# ST. JOSEPH'S COLLEGE OF PHARMACY

## DHARMAGIRI COLLEGE CAMPUS

## CHERTHALA

## BACHELOR IN PHARMACY
**SIXTH SEMESTER**

**PRACTICAL RECORD**

## PHARMACOLOGY III

Certified that this is a bonafide record of the practical done by

NAME…..……

ROLL NO…..……

REG NO……

PERIOD ……..……

College seal

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Submitted for the practical examination held by Kerala University Of Health science

**External Examiner**

**Internal Examiner**

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# DOSE CALCULATIONS IN PHARMACOLOGICAL EXPERIMENTS

**OBJECTIVE :**

To learn the dosage calculation and stock solution preparation in experimental animals' studies

**PRINCIPLE**

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 for scientific purposes in almost every aspect of biomedical, behavioural researches and testing conducted in universities, medical schools, pharmaceutical companies, research institutes, etc. Experiments on animals are necessary in drugs discovery and development as well as to advance medical and biological knowledge. Dosage calculation and stock solution preparation based on dosage rationale formula are prerequisites to drug administration in experimental animals.

The dose of drug for animals is expressed as dose (mcg/mg/g) per kilogram of animals. From this experimenter should calculate the dose required for each animal.

Dose required for each animal =

$$\frac{\text{Dose (mcg/mg/g) per kilogram}}{1000} \times \text{Animal Body weight (g)}$$

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

Dosages are usually calculated from stock solution of the test drugs dissolved in appropriate volume of solvent (vehicle). 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, for aqueous solvents, 20 ml/kg (2 ml/100g) body weight can be considered. Large dose cause unnecessary stress to animals and can also overload the stomach capacity. Lower volume (5 ml/kg) can be considered to dissolve highly soluble solute drugs. Such low volume would ease the administration of drug in solution.

Volume required for animal =
(Dose volume ml / 1000 g ) X Animal body weight in grams

# CALCULATIONS

Experiment No. : Date : / /

# EFFECT OF DRUG ON CILIARY MOTILITY OF FROG OESOPHAGUS

**OBJECTIVE :**

To find out the effect of drug on ciliary motility of frog oesophagus

**PROCEDURE:**

Pith a frog. Slit open the oesophagus from the buccal cavity to the stomach. Wipe the blood gently using a cotton swab dipped in Frog's Ringer solution, proceeding from cephalic to caudal end. Moisten the surface with Ringer solution. Place two pins at a distance of 2-3 cm. Place one seed on the groove near the pin at cephalic end. Start the stopwatch and observe the time taken for the seed to reach the pin at the caudal end. Take 2 such readings and calculate the average.

INSTRUCTIONS:

The interface shows two groups of frog (containing six frog in each group) which have been allotted to two groups. select the groups to be treated with the test drug (Acetylcholine) and the vehicle.
Administer the respective treatment to individual animals and then put poppy seed st intestine part of frog.
Click the frog to observe the selected frog.
Record the responce time at which the seed move.
Record the responce time at which the seed move at the caudal end of frog intestine.
The frog in test group are injected Acetylcholine and the frog in control group are administered with the normal saline.

**PRINCIPLE:**

**REPORT:**

Experiment No. : Date : / /

# RABBIT PYROGEN TEST

**OBJECTIVE :**

To test the sterility of test drug

**PROCEDURE:**

1. Animals are divided into two groups (3 animals in each)
2. Administer one group with the drug to be tested and other with vehicle by intravenous route.
3. Temperature of each animal is recorded with tele-thermometer.

**INSTRUCTIONS:**

1. Rabbits must be healthy and mature
2. New Zealand or Belgian Whites used mostly
3. Either sex may be used but males are preferred
4. Must be individually housed between 20 and 23°c
5. Their temperature should not vary more than ± 3° c.
6. Atmosphere should be free from disturbances which likely to excite them
7. Equipment and material used in test (glassware, syringes, needles etc) must be made free from Pyrogens by heating at 250° c for not less then 30 minutes or any other method.
9. Retaining boxes (should be comfortable for rabbits as much as possible)
10. Thermometers or thermistor probe (should be inserted in standardized position in rectum, precision of ± 0.1° C)

**PRINCIPLE:**

The Rabbit Pyrogen Test in an in vivo test to detect pyrogens qualitatively. Rabbits have a similar pyrogen tolerance to humans, so by observing a change in body temperature in rabbits it is possible to make a determination of the presence of pyrogens. This method can detect non-bacterial endotoxin pyrogens as well as bacterial endotoxins.

**REPORT:**

Experiment No. : Date : / /

# Effect Of Drug On Skeletal Muscle Relaxation Using Rota-Rod Apparatus

OBJECTIVE :

To study the effect of CNS suppressant and skeletal muscle relaxant drug on mice using rotarod apparatus.

PROCEDURE:

1. Animals are divided into two groups (3 animals in each)
2. Administer one group with the drug to be tested and other with vehicle by intraperitoneal route.
3. Adjust the speed of instrument (25 rotations/60 sec).
4. Click on the vehicle treated animal and note down the free ridings and fall off time (Sec).
5. Click on the drug treated animal and note down the free ridings and fall off time (Sec).
6. When the animal fall off respective time will be displayed on the timer.

PRINCIPLE:

Rotarod apparatus has a horizontal grooved rod rotating at a fixed speed. The mice are made to balance on this rod. Dependent upon their motor co-ordination, Central nervous activity and grip strength the animal either stay on the rotating rod for specific time and after that fall down on the platform of each compartment. The floor of each compartment has sensors that deactivate the timers and the exact fall off time for each rat is displayed on the respective display.

Reduction of motor co-ordination, CNS depression and skeletal muscle relaxation lead to decrease in the fall off time and decrease in number of free ridings of animal balancing on the rotarod. Thus lesser fall off time and less number of free ridings indicate that the administered drug has CNS depressant or muscle relaxant activity that either lead to decrease in the motor co-ordination or decrease in the gripping power

REPORT:

Experiment No. : Date : / /

# Effect Of Drug On Locomotion Using Actophotometer

**OBJECTIVE :**

To study the effect of drug on locomotion using actophotometer.

**PROCEDURE:**

1. Animals are divided into two groups (6 animals in each).
2. Administer one group with the drug to be tested and other with vehicle by oral route.
3. Put one animal at a time in the Actophotometer
4. Start the instrument
5. Count the locomotor activity for 10 minutes.
6. Record the observations.

**PRINCIPLE:**

Actophotometer has a central chamber with arrangement of light sources and photocells at the base of two opposite walls.The light of each source is focused on a photocell. Any Interruption in the path of light activates the photocells and this is counted as a measure of horizontal locomotor activity of the mice kept in the chamber.

The drug acting on central nervous system as stimulants or suppressants affect the locomotor activity in experimental animals. Such changes in the motor activity can be easily determined by using simple techniques in which the animal treated with drugs is kept in an open compartment having square marking on its floor . The lines of such squares crossed by the animal are counted by the observer. The number on lines crossed correlates with the locomotor activity. Alternatively, the locomotor activity can be determined using actophotmeter or more sensitively by using automated video tracking system in addition to the horizontal movement the vertical movement(rearing -standing standing on hindlegs and exploration of surroundings) is also considered as a parameter associated with locomotor activity.

**REPORT:**

Experiment No. : Date : / /

# Effect Of Drug On Convulsion By Electro Covulsiometer Method

**OBJECTIVE :**

To study the anti-convulsive effect of the drug in mice using electro covulsiometer.

**PROCEDURE:**

1. Animals are divided into two groups (6 animals in each)
2. Administer one group with the drug (Phenytoin 25 mg/kg) to be tested and other with vehicle by intraperitoneal route.
3. After 30 minutes, attach electrodes of the convulsiometer on the ears of the mouse.
4. Give shock 30mA intensity for 0.2 seconds duration and measure tonic seizures, clonic seizures and stupor. Also report the survival/ death of animal.
5. Determine average duration determine whether treatment with phenytoin reduces duration of these stages of epilepsy.

**PRINCIPLE:**

Electroconvulsiometer is used to deliver the electric shock of required intensity for required intensity for required duration. This instrument is used to evaluate the anticonvulsant effect of pharmacological agents against electro shock induced convulsions in experimental animals. An electrical stimulus with an intensity that induced characteristic convulsion is applied to the animals through the electrode placed on ear pinna. The duration of tonic and clonic seizures are measured. The drug to be tested is administered to separate group of animals and its effect on such duration on such convulsions is measured. Anticonvulsant pharmacological agents reduce the duration of seizures induced by electrical shocks.

Electrical shock given through the electrodes applied on the ear pinna results into a burst of exitatory neurotransmitters from the brain. This activate the brain activity during grand-mal epilepsy. Prior treatment of animals with the drugs, reduces the exited activity of brain.

**REPORT:**

Experiment No. : Date : / /

# Effect Of Drug On Convulsion By PTZ Method

**OBJECTIVE :**

To study the anti-convulsive effect of the drug in mice using pentalin tetrazole induced convulsion method.

**PROCEDURE:**

1. Select albino mice having body weight between 20 to 25 gm.
2. Divide the mice into 2 groups of 5 animals
3. Weigh the mice in each groups, do the marking and keep in mice cage
4. Administer the drug solution as shown below
5. Group 1- Pentylenetetrazole 80 mg/kg, ip or picrotoxin 4 mg /kg, ip
6. Group 2- inject diazepam 5 mg /kg, ip. After 30 minute, inject pentylenetetrazole 80 mg/kg, ip or Picrotoxin 4 mg /kg, ip
7. Record the onset and duration of convulsion and their type (tonic or clonic)

**PRINCIPLE:**

Central nervous system stimulants at higher doses produce convulsion. Pentylenetetrazole is a central nervous system stimulant. It produces jerky type of clonic convulsion in rats and mice. Pentylenetetrazole closes the GABA- linked chloride channel in the brain. Therefore, it produces clonic convulsion. Diazepam and sodium valporate open the GABA linked chloride channels and produce hyperpolarization. Therefore, they prevent the convulsion induced by pentylenetetrazole or picrotoxin. Compounds which prevent chemoshock, are found to be useful in treating absence-seizure (petitmal epilepsy)

**REPORT:**

Experiment No. : Date : / /

# Effect Of Drug On Catatonic Activity

**OBJECTIVE :**

To study the stereotype and anti-catatonic activity of drugs on mice

**PROCEDURE:**

1. Animals are divided into two groups
2. Administer one group with the stereotypy OR anti- catatonic behaviour inducer to be tested and other with vehicle by oral route.
3. Evaluate one animal at a time
4. Start the Experiment
5. Count the jumping activity for specific duration.
6. Record the observations.

**PRINCIPLE:**

This experiment presents a quantitative, observational method for the assessment of rodent stereotyped and anti catatonic behavior which consists of motor responses that are repetitive, invariant, and seemingly without purpose or goal. The most classic behavioral pattern that is characteristic of stereotypy and anti catatonic behaviour is that elicited by high doses of CNS stimulants, in rodents, although it can also occur in response to other drugs or neurotoxic treatments affecting the basal ganglia.

Measurement of stereotypy,can be adapted to sampling many forms of spontaneous behaviors, including rearing, grooming & jumping responses of behavioral checklists and scoring the same.

**REPORT:**

Experiment No. : Date : / /

# Effect Of Drug On Anxiolytic /Anxiogenic Activity Using Elevated Plus Maze

**OBJECTIVE :**

To study the anxiolytic /anxiogenic activity of drug using elevated plus maze apparatus test.

**PROCEDURE:**

1) Weigh and mark the animals and divide them into groups.
2) Place the animal in center square of plus maze.
3) Record the number of entries made by animals into the closed or the open arms, as well as the time spent by animals in each type of arms for 5 minute.
4) Administer freshly prepared drugs solutions/vehicle to each group.
5) Thirty minutes after the treatment, place the animal in center square of plus maze and monitor the number of entries and time spent by animals in arms.
6) Calculate their percentage ratio of open/total arm entries and time spends in open arms.

**PRINCIPLE:**

The elevated plus maze task is a simple method to assess anxiety like behaviours in rodents. The test is performed on a plus shaped apparatus with two open and two closed arms. The animal is allowed to freely explore the maze for 5 min while the duration and frequency of entries into open and closed arms is recorded. The task is based on an approach-avoidance conflict, meaning that the animal is faced with a struggle between a propensity to explore a novel environment and an unconditioned fear of high and open spaces. Consequently, an anxiety-like state is characterized by increased open arm avoidance, compared to control animals. It has been able to detect both anxiogenic and anxiolyitic drug effects under the specified conditions. It is a very popular test, there can be considerable variations in the procedures applied across different laboratories.

This is an animal model of anxiety that usually uses rodents as a screening test for putative anxiolytic or anxiogenic compounds and as ageneral research tool in neurobiological anxiety research. The model is based on the test animal's aversion to open spaces and tendency to be thigmotaxic (a preference to remain near to, or touching, vertical surfaces).

The anxiety reduction is indicated in the plus-maze by an increase in the proportion of time spent in the open arms ( time in open arm/total time in open time in open or closed arms), and an increase in the proportion of entries into the open arms (entries into open arms/ total entries into open or closed arms. The total number of arm entries and number of closed-arm entries are used as measures of general activity.

**REPORT:**

Experiment No. : Date : / /

# Effect Of Drug On Analgesia Using Eddy's Hot Plate

**OBJECTIVE :**

To study of analgesic activity of the drug with the help of Eddy's hot plate apparatus.

**PROCEDURE:**

1. Animals are divided into two groups (6 animals in each)
2. Administer one group with the drug (Pentazocine 10mg/kg) to be tested and other with vehicle by intraperitoneal route.
3. After 60 minutes put the mice on the Hot Plate maintained at 550 C.
4. Record the response time at which the mouse licks its fore paws or jumps.

**PRINCIPLE:**

Eddy's hot-plate is a device used to give heat stimulus to the paws of animal. A metal plate at the top of hot plate apparatus can be heated in the controlled manner and maintained at the required temperature for the duration of an experiment.Generally, in testing of the analgesic activity of drug in animal, 55* C temperature is used as a thermal stimulus.

Analgesic drug increase pain threshold (ability to tolerate a painful stimulus). This effect can be evaluated in animal models of analgesia. To induce pain , following type of stimuli can be used:

Radiant heat projected on the tail, paw or shaved skin
Cold stimulus applied through a cold plate
Mechanical pressure on tail or paw applied using a clamp
Chemical stimulus (formalin)

The time taken by the animal to reveal pain sensation through vocalization or paw licking or effort to escape the painful stimulus (withdrawal of the body part on which pain is inflicted-paw or tail) is determined. The analgesics increase the time taken by the treated animal to reveal pain sensation

**REPORT:**

Experiment No. : Date : / /

# Effect Of Drug On Analgesia Using Tail Flick Method

**OBJECTIVE :**

To study of analgesic activity of the drug with the help of tail flick apparatus,

**PROCEDURE:**

1. The inerface shows two groups of mice (containing six mice in each group) which have been rendomly selected and alloted to two groups. select the groups to be treated with the study drug and the vehicle.
2. Administer the respective treatment to individual animals by intraperitoneal route.
3. Click the mouse to put the selected mouse on the Tail flick Apparatus.
4. Record the responce time at which the mouse move the tail.
5. The mice in test group are injected Morphine (10 Mg /kg Oral) and the mice in control group are administered with the vehicle in the same volume. The responce time are recorded after 1 hour of drug administration.
6. Step to be followed:
   Step-1:
7. Select the animal to be treated with vehicle or drug dependent upon its group and administer the respective treatments.
   Step-2:
8. After 1 hour of the treatments, Load the mice on the Tail-flick Analgesiometer

**PRINCIPLE:**

Introduction:The tail flick test is a test of the pain response in animals, similar to the hot plate test. It is used in basic pain research and to measure the effectiveness of analgesics, by observing the reaction to heat. It was first described by D'Amour and Smith in 1941.

Analgesic drug increase pain threshold (adility to tolerate a painful stimulus). This effect can be estimated in animal models of analgesia. To induce pain , following type of stimimuli can be used:

Radiant heat projected on the tail, paw or shaved skin
Cold stimulus applied through a cold plate
Machenical pressure on tail or paw applied using a clamp
Electrical stimulus on both pulp
Chemical stimulus (formalin)

The time taken by the animal to reveal pain sensation through vaocalization or paw licking or effort to escape the painful stimulus (withdrawl of the body part on which pain is inflicted-paw or tail) is determined. The analgesics increase the time taken by the treated animal to reveal pain sensation.

**REPORT:**

Experiment No. : Date : / /

# Effect Of Drug On Nociception Using Acetic Acid Induced Writhing

**OBJECTIVE :**

To study analgesic activity of the drug by using acetic acid induced writhing test

**PROCEDURE:**

1. Weigh, mark and divide the animals into group
2. To the control group, inject intraperitoneally 0.25 ml of 0.02 % Phenylquinone (suspended in a 1 % suspension of Carboxy Methyl Cellulose) or 0.1 ml of 0.6 % acetic acid and placed under bell jar for observing number of writhing in 10 minutes for every 30 minutes
3. Note the onset on writhe. Record the number of abdominal contraction, trunk twist response and the extension of hind limbs as well as the number of animals showing such response during a period of 10 minutes
4. The next groups of animals inject the test drugs. 15 minutes later, administer the noxious substance to these animals.
5. Note the onset and severity of the writhing response as done in control group.
6. Calculate the mean writhing score in control and drug treated groups, and note the inhibition of pain response by the drugs.
7. Anti-nociception is quantified as percent inhibition using the following formula:

% Inhibition = [(control responses - test responses)/control responses] X100

**PRINCIPLE:**

Writhing induced by acetic acid or phenylquinone is a painful reaction, which can well be characterized by clean observable signs such as constriction of abdomen, twining of trunk (ophistotonus) and extension of hind limb. While only narcotic analgesic produces positive response in mechanical, thermal and electrical methods, in writhing method, both narcotic and non-narcotic analgesic produce positive response.

Pain is induced by injection of irritants into the peritoneal cavity of mice/rat. The animals react with a characteristic stretching behavior, which is called writhing. This test is suitable to detect analgesic activity although some psychoactive agents also show activity. An irritating agent such as phenylquinone or acetic acid is injected intraperitoneally to mice and the stretching reaction is evaluated. The reaction is not specific for the irritant.

**REPORT:**

Experiment No. : Date : / /

# Study Of Anti-Inflammatory Activity Using Carrageenan Induced Paw Oedema Method

OBJECTIVE :

To study the anti-inflammatory activity of the drug using carrageenan induced paw oedema method

PROCEDURE:

Twelve healthy male albino rats weighing 100-200 gms will be selected and made into two groups of six animals each. All the animals will be kept on fasting for 18 hours. The hind paw of the rats will be marked at the level of tibio tarsal junction of hind leg, so that while measuring the volume, the dipping will be done to the same 22 level. 0.1 ml of 1% Carrageenan will be administered to the rats into the plantar surface of the right hind limb to induce paw oedema. The volume will be measured immediately and after 3 hours using plethysmometer. One group serve as control, 0.3 ml of Normal saline will be given orally. Another group will receive the test drug, Acetyl salicylic acid 300 mg/Kg. After 30 minutes of the administration of drug, The change in the paw volume was compared with the control animals. The percentage of oedema compared to the control by the test drug.

PRINCIPLE:

Plethysmometer is an equipment used to measure paw volume of rat while preforming anti-inflammatory activity.

Inflammation is a protective response to injury. It occurs in three phases:

(a) The first phase being oedema and swelling with accompanying pain. These effects are produced as a result of the dilation and increased permeability of the blood vessels (veins) due to the release of certain mediators such as histamine, serotonin and kinins etc.

(b) In the second phase, leukocytes migrate to this area and mopping up operations starts.

(c) Second phase is followed by repair, which is ushered in by the proliferation of fibroblasts and synthesis of connective tissue.

The ability of a compound to reduce the local oedema induced in rat paw by various irritants is the most widely used test to screen new non-steroidal anti-inflammatory drugs. Many compounds like formalin, carrageenan, Kaolin, yeast and dextran have been used as irritants to produce oedema

REPORT:

Experiment No. : Date : / /

# Study Of Anti Ulcer Activity - Using Pylorus Ligation Method

OBJECTIVE :

To study the anti ulcer activity of the drug using pylorus ligation method.

PROCEDURE:

1. Rats fasted for 24 hrs prior to pyloric ligation.
2. Randomly divided into 2 groups of 3 animals each.
3. Drugs administered once for 2 days and 30 mins prior to ligation
4. Rats anesthetized with ether.
5. Pyloric ligation procedure done.
6. Rats placed in separate cages and allowed to recover.
7. 19 hrs after pyloric ligation, animals sacrificed by decapitation.
8. Abdomen opened and stomach dissected out.
9. Contents of the stomach collected in a centrifuge tube.
10. Stomach opened along greater curvature and ulcers observed under 10x magnification.

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.

REPORT:

Experiment No. : Date : / /

# Biostatistics Methods In Experimental Pharmacology (Student's t Test)

OBJECTIVE :

To study the student's t test biostatistics method of calculation in experimental pharmacology

PRINCIPLE:

Statistics is basically a way of thinking about data that are variable, Statistics implies both, data and statistical methods. It can be considered as an art as well as science. Statistics can neither prove not disapprove anything. It is just a tool. Statistics without scientific application has no roots. Thus, statistics may be defined as the discipline concerned with the treatment of numerical data derived from group of individuals. These individuals may be human beings, animals, or other organisms.

Mr. W. S. Gosset introduced 't' distribution of small samples and one of the most widely used tests in pharmacological investigations, involving the use of small samples. The 't' test is always applied when the number of sample is 30 or less. There are two types of 't' test, paired and unpaired. When comparison has to be made between two measurements in the same subjects after two consecutive treatments, paired 't' test want to use, example, when we want to compare effect of drug A before start of treatment (baseline) and after the treatment with drug A. When comparison is made between two measurements in two different groups, unpaired 't' test is used. For example, when we effects of drug A and B after one month from baseline in both groups, unpaired 't' test' is applicable.

PROCEDURE:

STUDENTS-T-TEST OF UNPAIRED SAMPLES

1) Calculate the mean of first series ($\ddot{X}1$) and mean of second series ($\ddot{X}2$)
2) Calculate the deference between values and mean, first series ($X'_1 - \ddot{X}_1$) and second series ($X'_2 - \ddot{X}_2$)
3) Calculate the squires of deferences, $(X'_1 - \ddot{X}_1)^2$ and $(X'_2 - \ddot{X}_2)^2$
4) Calculate the sum of the squire of deference, $\sum(X'_1 - \ddot{X}_1)^2$ and $\sum(X'_2 - \ddot{X}_2)^2$
5) Calculate the pooled standard deviation ($S^2$) using the formula

$$s^2 = \frac{\sum_{i=1}^{n_1}(x_i - \bar{x}_1)^2 + \sum_{j=1}^{n_2}(x_j - \bar{x}_2)^2}{n_1 + n_2 - 2}$$

Where n1 is the Sample of size of first series and n2 is sample size of second series

6) Calculate the t value

$$= \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{s^2\left(\frac{1}{n_1} + \frac{1}{n_2}\right)}}$$

7) Calculate the degree of freedom = n1 + n2 - 2

8) Check the t table value with respect to the degree of freedom at 5% significance level

9) Compare the calculated t value with the t table value, if calculated t value is grater than t table value then there will be difference between both means

STUDENTS-T-TEST OF PAIRED SAMPLES

1) Calculate the difference between paired samples (d)
2) Calculate the sum of difference between paired samples ($\sum d$)
3) Calculate the mean of difference between paired samples ($\bar{d}$)
4) Calculate the square of sum of difference between paired samples $(\sum d)^2$
5) Calculate the sum of the squire of difference between paired samples $(\sum d^2)$
6) Calculate the standard deviation of paired differences (Sd) using the formula

$Sd = \{\sum d^2 - [(\sum d)^2 / n] \} / n-1$

Where n is the sample of size

7) Calculate the t value

Paired data samples $t = |\bar{d}| / \sqrt{(Sd/n)}$

Where $|\bar{d}|$ is mean of difference between paired samples with out sign

8) Calculate the degree of freedom = n-1
9) Check the t table value with respect to the degree of freedom at 5% significance level

Compare the calculated t value with the t table value, if calculated t value is grater than t table value then there will be difference between both means

CALCULATIONS

Experiment No. : Date : / /

# Biostatistics Methods In Experimental Pharmacology ( ANOVA)

OBJECTIVE :

To study the ANOVA biostatistics method of calculation in experimental pharmacology

PRINCIPLE:

To compare two sets of unpaired or paired data, the student's 't' test is applied. For analyse 3 or more sets need well-designed and multi-talented method called as analysis of variance (ANOVA). This test compares means of more number of means one time. It draw assumption that each sample is randomly drawn from the normal population, and also they have that of population. There are two types of ANOVA, one way ANOVA and two way ANOVA

One way ANOVA: It compares three or more unmatched groups when the data are categorized in one way. For example, we may compare a control value with three different doses of aspirin in rats (four unmatched groups). Choose repeated measures ANOVA test when the trial uses matched subjects. For example, effect of supplementation of vitamin C in each subject before, during, and after the treatment. Matching should not be based on the variable you are comparing. For example, comparing blood pressures in two groups, it is better to match based on age or other variables, but it should not be to match blood pressure. The term repeated measures applies strictly when you give treatments repeatedly to one subjects. The P value is calculated from the ANOVA table.

Two way ANOVA: Also called two factors ANOVA, determines how a response is affected by two factors. For example, measure a response to three different drugs in both men and women.

PROCEDURE:

1.

REPORT:

Experiment No. : Date : / /

# Biostatistics Methods In Experimental Pharmacology (Chi square test)

OBJECTIVE :

PROCEDURE:
1.

INSTRUCTIONS:

PRINCIPLE:

REPORT:

Experiment No. : Date : / /

## Biostatistics Methods In Experimental Pharmacology (Wilcoxon Signed Rank test)

OBJECTIVE :

PROCEDURE:
1.

INSTRUCTIONS:

PRINCIPLE:

REPORT:

Experiment No. : Date : / /

# Estimation Of Serum Total Cholesterol

OBJECTIVE :

To determine the amount of cholesterol present in the given sample of serum.

PRINCIPLE:

In this method, proteins in serum are precipitated with ferric chloride-acetic acid reagent. The protein-free filtrate containing cholesterol is treated with concentrated sulphuric acid. The cholesterol in the presence of sulphuric acid undergoes dehydration to form 3,5 - cholestadiene. This intern oxidise and sulphonated to form red coloured cholesta- polyene. The intensity of red color formed is proportional to the amount of cholesterol present in the serum.

Reagents Required

1. Ferric chloride acetic acid reagent
2. Concentrated sulfuric acid (analytical grade)
3. Cholesterol standard (working) – 0.04 mg/ml.

PROCEDURE:

1) Pipette 0.2 ml serum in a 15 ml stoppered centrifuge tube and add 9.8 ml of working ferric chloride reagent, stopper the tube and shake well carefully and keep it for 15 minutes for flocculation of protein.
2) Centrifuge the solution and prepare the test, standard and blank solution
3) Test solution: 5 ml protein free supernatant +3 ml concentrated sulphuric acid, mix well
4) Standard solution : 5 ml working standard +3 ml concentrated sulphuric acid, mix well
5) Blank solution: 5 ml working ferric chloride acetic acid reagent +3 ml concentrated sulphuric acid, mix well
6) Keep the solutions for 30 minutes.
7) Read the absorbance of test and standard at 540 nm using green filter.

**Calculation**

Concentration of standard in 5 ml std solution = 5 × 0.04 mg/ml = 0.2 mg/ml

Serum cholesterol in 100 ml serum (mg%) =

$$\frac{\text{Reading of test}}{\text{Reading of standard}} \times \text{Concentration of standard} \times \frac{100}{\text{Volume of serum taken}}$$

$$= \frac{\text{Reading of test}}{\text{Reading of standard}} \times 0.2 \times \frac{100}{0.2}$$

$$= \frac{\text{Reading of test}}{\text{Reading of standard}} \times 100$$

REPORT:

Experiment No. : Date : / /

# Estimation Of Creatinine In Given Sample

OBJECTIVE :

To determine the amount of creatinine present in the given sample using Jaffe' reaction method.

PRINCIPLE:

Creatine, methyl guanidoacetic acid is synthesized in the liver and kidney and carried by the blood to muscular tissues and brain and converted to creatine phosphate. Energy needed for muscular contraction is provided by ATP break down to form ADP. The ATP is regenerated from ADP by the action of creatine kinase. This regeneration of ADP by the hydrolysis of creatine phosphate (Lohmann's reaction). During this process creatine phosphate is converted to creatine. Creatine in turn converted by spontaneous dehydration into creatinine. About 2% of the total creatine is converted to creatinine per day so that the rate of creatinine formation is constant in an individual as it is related to muscle mass. Creatinine is filtered at the glomerulus and reabsorbed by the tubules. So that at low plasma concentrations, as in a normal individual, no creatinine appears in urine. Creatinine is also filtered at the glomerulus. It is reabsorbed at PCT in very small amounts and secreted in the tubules to a minor degree.

The plasma/serum creatinine increases with renal diseases – nephritis, nephrotic syndrome, acute and chronic renal failure and other types of renal insufficiency caused by drugs and toxins.

The method used for the determination of serum creatinine is based on the Jaffe' reaction. In Jaffe' reaction creatinine is react with picric acid solution in alkaline sodium hydroxide solution, a yellow-red coloured picramic acid is formed. The absorbance of the complex is measured with green filter in a colorimeter or at 505 nm in a spectrophotometer

PROCEDURE:

1) Take 3 ml of the sample into the test test tube, 3.0 ml of distilled water into the blank test tube and 0.5 ml of working standard (1 mg/dL) plus 2.5 ml distilled water into standard test tube.
2) Add 1.0 ml picric acid solution to each test tube and mix well
3) Add 1.0 ml sodium hydroxide (0.7 mol/L) and shake well
4) Keep all the tubes at room temperature for 15 minutes.
5) Adjust the reading in the colorimeter to zero with the blank, using green filter in the colorimeter (520 nm)
6) Read the absorbance of test and standard against the blank.

Calculations

Concentration of creatinine in 100 ml (mg%) =

$$\frac{\text{OD of Test}}{\text{OD of Standard}} \times \text{con. of std in mg\%} \times \frac{100}{\text{volume of sample}}$$

$$= \frac{\text{OD of Test}}{\text{OD of Standard}} \times 0.05 \times \frac{100}{0.5} = \frac{\text{OD of Test}}{\text{OD of Standard}} \times 10 \text{ mg\%}$$

REPORT:

Experiment No. : Date : / /

Effect Of Drug On Analgesia Using Tail Immersion Method

OBJECTIVE :

PROCEDURE:

PRINCIPLE:

REPORT:

Experiment No. : Date : / /

Effect Of Drug On Analgesia Using Tail Immersion Method

OBJECTIVE :

PROCEDURE:

PRINCIPLE:

REPORT:

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Effect Of Drug On Analgesia By Haffner's Tail Clip Method

OBJECTIVE :

PROCEDURE:
1.

INSTRUCTIONS:

PRINCIPLE:

REPORT:

Experiment No. : Date : / /

Effect Of Drug On Nociception Using Formalin Induced Pain

OBJECTIVE :

PROCEDURE:
1.

INSTRUCTIONS:

PRINCIPLE:

REPORT: