S.A. RAJA PHARMACY COLLEGE

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VI – SEMESTER – (III-B.PHARM)

PHARMACOLOGY – III

PRACTICAL LAB MANUAL

Exp No : I

GUIDELINGS ON DOSAGE CALCULATION AND STOCK SOLUTION PREPARATION IN EXPERIMENTAL ANIMALS STUDIES

Aim – To prepare stock solution and calculate dosage calculations in experimental animals studies (or) Preclinical studies

Introduction:

Experimental animals are very important tool in non-human research models

Dosage calculations and stock solution preparation based on dosage rationable formula are prerequisites to drug administration in experimental animals

However, drug dosage calculations and stock solutions preparations are not clearly explained in most scientific literature.

Vehicle of choice, drugs dissolutions & volume selections rationale:

A vehicle is any substance that acts as when drug is administered

Vehicle which is an essential consideration in all animal research should be biologically inert, it has no toxic effects on animals influence the results obtained for the compound under investigations.

Eg-water, normal saline (0.9% nacl), 50% polyethylene glycol, 5-10% tween 80,0.25% methyl cellulose (or) carboxy methyl cellulose.

In most researches involving experimental animals dosage are usually calculated from stock solution of the test drugs dissolved in vehicle.

According to OECD's (organization of economic corporation and development) guidelines, dosage of drug should be constituted in an appropriate volume not usually

exceeding 10 ml/kg body weight of experimental animals for non aqueous solvent in oral route of administration.

however the aqueous solvents 20ml/kg body weight can be considered large dose volumes. Can cause unnecessary stress to animals large dose volumes. Can cause unnecessary stress to animal can also overload the stomach capacity and pass immediately into the small bowel or can result in passive reflux in stomach, aspiration, pneumonia, pharynageal, esophageal and gastrin irritation or injury with structure formation.

However, highly viscous drug solutions should be diluted whenever possible, for ease of adininistation.

However, final dilution valume should be not exceed 20ml/kg. based on 10ml/kg valume selection, required dose valume for a 100gm rat can be calculated as follows 100g/1000gx10ml=ml

NB-1g=100g

Based on 20ml/kg volume selection, required dose volume for a 100g not can be calculated as follows.

100g/1000gx20ml=2ml

Exp No : II

ANTI – ALLERGIC ACTIVITY BY MAST CELL STABILIZATION ASSAY

Requirements

Animals – guinea pigs = 400 – 600 gms

Albino rats – 175 – 200 gms

Drugs – histamine dihydrochloride aerosol (0.2% W/V)

Chlorpheniramine maleate (2mg / kg)

Disodium chromoglycoate (50mg / kg)

Reagents – Saline solution (0.9%)

Egg albumin (100 µg / ml)

Toludine blue solution (1%)

Instruments – microscope with 10x magnification lens

Procedure-

Evaluation of bronchoconstriction in guinea pigs by using histramine aerosol-

To take two groups of guinea pigs. Each group containing 3 animals. Animals have to be fasted over night. Normal saline gives to the group-I animals Chlorpheniramine maleate (2mg/kg) gives to the group-II animals. Before the drug administration animals should be exposed to the histamine aerosol (0.2%) in histamine chamber. Then determine the end point. The pre-covulsion dyspea (PGD) is the time of exposure of histamine aerosol to onset of dyspnea that leads to convulsion. As early air & time of onset of PCG is to be noted on day zero. Then animals have to treat with drug after 24 hrs. After 1 hr of drug admonition once again are exposed to histamine aerosol & PCD is determined.

Percentage (%) of protection offered by the drug can be calculated by the below formula.

Formula:

Percentage protection – $(1-T_1/T_1)x100$

Where T_1=mean value of PCD before drug administration

T_2=mean value of PCD after drug administration.

Observations:

Group	Percentage protection
Group – 2 Chlorphemniramine maleate (2mg/kg)	

Mast cell stabilization activity:

Albino rats are divided in to 2 groups. Each containing 3 animals. Group-1 receives normal saline. Disodium chromoglycalate (50mg/kg) gives group-2 for 3 days. Inject 10ml/kg of 0.9% saline into peritoneal cavity on 4^{th} day to each animal. Massage the peritoneal region of the animal gently for 5min, then collect the peritoneal fluid and transfer to the test tube which is carrying 7-10ml of PRMI buffer. Centrifuge the fluid for 400-500RPM. Discard the supernatant & wash the pellets of mast cells twice with same buffer by centrifugation. Add egg albumin to the above cell suspension & incubate at 37^0C for 10 min. Later the suspension has to stain with 1% toluidine blue solution & observer the slide under microscope for calculating number of granulated and degranulated mast cells in each group.

Observations

Group	Total number of cells (n=100)	
	Granulated	degranulated
Group-1 (saling)		
Group-2 Disodium chromoglycate 50mg/kg		

Exp No : III

STUDY OF ANTI-ULCER ACTIVITY OF A DRUG UNING PYLORUS LIGANTION (SHAY) RAY MODEL

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

REQUIREMENTS: Animals: Albino Wister rats of 150-250g are selected for the study.

Drugs: Ether (anesthetic), Ranitidine 20mg/ kg, P.o, 0.9% normal saline

Reagents: 0.1N NaOH, Phenenolphthalin, Toper's reagent,

Instruments: Dissecting microscope (10X magni-cation lens), Burette, P meter, Surgical instruments.

Procedure:

Pyloric ligation method: Fasted the albino rats 24 hours before the experiment. Then administer the reference drug and control vehicle before 1 hour of pylorus ligation. Then anesthesia given to animal with ether. Then open the abdomen by small midline incision below the diploid process. Then stomach pylori portion was lighted without causing damage to blood vessels, then stomach was isolated and abdominal wall was sealed with sutures. After 48hours of ligation, stomach was ,dissected out and collected the contents into the clean tubes. The volume , pH and total, acid content of juice were certrifused, filtered and titrated for estimation of total acidity. Then number of ulcers were based on following below formula.

FORMULA:

$$\mathbf{Ui = UN + Us + Up \times 10^{-1}}$$

Where,

Ui = ulcer index

UN = average number of ulcers per animal

Us = Average no. Of severity score

Up = Percentage of animal a with ulcers

Percentage inhibition of ulceration was ,calculated and compared with standard or control.

CONCLUSION

Comparison of ulcer index between study groups estimates the potency of antiulcer activity of test drug. Decrease in volume and total acidity determines anti-secretary activity of test drug and rise in P evaluates acid naturalizing action of the drug.

Exp No : IV

STUDY OF ANTI-ULCER ACTIVITY OF A DRUG USING NSAIDS

BACKGROUND

Peptic ulcer defined as an ulceration of the mucous membrane of the stomach , duodenum or esophagus. The imbalanced secretions of gastric acid , pepsin and duodenal mucosal defense mechanisms are the causes of such ulcers. Excessive non- steroidal and anti-inflammatory drugs (NSAIDs) consumption of can cause damage of gastrointestinal mucosa leading to ulcers. Aim of this experiment is to screen the drugs for their antiulcer activity.

REQUIREMENTS

Animals: Albino Westar rats of (200-300g) either sex

Drugs: Aspirin – 400 mg/kg, p.o

Ranitidine 30 mg/ kg, p.o.

Dissolve both drugs in 1% CMC.

Chemicals: 1% CMC.

Instruments: Dissecting microscope (10X magnification lens),

Surgical instruments.

PROCEDURE: Select albino rats weighting 200-300g and divide them into the two groups consisting of 3 animals in each group. Group-1 receives 1% CMC. Group -2 receives Ranitidine (30mg/kg, p.o). Administer Aspirin (200mg/kg)/ Diclofenac sodium (100mg/kg) suspended in 3 ml of CMC after 30 minutes of antivcular drug administration in both groups. Avoid access to feed and water to animals. After 6 hours sacrifice the animals by cervical decapitation. Open along the greater curvature of the

stomach, remove the stomach contents and wash with 0.9% saline. Observe for the ulcers formed and measure the length of each ulcer and calculate ulcer index.

Ulcer score: 1mm (pin point)=1; 1-2mm =2; >2mm =3;>3mm =4

$$\textbf{ULCER INDEX} = (U1 + U2 + U3) \times 10^{-1}$$

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 and 3- perforations.

CONCLUSION

Comparison of ulcer index between study groups estimates the potency of anticular activity of test drug.

REFERENCES

1. Wallace JL. Fallieres Best Pact Res Clan Gastroenterology. 2000; 14 (1): 147-59

Exp No : V

STUDY OF EFFECT OF DRUGS ON GASTRO INTESTINAL MOTILITY

IMDRODUCTION

The motility function gastrointestinal tract (GIT) is associated with

1. Forward propulsion of ingested food
2. digestion
3. absorption of nutrients and
4. expulsion of unabsorbed food material.

These function are adequately ,supported by cyclic motor activity occurring in almost all parts of the GIT which is due to migrating my electrical complex (MMC) through electrical activity of the GIT. Any discrepancy in motility patter can affect ,functionality of the GIT. A decrease in motility can lead to the stasis of food / chime in the intestine which favors the increases in the quantum of bacterial growth and cause constipation. Sometimes such situation may cause medical emergency when the barrier is breached, leading to bacterial translocation to other organs of the body. On the other hand, increased motility interferes with the digestion and absorption process and can lead to diarrhea and the malabsorption syndrome. The clinically known conditions of motility disorders such as achalasia, gastric stasis, outlet obstruction, acute intestinal ileum, chronic intestinal pseudo obstruction, magacolon, and ,generalized disorders of motility deserve treatment with safer drugs. The evaluation of gastrointestinal (GI) motility is helpful in

1. determining the therapeutic potential of new drugs in motility disorders,

2. determining alteration in motility secondary to physiological or pharmacological 42 stimuli

3. Evaluating the effect of pathological condition on GI transit.

In vivo methods in animals exhibit true effects of investigational drugs in biological milieu. Studies of motility of GI are associated with observation of marker in immediately excised sections of GIT or observation of motility in conscious animals using electrical gadgets. The following are the most popular in vivo methods to study GI motility in experimental animals.

1. Assessment of intestinal transit
2. Assessment of gastric motility
3. Measurement of gastrointestinal transit
4. Measurement of colon motility
5. Long term recording of intestinal mechanical and electrical activity.
6. Assessment of GIT motility in dogs.

Assessment of gastric motility

The function of the stomach includes initiation of digestion by exocrine secretions such as acid and pepsin, which are under the control of the endocrine secretion of hormones that also coordinate intestinal motility. Various techniques have been developed to assess gastric motility causing the gastric emptying (GE). The influence of drugs on gastric mechanical action on the bioavailability of novel compounds is of critical importance in drug development. Disturbed gastric my electric activity leading to gastro paresis can cause delayed GE, often found in patients with diabetics mellitus. Electro astrograph (EGG) may be used to evaluate the influence of prokinetics and other drugs on this condition 8 and aid in determining effective therapy.

Exp No : VI

EFFECT OF DRUGS ON GASTRO INTENSTINAL MOTILITY

BACKGROUND

Intestinal motility is regulated by the enteric nervous system of the gut (Auerbachs and messengers plexuses) and the activity of this system can be modified by atomic nervous system. Hence effect of sympathomimetic and parasympathomimetic drugs on intestinal motility can be studied by using isolated piece of intestine. parasymimetic drugs stimulate enteric neurons to release acetylcholine at neuromuscular junctions and enhance muscle tone and rhythm city. Many animal models can be employed to study intestinal motility of sympathetic and parasympathetic drugs. Guinea pig ileum is advantageous for assay purpose as it produces steady baseline for studying effects of drugs. Rabbit intestine (ileum, duodenum, jejunum) usually jejunum is used for the effects of pendulum movements (continuous contraction and relaxation – Finke man method). 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 – 10ug/ml, Atropine

Sulphate – 10ug/ ml, isoproterenol/ isopernaline 10ug/ml, Propranolol – 10mg/ml,

Solutions: Tyrone 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 fink leman method developed by walker and scott. Select a medium sized rabbit for the study. Fast the animal for 24 hours prior to experiment asfood in gut results in messy dissection and flushing or gut contents may damage the intestine. Before sacrificing the rabbit, prepare Thyroids Ringer solution and place about 250ml 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 10cm away from ileocaecal value. An optimal length of tissue (5-6cms) is cut carefully and tie the thread to ant mesenteric border on both sides and place them in Thyroid solution (extra pieces of ileum can be stored in ice cold Thyroid solution so that they are viable for hours. In ice cold solution the motility will ceases but after placing them in warm solution the tissue gets relaxed and shows motility within 5-10 minutes). Record the rhythmic activity of the ileum by using frontal writing lever and kymograph. Suspend the tissue in organ bath of Thyroid solution (100ml) at 37c with adequate oxygen supply (mixture of 95% O and 5% of Co). Tie one end of the thread of tissue of fixed point inside the organ bath and the other end to the lever for recording constructions on the kymograph. Stabilize the tissue in the solution to the conditions for about 30 minutes. Ensure the lever should be placed horizonontally and record the normal constructions followed by effects of drugs on muscles. After recording normal constructions inject the drugs one by one and observe for force of contraction and tone (normal, increased or decreased), frequency of constructions (per minute) before and after drug administration. Inject 0.1ml of drugs in the succession order in the organ bath and the responses are recorded. After nothing the effect of every drug, drain the muscle bath and refill with fresh warm thyroid solution (100ml). Take the control (without drug) reading before and after each drug response. Maintain washout 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.

Exp No : VII

ESTIMATE OF SERUM BIOCHEMICAL PRAMETERS BY USING SEMI AUTOANALYSER

a) Almandine Aminostransferease(ALT)

α cetoglutarate reacts with L- almandine in the presence of ALT to form L- glutamate plus pyruvate. The pyruvate is used in the indicator reaction for a kinetic determination of the reduced from of nicotiamide adenine dinucleotide (NADH) consumption. The international Federation of Clinical Chemistry (IFCC) has know recommended standardized procedures for ALT determination, including 1) Optimization of substrate concentrations, 2) the use of tries buffers, 3) Preincubation of a combined buffer and serum solution to allow side reactions with NADH to color, 4) Substrate start (α-katolgutarate) , and 5) Optimal pyridoxal phosphate activation. As a group, the transmineasess catalyze the into conversion of amino acid and α-Kato acids by transferring the amino groups. The enzyme ALT been found to be in highest concentration in the liver, with decreasing concentrations found in kidney, heart, skeletal muscle, pancreas, spleen, and lung tissues. Almandine amino transferees measurement are used in the diagnosis and treatment of certain liver diseases (e.g. Viral hepatitis and cirrhosis) and heart disease. Evaluated levels of the transaminases can indicate myocardial infraction, hepatic disease, muscular dystrophy, or organ damage. Serum elevations of ALT activity are rarely observed except in parenchyma liver disease, since ALT is a some more liver- specific enzyme than aspirate amino transfers(AST).

a) Reagent 1 (R1) working solution :

(Bottles 1 and 1 a) Tries buffer: 125mmol/1, pH 7.3; L-almandine: 625mmol/1; NADH: 0.23mmol/1(Yeast); LDHS 1.5 U/ml (microorganisms); preservative connect bottle 1to Bottle 1a and dissolve the granule into the buffer.

b) Reagent 2 (R2) Working solution:

Ketoglutarate: 94mmol/1; preservative C until the use α-catalogue solution, supplied "ready to use". Store capped at 2-8 expiration date on the package.

b. Albumin

At the reaction pH, the bromcresol purple(BCP) in the Roche Diagnostics (RD) albumin system reagent binds selectivity with albumin. This reaction is based on a modification of a method described y Dumas (4). Although BCP is structurally similar to the conventional bromcresol green (BCG), its pH color change interval is higher (5.2 - 6.8)than the color change interval for BCG (3.8-5.4), thus reducing the number of weak electrostatic dye/protein interactions. The BCP system eliminates many of the nonspecific other serum proteins as a result of the increased pH. In addition, the use of a sample blank eliminates background spectral interferences not completely removed by dichromatic analysis. Albumin constitutes abut 60% of the total serum protein in normal, healthy individuals. Unlike most of the other serum proteins, albumin serves a number of functions which include transporting large insoluble organic (e.g., long0 chain fatty acids and bilirubin), binding toxic metal ions, transporting excess quantities of poor's soluble hormones (e.g., cortical, aldosterone, and thyroxin), maintaining serum osmotic pressure, and provisioning a reserve store of protein. Albumin measurements are used in the diagnosis and treatment of numerous diseases primarily involving the liver or kidneys.

a) Reagent 1(R1) working solution:
Citrate buffer: 95mmol/1, pH 4.1; preservative
b) Reagent 2 (R2) working solution:
Citrate buffer: 95mmol/1, pH 4.1; bromcresol green :0.66mmol/1; Use supplied ready to use. Transfer the contents of BCP chromate to an analyzer bottle.

Exp No : VIII

EFFECT OF SALINE PURGATIVE ON FROG INTESTINE

BACKGROUND

Saline purgatives are the salts comprising of highly charged ions and not crosses cell membrance 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, Pitching needle, dissecting instruments, needle with thread, and 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 colors 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, 0.2ml of hypertonic solution to second compartment and 0.2ml of isotonic solution into third compartment. Wait for 20 minutes and the observations are to be recorded.

OBSERVATION

Drug Compartment Effect, Hypotonic solution (0.2ml of0.9% of saline) First compartment Shrunken, Hypertonic solution (0.2ml of 27% magnesium sulphate), Second compartment Swollen, Isotonic solution (0.2ml 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.

Exp No : IX

INSULIN HYPOGLYCEMIC EFFECT IN RABBIT

Aim: To determine the insulin hypoglycemic effect in rabbit.

Animal required: Healthy rabbits weighing 1800-3000gms.

Drugs : 20 units of insulin preparation. One unit contains 0.04082mg of insulin.

Reagents: Normal saline, HCL, 0.5% phenol, 1.4-1.8% glycerin.

Procedure:

Select healthy rabbits weighing 1800-3000gems for the study. They should be maintained in uniform diet for 7 days. Fast the animals for 18hrs 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% unit/ml of solution.

Withdraw 2ml 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 an initial blood glucose level. Then injection (1unit/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 find blood glucose levels.

Report:

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

Exp No : X

TEST FOR PYROGEN RABBIT

Aim

To prepare and submitted preliminary test (sham test) and rabbit pyroxene test.

Methodology

Groups of three healthy nature rabbits are chosen. Accurate thermometer are inserted into the rectum of the rabbits to record their body temperature. Test solutions are warmed to 37^0C prior to injection. Rabbit temperature are recorded at 30min intervals between 1 and 3hr.

Procedure

Inject the solution (38.5^0C) under examination slowly into the marina (vein of the ear of each rabbit) over a period of exceeding 4 minutes unless otherwise prescribed in the monograph. Record the temperature of each animal at half- hourly intervals for 3 hours after the injection.

Observation

The different between the initial temp and the maximum temperature which is the highest temp recorded for a rabbits taken to be its response.

ACUTE ORAL TOXICITY

Aim: To determine the therapeutic index line ratio between the lethal dose and the pharmacologically effective dose of AOT.

Introduction:

An Acute oral toxicity study was performed according to the organization, of economic cooperation and development (OECD) guidelines L/20 for testing a chemical.

- Five female rats were used
- A test suspension of 20% (200mg/ml) in purified water was orally admixture only once at a single dose of 2000mg/kg.

Principle:

It is the principle of the test unit that, based on a step wise procedure with the minimum number of animals per step.

- Sufficient information is obtained on the actuate toxicity of the test substance or enable to its classification.
- Absence or presence if compound – related mortality of the animals dosed at one step will determine the next step I.e.,
    - No further testing is needed
    - Dosing of three additional animals with same dose
    - Dosing of three additional animals at the next higher or lower dose level

Preparation of animals:

The animals are randomly selected, marked to permit individual identification and kept in their cages for at least 5days prior to dosing to allow for acclimatization to the laboratory conditions.

Procedure:

Administration if doses:

The test substances is administered in a single dose by manage using a stomach tube or a suitable intubation canola

- In the unusual circumstance that a single dose is not possible, the dose may be given in smaller fractions over a period not exceeding 24hrs.

- Animals should be faster prior to dosing

  Ex: Rat, food but not water should, be with hold overnight.

Results:

- Response data and ,dose level for each animal (I.e., animal shows, mortality, nature, severity).
- Tabulation of body weight and body changes.

Exp No : XI

ACUTE SKIN IRRITATION

Aim:

To perform the acute skin irritation

Principle:

The test chemical to be tested is applied in a single dose to the skin of an experimental animal, untreated skin areas of the test animal serve as the control. The degree of irritation/ corrosion is read and scored as specified intervals. Animals showing continuening signs of serve distress and/or pain at any stage of the test should be humanely killed, and the test chemical assessed accordingly.

Procedure

The test chemical should be applied to a dorsal/ flank (approximately $6cm^2$) of skin and covered with a gauze patch. In cases in which direct application is not possible (eg: liquids or some pastes) the test chemical should first be applied to the gauze patch, which is then applied to the skin. The patch should be loosely held in contact with the skin by means of a suitable semi- occlusive dressing for the duration of the exposure period . If the test chemical is applied to the patch, it should be attached to the skin in such a manner that there is good contact & uniform distribution of the test chemical on the skin. Liquid test ,chemicals are generally used undiluted. When testing solids, the test chemical should be diluted with the smallest amount if water (or another suitable vehicle) sufficient to ensure good skin contact. At the end of the exposure period, which is normally 4 hours, residual test chemical should be removed.

Dose level:

A dose of 0.5ml of liquid or 0.5g of solid or paste is applied to the site.

Observation:

The degree of irritation/ corrosion is read and scored at specified intervals.

Exp No : XII

ACUTE EYE IRRITATION TEST

Aim:

To perform the acute eye irritation test.

Methodology

Following pretreatment with a systemic analgesic and induction of appropriate topical anesthesia, the substance to be tested is applied in a single dose of the eyes of the experimental animal the untreated eye serves as the control. The degrees of eye irritation/ corrosion is evaluated by scoring lesions of conjunctiva, cornea, and its at specific intervals.

Procedure

Sixty minutes prior to test substances application TSA, baprenorophine 0.01mg/kg is administered by subcutaneous injection (sc) to provide a therapeutic level of system analgesia. Five minutes prior to TSA, one (or) two drops of a topical ocular anesthetic Ex- 0.5% proparacaine hcl.

In order to avoid possible interference with the study, a topical anesthetic that does not contain preservations is recommended. The eye of each animal that is not treated with test articles but which is treated with topical anesthetics serves as a control. If the test substance in anticipated to cause significant pain and distress if should not normally be tested in vivo. However in case of doubt (or) where testing in necessary consideration should be given to additional applications of the topical anesthetic at 5 minutes intervals prior to TSA. If an animal shows signs of pain and distress during the study a recue dose of buprenophine 0.03mg/kg would be given immediately and repeated as often as every 8 hours. Melocicam 0.5mg/kg sc would be administered every 24 hrs injection with recue dose of buprenorphine but not until at least 8 hours.

Observation:

Duration of the observation period should ,be sufficient to evaluate fully the magnitude and reversibility of the effects observed. Animal shows signs of serve pain (or) distress. Observed normally for 21 days. If visibility is seen before 21 days the experiment should be terminated at that time.