm

Plant extract - 91, 89, 83, 59, 81, 87 bpm

Preparation no. 7-12:

Basal heart rate- 75, 70, 61, 52, 73, 75 bpm

4. The analgesic effect of morphine (3 mg/kg; sc) and aspirin (10 mg/kg; sc) were compared using 0.6% acetic acid writhing. The data are as follows.

Saline (n=6) : 22, 25, 29, 30, 21, 32 writhings (in 15 min)

Aspirin (n=7) : 15, 18, 18, 20, 22, 17, 16 writhings (in 15 min)

Morphine(n=6) : 5, 8,11, 2, 10, 4 writhings (in 15 min)

Conclusion:

EXPERIMENT 14

Biostatistics methods in experimental pharmacology (student's t test, ANOVA)

Introduction to Biostatistics:
Introduction to Biostatistics It is the branch of statistics that deals with data relating to living organisms. A Biostatistician would be involved with carrying out research, devising experiments, and providing an in depth analysis of all results. This is a great opportunity to make a difference because by carrying out this crucial research, a Biostatistician can make a difference to health care and public health.

In experimental pharmacology we have to understand when to use and how to calculate and interpret different measures of central tendency (mean, median and mode) and dispersion (range, IR and standard deviation) We have to identify the types of error encountered in statistical analysis, the role of sample size and implications for decision making. Describe basic assumptions required for utilization of common statistical tests including the student's t-test, Paired t-test, chi square analysis, wilcoxon signed rank etc.

Student's t-Test : Two types

1. Independent
2. Paired

$$T = \frac{\bar{X} - \bar{Y}}{S_p \sqrt{\frac{1}{n_1} + \frac{1}{n_2}}}$$

X and Y are the two populations. The bar above it means sample mean.
The n 1 and n 2 are the sample sizes. Sp = pooled standard deviation
Analysis of Variance (ANOVA) Is a technique whereby the total variation present in a data set is partitioned or segregated into several components.

For example, if four drug levels with their six possible combinations are to be compared, and each comparison is made by using Alpha = .05,
-there is a 5% chance that each comparison will falsely be called significant.
So the recommended use of ANOVA protects the researcher against error inflation by first asking if there are differences at all among means of the groups. Some basic concepts in experimental designs are the minimum requirements to appreciate the approach of ANOVA in estimating and testing the hypotheses about - population means or about - population variances.

It may be pointed out that when experiments are designed with the analysis in mind,researchers can, before conducting experiments, identify those sources of variation that they consider important and choose a design that will allow them to measure the extent of the contribution of these sources to total variation.

Conclusion:

EXPERIMENT 15

Biostatistics methods in experimental pharmacology (Chi square test, Wilcoxon Signed Rank test)

Chi-square test

The Chi-square test is a non-parametric test of proportions. This test is not based on any assumption or distribution of any variable. This test, though different, follows a specific distribution known as Chi-square distribution, which is very useful in research. It is most commonly used when data are in frequencies such as number of responses in two or more categories. This test involves the calculations of a quantity called Chi-square (x^2) from Greek letter ′Chi′(x) and pronounced as ′Kye.′ It was developed by Karl Pearson.

Applications

1. Test of proportion: This test is used to find the significance of difference in two or more than two proportions.
2. Test of association: The test of association between two events in binomial or multinomial samples is the most important application of the test in statistical methods. It measures the probabilities of association between two discrete attributes. Two events can often be studied for their association such as smoking and cancer, treatment and outcome of disease, level of cholesterol and coronary heart disease. In these cases, there are two possibilities, either they influence or affect each other or they do not. In other words, you can say that they are dependent or independent of each other. Thus, the test measures the probability (*P*) or relative frequency of association due to chance and also if two events are associated or dependent on each other. Varieties used are generally dichotomous e.g. improved / not improved. If data are not in that format, investigator can transform data into dichotomous data by specifying above and below limit. Multinomial sample is also useful to find out association between two discrete attributes. For example, to test the association between numbers of cigarettes equal to 10, 11- 20, 21-30, and more than 30 smoked per day and the incidence of lung cancer. Since, the table presents joint occurrence of two sets of events, the treatment and outcome of disease, it is called contingency table (Con- together, tangle- to touch).

How to prepare 2 × 2 table

When there are only two samples, each divided into two classes, it is called as four cell or 2 × 2 contingency table. In contingency table, we need to enter the actual number of subjects in each category. We cannot enter fractions or percentage or mean. Most contingency tables have two rows (two groups) and two columns (two possible outcomes). The top row usually represents exposure to a risk factor or treatment, and bottom row is mainly for control. The outcome is entered as column on the right side with the positive outcome as the first column and the negative outcome as the second column. A particular subject or patient can be only in one column but not in both. The following table explains it in more detail:

Even if sample size is small (< 30), this test is used by using Yates correction, but frequency in each cell should not be less than 5. Though, Chi-square test tells an association between two events or characters, it does not measure the strength of association. This is the limitation of this test. It only indicates the probability (*P*) of occurrence of association by chance. Yate′s correction is not applicable to tables larger than 2 X 2. When total number of items in 2 X 2 table is less than 40 or number in any cell is less than 5, Fischer′s test is more reliable than the Chi-square test.

2) Wilcoxon-Matched-Pairs Signed-Ranks Test

This is a non-parametric test. This test is used when data are not normally distributed in a paired design. It is also called Wilcoxon-Matched Pair test. It analyses only the difference between the paired measurements for each subject. If *P* value is small, we can reject the idea that the difference is coincidence and conclude that the populations have different medians.

Conclusion: