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A REVIEW ON PRECLINICAL PHARMACEUTICAL RESEARCH: PRINCIPAL AND COMMON ROUTES OF ADMINISTRATION IN SMALL RODENTS

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ABSTRACT: Preclinical drug or pharmaceutical research in mouse and rat species is inevitable in the scientific investigations of a chemical substance or lead entity. The administration routes are the experimental study design, and regulatory requirements may influence the study of humans' intended therapeutic/clinical route. Specific route selection in the investigation of test substances must be profoundly well-versed to the research scientists and approved by local ethics committee members. Every route has advantages and disadvantages in absorption, bioavailability, metabolism, and distribution of substances, which should be carefully considered while selecting a route. Routes of administration can be performed painlessly if proper heed is given to the restraint and holding of animals and using adequate technical skills by technical persons and research scientists. This review describes the principal and common routes used in early drug discovery programs and preclinical research, including guidelines for safe injection volumes, sites of administration, preparation of sites, advantages & disadvantages, limitations, handling and restraint techniques, vehicle selection, and formulation strategies. **Conclusion:** This can be concluded knowledge of available methods for administration and handling techniques and the disposition pattern as well as formulation strategies will aid the scientist in choosing the most suitable route for the compound to be investigated.

INTRODUCTION: Scientific investigation of the test substance or lead molecule in rodents, particularly mouse and rat species, is indispensable in preclinical pharmaceutical research or biomedical research [1]. Test substance administration in rodents is one of the major methods for evaluating biological activity.

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Further, the safe, effective, and humane restraint of mice and rats is warranted in research. In *in-vivo* experimental study design related to efficacy, pharmacokinetics, or regulatory toxicology in small animals, the route of substance administration is an essential consideration.

The ethical protocol specific to each study commonly demanded to mention of the route-related requirements like the route of study, dose or doses used for the particular study, dose concentrations, dose intervals such as in repeated dose study, and most importantly, the dose volume, which is variable for most of the route. Further, the administration route under study largely depends

on the specific study objective; for instance, it may be a clinical or the predominant route of anticipated human exposure [2]. In drug discovery as in early animal studies, the principal routes are i.v, s.c, i.p., and oral gavage, which are mostly explored to know the compound PK-PD relationship or proof concept studies. All routes have advantages and limitations regarding absorption, bioavailability, metabolism, and substance distribution that should be known to study scientists or research scientists before planning an *in-vivo* experimental protocol. The rate of absorption from the site of the route of administration particularly depends upon the absorbing surface size, blood flow to the site of administration, physical and chemical nature of the substance, substance concentration, and its solubility in the tissue fluids [3].

The fundamentals related to sterility, viscosity, and physiologic compatibility of the test formulation or vehicle in which the test substance is dissolved / suspended must be addressed meticulously as these may influence the obtained results [3, 4]. Like humans, rodents have a wider range of pH tolerability for the Injectables. Usually, for all routes of administration, a pH working range of 4.5-8 is acceptable [5]. For parenteral routes, a very close pH range of 6.8-7.2 is recommended. However, an intravenous route has the widest pH tolerance because of the buffering capacity of blood and quick dilution in the blood pool [5, 6]. For oral solution, a pH of 3 tolerates fairly high buffer capacity. An order of degree of tolerance of pH for routes is oral gavage >i.v. > i.m. > s.c. > i.p. [6].

The parenteral formulation must be sterile, preventing pathogens into the animal [3]. Gauge needle selection for injections is based on the route of administration, the viscosity of the solution/suspension, and the animal size [6, 7]. The smallest gauge diameter feasible to administer the solution should be chosen. Further proper restraint technique is essential, reducing the distress to animals and increasing the success of treatment. Therefore, personnel handling and restraining experimental animals should be well-trained and attain a scientifically high standard [3]. Knowledge of these will help the scientists or researchers select a suitable route for the substance which may be investigated. Thus, the purpose of the review is to describe the most common and principal routes of administration and its techniques, advantages and disadvantages, and disposition pattern. Further, formulation strategies in early drug discovery stages have also been discussed. Moreover, some restraint techniques and gentle handling of animals, which are essential for the painless administration of substance formulations resulting in successful treatment, have also been specifically demonstrated.

Basic Terminology:

A. Parenteral: Administering the substance outside of the gastrointestinal tract.

Routes of Parenteral Administration:

- Intravenous (i.v./IV): Administering the substance into the venous circulation.
- Intraperitoneal (i.p./IP): Administering the substance into the abdominal cavity.
- Transdermal (Percutaneous): Applying the substance directly to the skin for systemic effect.
- Subcutaneous (s.c./SC): Administering the substance under the skin (between the skin and muscle layers).
- Intradermal (i.d./ID): Administering the substance into the dermis.
- Intramuscular (i.m./IM): Administering the substance into the muscle.
- Intraosseous (i.o./IO): Administering the substance into the bone marrow.
- Intratracheal (i.tr./ITr): Administering the substance within the trachea.
- Intracranial: Administering the substance into the brain.
- Epidural (e.d./ED): Administering the substance into the epidural space of the spinal cord.
- Intrathecal (i.t./IT): Administering the substance into the subarachnoid space (in the spinal canal but not within the spinal cord).

B. Enteral: Administering the substance into the gastrointestinal tract.

Routes of enteral administration:

- Per os: Administering the substance by mouth.
- Gavage**:** Administer the substance *via* a tube passed through the mouth into the esophagus or stomach.
- Rectal**:** Administering the substance into the rectum.

C. Topical (Epicutaneous): Applying the substance directly to the skin for topical effect.

- Intranasal (i.n./IN)**:** Administering the substance into the nose.
- Dermal: Applying the substance directly to the skin for topical effect.

D. Other:

- PPE and Hygiene**:** It is defined as personnel protective equipment, which includes gloves, face mask, head cover, eye protection, apron, and others as per protocol requirement, shall be donned during the laboratory conduct, especially during animal handling and administration techniques. This is to ensure the protection of technicians/scientists from accidental exposure to blood and other body fluids. Gloves should be changed between animals and sharps used and properly disposed of in the provided leak-proof, puncture-resistant sharp container.
- **Gauge (G):** referred to as the diameter of the needle. E.g. 25 G needles have a smaller diameter than 23 G needles.
- **MMAD:** Mass median Aerodynamic Diameters.
- **GSD:** Geometric standard deviation (σg).

Common and Principal Routes of Administration & Injection Techniques: The local ethic committee (IAEC) approval is necessary before commencing any route of administration procedure in rodents for a test substance [1].

Imperative Observation for Parenteral Administration: Parenterally administered substance/s should be:

1. Isotonic.
2. Close to physiologic pH i.e. 6.8-7.2 pH, for out-of-range pH, administered through a central vessel (eg. jugular or femoral vein) or buffer the solution [8].
3. Considered **v**iscosity, concentration, stability, pH, buffering capacity, and formulation biologic inertness when approaching the volume limits for solution administering i.p., s.c., or i.m. [9-11].
4. Sterile and aseptically delivered.
5. Prepared in biosafety cabinet followed by filtration through a 0.2-micron filter [4].

The principal routes and most common routes of administration in rodents used in preclinical studies during drug discovery and development program, along with injection techniques, vehicle selection, formulation strategies, and some common restraint techniques, have been discussed and demonstrated; Which are as follows:

TABLE 1: DIFFERENT ROUTES OF ADMINISTRATION: COMMON AND LESS COMMON ROUTES [12]

Parenteral Route:	i.v. (CR) 2.i.p. (CR) 3. Administered to the skin or muscle ➢ i.d. (CR) ➢ s.c. (CR) ➢ i.m. (CR) ➢ Intracarnial (LCR) ➢ e.d or i.t (LCR) ➢ Transdermal (CR)(percutaneous)
Enteral Route	Oral (Gavage)route (CR) Rectal (LCR)
Topical	➢ Nasal (CR) ➢ Skin-Dermal (CR) ➢ Eye (LCR)
Pulmonary Route	➢ Inhalation into lungs (CR)

CR- Common Route, LCR-Less common route.

Subcutaneous Route: Mouse and Rat

About Route (Site, Advantages, Disadvantages & Limitations): This is a frequently used route. The absorption rate and extent depend on the compound type and its formulations. This route is oftenly explored in early drug discovery program to establish pharmacokinetic-pharmacodynamic (PK-PD) relationships over the oral route as it bypass hepatic first pass metabolism and intestinal barrier, which are the oral absorption limitations [13].

Usually, subcutaneous injection is made deeper than intradermal injection and placed in the more vascular space between the skin and the underlying muscles. These injections are rarely painful [14]. An injection is made in loose skin at the interscapular (scruff of the neck) (refer **Fig. 1**) or in the lingual area (lower left or right quadrant of the abdomen) (ref **Fig. 2**.). Base of the scruff or dorsolateral area of the neck is the preferable site in rodents. The rate of absorption is lower than from intraperitoneal or intramuscular injections [15]. By this method of administration, a substance depot (viscous solutions or suspensions) can be produced. This route is the best option when the repository injection site's relatively long absorption period is necessitated. This is a preferred method of administration due to technique simplicity, availability of multiple injection sites, and depositing large volumes [13, 16, 17]. Refer to table 3 for volumes and gauge size.

Limitations: A) An irritant drug cannot be injected, B) less tolerant of non-physiological pH [5].

Injection Technique: The mouse/rat can be restrained by grasping the skin along its back or at the nape of the neck with the left hand (if right-handed). (refer **Fig. 1**). Injection site (Scruff of the neck) can be cleaned with alcohol (70% ethanol). Gently but firmly place a free hand over the shoulders, and the scruff of the neck secures the animal, and the skin can be elevated to produce a tent. An injection can be made by inserting a needle at 30-45^0 at the Base of the skin fold between the thumb and forefinger.

Usually, raising a tent of skin exposes a large area of subcutaneous tissue of injection. Before injecting formulation, it should be ensured for needle placement by pulling back a syringe plunger for aspiration. The appearance of any blood in the needle hub indicates improper needle placement. Hence, the needle must be repositioned, and an injection can be made.

The formulation should be administered in a steady, fluid-motion manner. After injection, a bulge at the injection site may represent a large number of solutions. At the same time, gentle pressure can be applied to prevent backflow of the material [18-20].

FIG. 1: MOUSE (INTERSCAPULAR AREA): S.C. INJECTION AT THE AREA OF THE NECK WITH 26 G×1 / 2 IN. NEEDLE

FIG. 2: MOUSE (INLINGUAL AREA)- S.C. INJECTION AT LOWER LEFT QUADRANT WITH INSULIN SYRINGE 27 G×1/2 INCH., 1ML

Intramuscular Route:

Mouse and Rat:

About Route: Intramuscular injections are generally given in the hindlimbs, particularly in the quadriceps muscle group (anterior portion of thigh) or biceps femoris muscle (refer **Fig. 3** & **4**) [14]. It can also be given in to the area of gluteal muscles of hind legs. This route is occasionally used in early animal studies in drug discovery. IM injections are frequently painful due to the occurrence of distension of muscle fibers. Therefore, the appropriate dose volume and restraint technique is required for mouse/rat [14, 21], refer to **Table 3** for volumes and gauge size.

If necessary, then only performed in rodents because of reduced muscle size. Injection should not be made into the posterior muscle mass as there may be a possibility of damage to the sciatic nerve. This route usually results in more rapid absorption (aqueous drug solutions) than subcutaneous routes [5]. For most fluids, absorption usually takes 45-60 min. Depot preparations (aqueous and oily suspension) can be injected by this route, presenting a prolonged action. Absorption from the repository may remain for days and weeks [22].

Precaution: It is necessary to follow an alternate site for repeated injections. The triceps muscles of the front leg or the lumbar muscles may be used as an alternative site.

Injection Technique: The mouse/rat can be restrained by grasping the skin along its back with its left hand (if right-handed). Most importantly, this technique should be performed by well-trained personnel. (refer **Fig. 3** & **4**) [5, 19, 3]. The site of injection must be clipped in advance. During injection, the site can be cleaned with 70% ethanol. An injection needle can be inserted (5 mm deep is sufficient for a rat) into the bicep femoris (lateral thigh muscle mass). It should be directed away from the femur to avoid the sciatic nerve [14]. A syringe plunger can be slightly pulled back for aspiration to ensure that the needle has not entered a blood vessel. An appearance of any blood in the needle hub indicates improper needle placement. Thus, the needle must be repositioned for injection. The formulation can be administered in a steady, fluid motion. It is necessary that fluid should not be administered too rapidly. To ensure the dose dispersibility, the site should be massaged after injection [18].

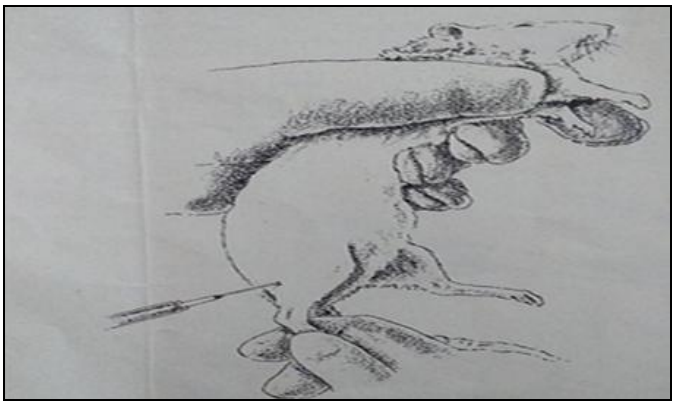

FIG. 3: RAL: BICEPS FEMORIS MUSCLE

Precautions: Care must be adopted to avoid injury to the sciatic nerve located close to the femur [21]. An Injection may be ensured for penetration into the blood vessel by checking aspiration. The injection may proceed if no blood is drawn into the syringe [14].

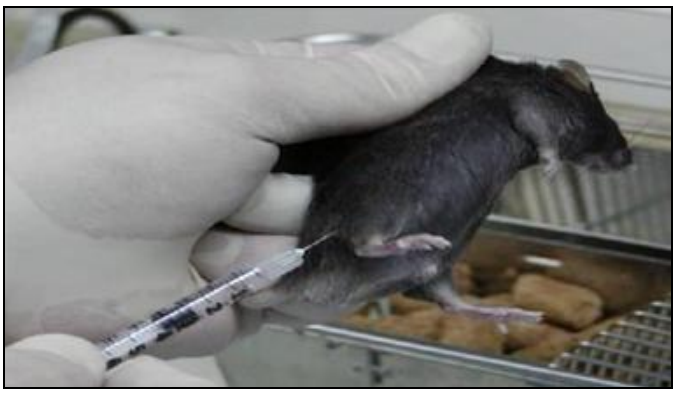

FIG. 4: MOUSE: THIGH MUSCLE I.M INJECTION WITH 27 G×1/2 IN. NEEDLE

Intradermal Route:

Mouse & Rat:

About Route: An intradermal injection is administered between the layers of the skin, just under the superficial layer of the epidermis (refer **Fig. 5**). This is typically used for an assessment of immune, inflammatory, or sensitization response [23, 24]. The recommended volume of 0.05-0.1 mL can be used, dependent on the thickness of the skin; refer to **Table 3** for volumes and gauge size for mouse and rat. A distinct bleb or pockets of fluid result upon injection (refer to **Fig. 6**).

Injection Technique: Hair can be clipped on Animal's back by an electric clipper or wet shaver, prior administration of 24 hr [18]. After clipping, the

hairless area should be cleaned of fat, grease, or debris before application. During injection, the animal can be anesthetized (light anesthesia), for which a procedure must be described in an approved animal study proposal. Clean the injection site with alcohol (70% ethanol). The skin is held tautly with the thumb and index finger. Insert a needle between the layers of skin at a 30° angle, as shown in **Fig. 5**. A very slight pullback is given to the syringe plunger for aspiration. If blood appears in the needle hub, then the needle must be repositioned for injection. The substance can be administered slowly to avoid tissue trauma. Proper injection results in a small, circular skin welt, as shown in the picture below [20]. After an injection, gentle pressure can be applied to prevent backflow.

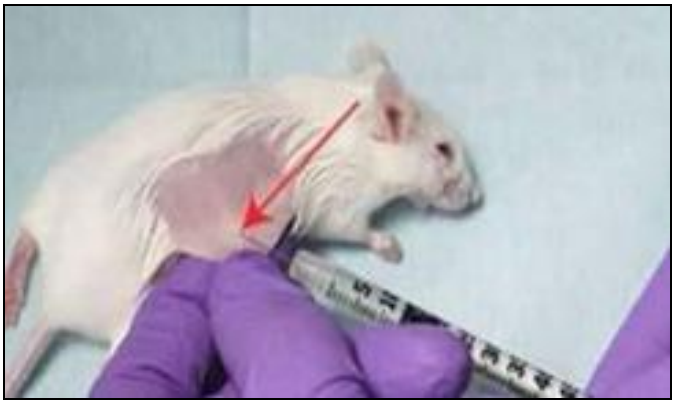

FIG. 5: MOUSE: INTRADERMAL INJECTION WITH INSULIN SYRINGE 29 G×1/2 IN., 0.5 ML

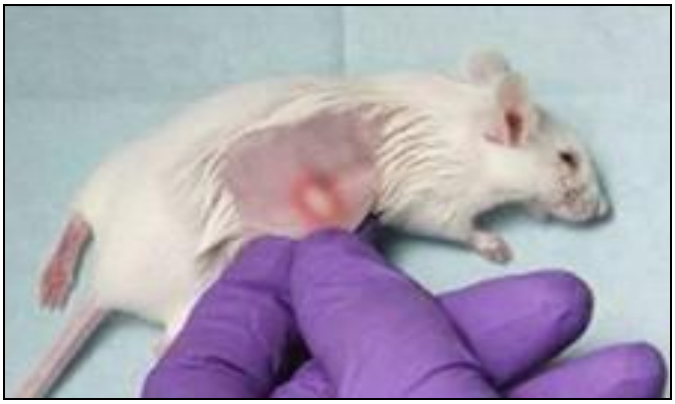

FIG. 6: MOUSE: AFTER INJECTION, SMALL CIRCULAR SKIN WELT

Intraperitoneal:

Mouse and Rat:

About Route: This is the most frequent and preferred injection route for nonirritant or isotonic solutions. The large surface area of an abdominal cavity and its abundant blood supply facilitate rapid absorption, usually one-half to one-quarter as rapid as the intravenous route. The peritoneal surface area in the rat (200g) is estimated to be 125 cm^2. Upon intraperitoneal administration, usually, the drug diffuses from the peritoneal cavity into the surrounding tissues; after that, the compound may be carried away by capillary blood or lymph, metabolized by tissue enzyme or bound to tissue proteins. The transport may occur by A) The mesenteric-portal vasculature B). The mesentrical-extraportal and the extramesentrical vasculature C) Lymphatic vessels. By this route, the compound may be absorbed rapidly, but partial biotransformation may take place in the liver before systemic circulation [5, 25, 26]. This lead to quite difference in bioavailability to that of an intravenous route. Sometimes, it has been preferred over to oral route by scientists during early drug discovery as it avoids the intestinal barrier, therefore, it has been used to evaluate the effects of target engagement rather than properties of a drug formulation or its pharmacokinetics profiling [27].

Relatively large volumes can be given (refer to **Table 3**. Further, the drug is generally administered in solution form compared to suspension because the fluid supply to the peritoneal cavity is limited for solubilizing the drug from suspension. Further, a formulation vehicle, most likely cosolvent, *i.e.*, polyethylene glycol-400 (PEG-400), is used, which is known to extract water from surrounding tissues, and this may result in precipitation of the drug due to the dilution of cosolvent; hence, a higher concentration of PEG-400 should be used cautiously [27].

An intraperitoneal injection is generally made in the lower left quadrant of the abdomen. There are no vital organs in this area except for the small intestine, as the small intestine poses low risk mainly due to its mobility. In contrast, the right quadrant contains much of the large bowel and upper abdomen, which is hazardous to inject because the liver, stomach, and spleen are situated here [18, 28]. Administration of an intraperitoneal injection requires extra caution to avoid penetrating various organs within an abdominal cavity.

Limitations: This route is used infrequently for multiple dose studies because of the possibility of drug injecting into the intestinal tract, irritant

materials may cause peritonitis and solutions of non-physiological pH are not tolerable.

Injection Technique: Restraint the mouse by scruff method with the left hand (if right-handed) or by forelimb crisscross method for rat [18]. Tip an animal's nose towards the floor, exposing an abdomen for injection. Locate the animal's midline and mentally divide the abdomen into quadrants as shown in **Fig. 7** & **9**. The lower left quadrant of the abdomen, is the appropriate site for the intraperitoneal injection [28]. The lower left quadrant of the abdomen, lateral to the midline, is chosen due to the lack of anatomically important structures. Clean the injection site with alcohol. Use the appropriately-sized syringe and needle for rat or mouse, and insert a needle with the bevel facing "up" (refer **Fig. 8**) into the lower left quadrant of the abdomen towards the head at 20-45^{o} angle (for rat) or 10^{o} angle (for mouse). This is to avoid the risk of damage to the kidney [15, 29, 30]. The material can be injected into the in-lingual area of the animal; refer to **Table 3** for volumes and gauge size. To avoid the intestine or urinary bladder injection, inserting only the cannula tip into the peritoneal cavity is essential. No resistance should be encountered to the passage of the needle [14]. For ideal volume, an injection can be made in 1-2 seconds and a few seconds more if injecting viscous formulation. If injecting daily for multiple days, it is acceptable to vary the side injected between the right and left quadrants of the abdomen.

Precautions: It is very important to retract a syringe plunger slightly prior to injection for negative pressure and that nothing to be aspirated. If a yellowish fluid is withdrawn into the syringe, which indicates the needle could be in the bladder. If a withdrawn is brown, the needle could be in the intestine or the cecum [18, 26]. If an appearance of blood in the syringe, it indicates a blood vessel is being entered. All of these indicate the contamination, and the syringe must be discarded. The procedure can be initiated again.

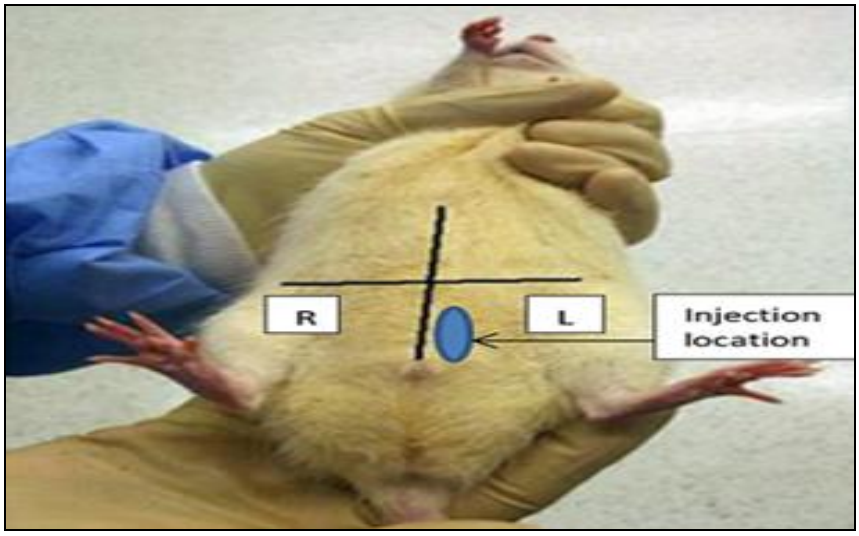

FIG. 7: RAT: QUADRANTS OF THE VENTRAL ABDOMEN, ONLY INJECT INTO THE LOWER TWO QUADRANTS, PREFERENTIALLY INTO THE LOWER LEFT QUADRANT

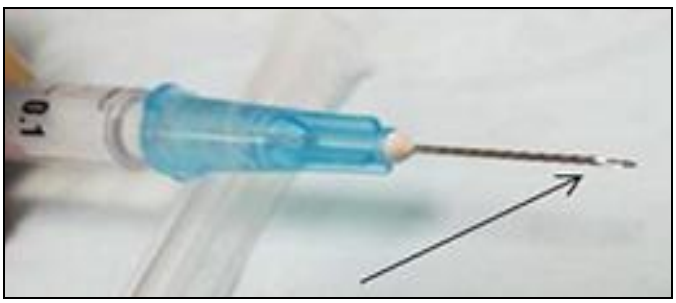

FIG. 8: BEVEL OF NEEDLE FACING "UP''

FIG. 9: MOUSE: IP INJECTION AT LOWER LEFT QUADRANT INSULIN SYRINGE 27G×1/2 IN., 1.0ML

Intravascular:

Mouse and Rat:

About Route: Left and right lateral tail veins are the most common vascular access used in mouse and rat (refer **Fig. 10** & **11**) [31-33]. Beside s.c. route, this route is usually explored in early discovery to understand compounds' PK-PD relationship as the plasma the plasma concentration can be predicted better [13]. By this route, solutions and nanosuspensions can be administered mostly. The advantages of this route are as follows:

Solution at high concentration, low or high pH can be administered intravenously, provided that the rate of injection is kept low. Desired levels of constant plasma concentration can be attained by controlling the rate of administration. By stopping the injection, unintended side effects can be halted during administration. In case of repeated injection, venous puncturing can damage or block the vein; therefore, injection should be started from the distal end of the tail vein [3]. Refer to **Table 3** for volumes and gauge size.

Precautions: substances that may induce hemolysis, thrombosis, or vasculitis are inappropriate for intravenous administration.

Injection Technique: For an i.v. injection, the mouse/rat should be restrained in a mechanical restrainer [7, 18, 34].

Warm water around 40-45 °C or 70% ethanol is used on a gauze sponge to swab a tail for 1-2 min to dilate the blood vessels [35, 36].

Then, hold a tail under slight tension, as shown in the picture below, and insert a needle parallel to the lateral tail vein penetrating 2-4 mm into the lumen. While injecting, ensure that the bevel of the needle faces upwards (refer to **Fig. 10** & **11**).

As the needle is inserted into the vein, a syringe plunger can be very slightly pulled back if blood appears into the hub of the needle, which indicates the placing of the needle correctness. While performing an injection, a syringe should not be aspirated as this will collapse the vessel.

The solution is injected slowly, and also it is necessary to ensure that there should not be any resistance during the injection. The needle should be removed after administration, and pressure should be applied at the site to stop the bleeding before keeping the animal in cage [3, 20].

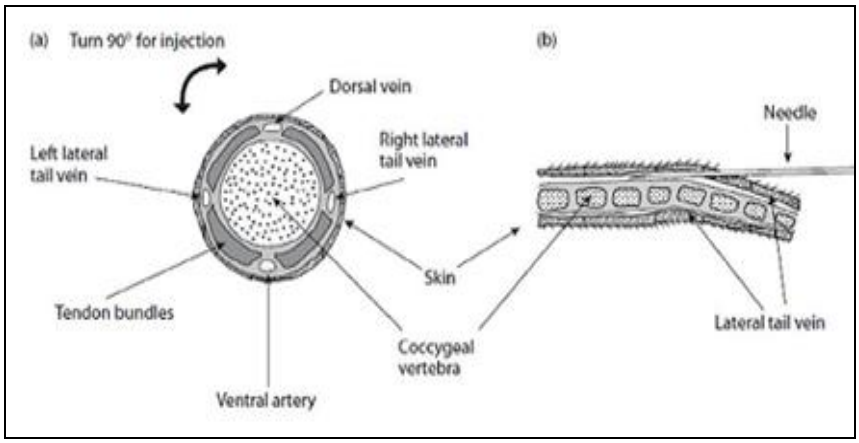

FIG. 10: (A) TRANSVERSE SECTION VIEW OF THE MOUSE TAIL (B) SAGITTAL VIEW OF THE MOUSE TAIL (TAIL IS TURNED 90 °)

Other Routes of Intravenous Administration are through the external jugular vein [37], the dorsal metatarsal vein [38, 39,] and the sublingual vein [31, 40] in mice and/or rats are situationally used.

Intravenous Injection or Infusion by Catheter [41, 42]:

Chronic Venous Cannulation: In biochemical toxicology study, if the test substance administration involves infusion or repeated intravenous injection, then chronic venous cannulation at jugular or femoral in rats is widely accepted. The method usually involves the surgical procedure with minimal trauma and speedy recovery, resulting in intact circulation and cannulation being maintained for longer. Appropriate anesthesia and analgesia should minimize the pain during surgery.

Commonly, a nose cone isoflurane vaporizer anaesthesia is provided with an anesthesia machine can be used. The said procedure should be described in the IAEC-approved protocol. The procedure for jugular cannulation involves limited isolation of muscle tissues at the neck region to expose the vein by blunt dissection. A poker needle of 18 G size is used to make a hole or bore from the surgical field site underneath the skin to the back of the neck that leads to the center of neck region, this creating a passageway for the other end of the catheter. Insert the cannula through this poker needle to the back of the neck region, and once it leads to the center region poker needle can be removed. Now again, the jugular vein is isolated, and the scalpel platform is placed underneath the jugular vein. Take two pieces of suture thread and place one above and the other below the insertion or catheterization point of the vein.

For cannulation, make a small cut with a fine curved scissor at the exposed jugular vein, and 22 G catheter beveled facing upside can be inserted with the guidance of a fine curved forcep. Slide the catheter until it reaches the bubble. Make sure the tubing is in the vein by pulling back the blood with a flusher from the catheter end on the back side of the neck. If blood appears without any resistance, it means the catheter is in the vein; once the blood flow is good, tie the top suture and bottom suture above and below the bubble, respectively or the vessel is sealed with surgical glue.

FIG. 11: MOUSE: INTRAVASCULAR (I. V) INJECTION (LATERAL VEIN) WITH INSULIN SYRINGE 27 G×1/2 IN.

Further, suturing the surrounding muscles with the suture needle. On the back of the neck, the incision at the catheter end is sealed with a Surgical staple or suturing with a surgical needle. The catheter is either closed with hoodie and metal cap or placed in velcro jacket. The intramuscular injection of cefazolin antibiotic; 160 mg/kg, to the right leg and flunixin analgesic; 2.5 mg/kg subcutaneously can be given and placed the rat in the recovery box. This procedure allows the free movement of the animal in the cage. Patency can be maintained for more than a week using sterile saline flushes and heparin. Refer to **Table 3** for volumes and rate.

Intragastric Administration (OG): Mouse & Rat

About Route: This is the most common route or an intended therapeutic route of choice for human, which explores in small molecule discovery programme. It provides ease of delivery and range of choices of vehicles to solubilize the compound. An oral gavage technique is used to administer the substance formulation directly into the stomach of animal [4, 18, 27, 33, 43]. Some substances are better absorbed when given orally in an empty stomach, therefore, it is essential to restrict an animal's food intake before dosing, the factor which may affect the absorption. Further, it was reported in rats that among fasting for 6, 12 and 18 hr, only 6 hr fasting indicated almost empty with no distress [44].

Usually, an absorption take place over the whole length of digestive tract but intestinal absorption is dominant due to large surface area of the intestinal villi [45, 46]. An absorption in the intestine is dependent on physicochemical state of the substance, absorbing surface and its metabolic activity, lipid solubility, molecular size of the substance and bile juice along with juices secreted from intestine and pancreas [3]. These Juices reduce the degree of ionization and increase an absorption rate of substance [47]. Nonionised form of orally administered substances absorbed quickly by diffusion, since the mucosal lining of the gastrointestinal tract is almost impermeable to ionized molecules but can only be absorbed if exists any affinity for a specific carrier. Thus, as per the ionic characteristics of the compound, substances absorption enhanced in acidic stomach, for instance, weak organic acids are in nonionized form, which is lipid soluble and suspected to be absorbed from this site or enhanced in the nearly neutral intestine, for instance, weak organic bases are in the Nonionised form, which is lipd soluble and tends to be absorbed from this site [46]. All substances which are absorbed through

gastrointestinal tract (GIT) are transported by portal vein to the liver. Some of the substances can be metabolized to a large extent in liver before reaching the systemic circulation, called as first pass effect and this has to be considered in selecting route of administration. As oral absorption is rate limited, therefore, some factors should be considered when selecting formulation for oral drug administration like rate limited solubility/ dissolution and variation in gastrointestinal physiological condition between species [43, 48, 49]. Gavage should be performed on restrained awake animals as anesthesia increases the risk of aspiration, in which material may advertently enter in to the lungs. Comparatively, non-sterile substance in suspension or solution form can be possibly administered in large amount by this route [5]. A pH as low as 3 can be tolerated for a solution of fairly high buffered capacity [5] whereas alkaline solutions are very poorly tolerated. Oral gavage needles should be of appropriate size, selected for use. These needles have ball tips at the end to prevent their passage into the trachea as shown in the image (refer **Fig. 12** & **14**). Refer table 3 for volumes and gauge size.

Injection Technique: Restraint the mouse by grasping the skin along its back from the scruff of the neck with left hand (if right-handed). Rat can be restrained by over the shoulder method (Refer common restraint technique section below). This restraint technique in mouse or rat will lead to immobilization of head, which is necessary for gavaging. Measurement of a needle is required against the animal's body to ensure proper needle length as shown in the following pictures (**Fig. 12** & **14**).

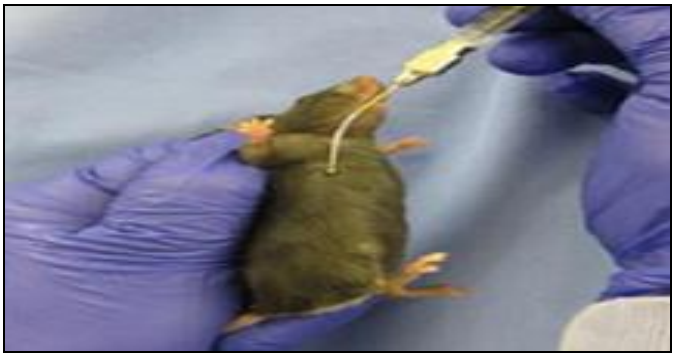

FIG. 12: MOUSE: GAVAGE NEEDLE MEASUREMENT FROM TIP OF THE ANIMALS NOSE TO THE MARK THE NEEDLE SHAFT AT THE LEVEL OF THE NOSE

Then, place the tip of the gavage needle in the animal's mouth as shown in the bottom images (refer **Fig. 13** & **15**). Slide the needle tip down the back of the mouth while moving tip towards the front in a fluid motion. (refer to **Fig. 15**) [20, 45]. Never force a gavage needle if any resistance indicates improper placement [50]. A syringe should not be aspirated. Once the needle is properly placed, administer the substance slowly. As soon as the administration is finished, the needle shall be withdrawn gently following the same angle as insertions [19, 20, 45].

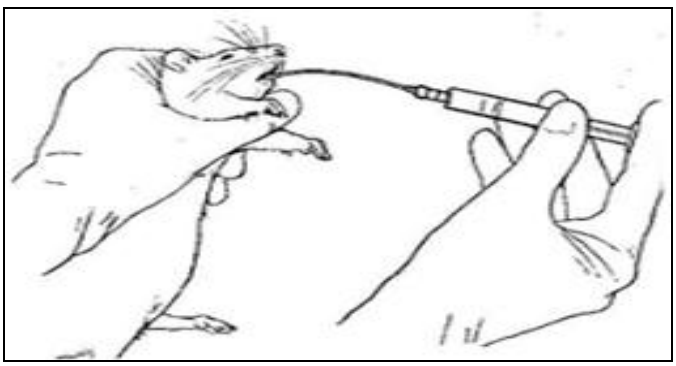

FIG. 13: ILLUSTRATION IN RAT: ORAL GAVAGE TECHNIQUE (RESTRAINT OVER THE SHOULDER METHOD)

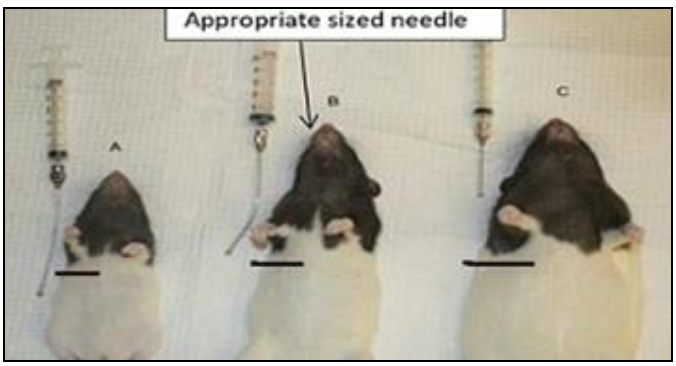

FIG. 14: GAVAGE NEEDLE SIZING IN RAT. (A) NEEDLE TOO LONG (B) APPROPRIATELY SIZED NEEDLE (C) NEEDLE MESUREMENT TOO SHORT

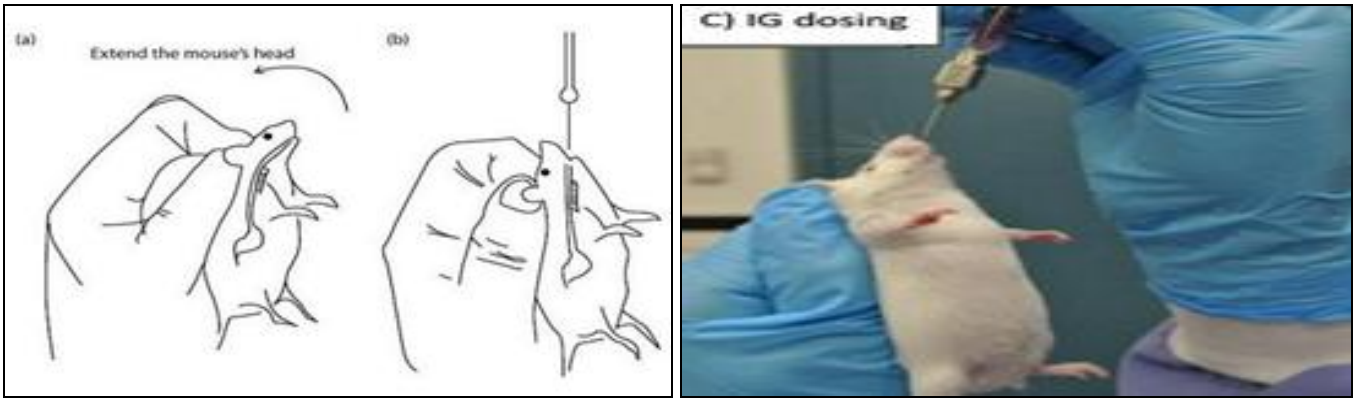

FIG. 15: PROCEDURE FOR INTRAGASTRIC ADMINISTRATION USING A BALL TIP NEEDLE-SCRUFF METHOD IN MOUSE (A) FIRST EXTENDED THE MOUSE HEAD (B) A STRAIGHT LINE IS FORMED BETWEEN THE MOUTH AND STOMACH; (C) INTRAGASTRIC (IG) INJECTION USING 1.0 ML SYRINGE WITH 22 G× 1.0 IN. A FEEDING NEEDLE IS MADE. 22G IS THE MOST COMMON FOR MOST ADULT MICE IN THE WEIGHT RANGE 15-20G; 20G FOR 20-25G; 18G FOR 25-30G; CURVED / STRAIGHT SHAPED NEEDLE FOR 18-22G WITH NEEDLE LENGTH 1.5 IN.

Topical (Dermal) Route of Administration:

About Route: This is also a convenient site for administration and to investigate both local (epicutaneous administration) and systemic (percutaneous absorption) effects following dermal absorption and metabolism. Mouse, Rat, Guinea Pig and Rabbit are the commonly used species in dermal testing of potential therapeutic agents for skin diseases or in checking the potential to cause skin irritation [8, 51]. Further, regulatory toxicology guidelines recommended conducting dermal toxicity test on the intact skin [52].

Particularly, after dermal application, systemic exposure and toxicity are measured in rats, while topical and systemic exposure and toxicity are measured in minipigs. The ethics committee must approve such regulatory studies. An anatomical structure of the outermost layer of the skin is lipoidal in nature, whereas a viable epidermis is fundamental of an aqueous environment [53]. Usually, the factors affecting the dermal and /or percutaneous absorption following topical application are the physicochemical properties of the substance, vehicle properties, and skin permeability [54]. Therefore, the extent of substance absorption through the skin and into systemic circulation (eg. patch) depends on the partition into lipid and water phases [3].

The dermal or percutaneous route is useful in targeting the drug to the site of action, minimizing the side effects, and achieving controlled drug delivery and can be explored as an alternative to oral route for drugs such as irregular absorption in the GIT, low bioavailability and heavy first pass effect [55, 56].

Administration (Site Application) Technique: The usual site is the skin covering the back and alike the intradermal site, skin preparations can be performed. It can be prepared by clipping or shaving a site 24 hours prior to dosing. For the rat, a close-to-head location is preferred as a site of application that prevents the animal from ingesting the material. This is particularly useful if the application is non-occluded [8].

Test substance can be applied with a dropper or smeared onto the skin in liquid form (may be diluted or undiluted) or paste form (with saline or appropriate solvent), of adequately restraint conscious mouse/rat [3]. The application of the test substance should be uniform and cover no more than 10% of the body surface (eg. for rat: 5 cm x 5 cm). Usually, animals that are clean, intact, and ungrazed skin are used for the application of test substances. An unintended skin abrasion due to improper clipping may increase the irritancy or introduce an error by increasing the absorption rate. Particularly, in regulatory toxicology studies, the maximum dose of a test material should be 2 g/kg. A dose in the volume of less than 500 µL is usually administered. Based on the exposure pattern, the application site may or may not be occluded or semi-occluded. The maximum time of occlusion should be 24 hr. Dressing material can be removed

carefully after the end of the exposure, and site can be washed clean with warm water and gently dried.

Intranasal Route:

About Route: In a research setting, this route is used for local or systemic delivery of substances. The nasal mucosal lining of the cavity is richly supplied with blood vessels, and the first pass effect can be avoided by this route, which is usually can be seen on oral delivery of most drugs. This route is useful for small lipophilic molecules, which can be absorbed more rapidly rather than highly polar substances. By this route, the blood levels are more or less similar to intravenous administration [57].

Therefore, quick systemic effects can be attained. Substances should be nonirritating by this route of administration. Interestingly, in pharmacological studies of certain drugs in rats, transnasal administration was found to be a promising administration route and an alternative to the oral route. It can be useful for the drugs targeted at the central nervous system (CNS) for treating certain diseases. The drugs that are difficult to cross blood brain barrier on oral or intravenously administration and drugs used for systemic diseases are usually targeted [58-61].

In the nasal cavity, the pathway mainly used for targeted delivery to CNS is olfactory neuroepithelium, in which the drug passes through *via* paracellularly and extracellularly into the olfactory bulb and further into the brain tissue or into the CSF [62, 63]. Further, it has been studied in rats that the absorption of water-soluble macromolecules, such as peptides and proteins, can be increased from the nasal cavity with the help of poly-L- arginine as an enhancer [64-66].

Administration Technique: It is performed in a lightly anesthetized animal. The scruff of the neck restrains the mouse or rat, and the tail is grasped between the small finger and palm for the administration of test substance [15, 67].

The animal is held in supine position with the head elevated. The micropipette or flexible polyethylene tube attached to a microsyringe can be used for the solution administration. The solution can be poured slowly by micropipette in the external nares of the nostril [68]. In rats, transnasal delivery can be performed by a flexible polyethylene tube inserted into the nostril about 8mm [69]. A small volume may be administered by this route refer to **Table 3.**

Inhalation Route (Mouse/Rat):

About Route: This route is frequently used to study asthma, air pollution, respiration, or when rapid absorption is needed [70, 71]. This route is mostly studied in toxicology when it is a route of human exposure for the test substance [72-74]. The most common species used for inhalation studies are rats, mice, and dogs [75, 76]. Usually, controlled conditions are required for the inhalation study of pharmaceutical or toxic materials under investigation. Anatomically, lungs are good for absorption and are characterized by large surface area, high blood flow and absorption close to blood [12]. Animals are usually exposed to test articles in liquid, solid or vapours aerosols form [73, 76, 77]. The particle size for all aerosols should be 1-4 µm MMAD with a GSD In the range of 1.5-3, is recommended [5, 77, 78].

Various systems have been used to house or restraint the test animals and provide a controlled atmosphere for exposure. Inhalation chamber methods like whole body and nose only (head only) are commonly used for exposure. Other specialized exposure methods are also reported, but technically, they are a little difficult [79]. An inhalation chamber should be selected based on the test article's nature and the test's objective. Usually, nose-only exposure is the preferred method. However, special objectives can be better achieved by the whole body chamber method with the recommendation that the volume of the animal should not exceed 5% of the chamber volume to maintain the atmosphere stability [77]. A vehicle of mostly water is preferred to generate an appropriate concentration and particle size of the test article in the atmosphere [73]. Commonly, exposure used may be of any duration upto 6 hr in rats and mice, and should not exceed 4 hr [77]. Chronic exposure 6 hr /day, 5 days /week can be employed in rats [80, 81].

Inhalation Method: The inhalation procedure can be performed by a well-designed inhalation chamber; it can be a specialized whole-body method and commonly by nose/head only method. Inhalation exposure of the substance to animals may be many hours per day, similar to normal cage

housing conditions. The whole body method provides a chamber atmosphere that is simulated to environmental exposures. By this method, test substance exposure to an individual animal can prevent the ingestion of test material due to the grooming of cagemates [73, 75, 80, 82, 83]. In contrast, the nose-only/head-only method minimizes skin or fur contamination, and a further amount of compound can be exposed only to a head or nose attached to a chamber [67, 84-86]. The commonly used method for exposure study is the nose or heady only, which is described further [84]. An animal, with the help of adjustable back restraint is inserted into tubular Holder, which can direct the atmosphere towards the animal nose only through the extension from the inlet manifold. Heat and humidity can be escaped to the atmosphere at the open site of the restraint. In case of toxic or hazardous compounds, tube sealing is used in restraint system to prevent the leak around the animals. The flow rate through each port should be more than a 1-minute ventilation rate (MV), so rebreathing and oxygen depletion can be prevented. This may vary further to various minimum flow recommendations as per the compound availability. For nose-only exposure chamber, commonly three flow rate recommendations are permitted, and they are, initial flow rate starts at 2.5 times the animal MV, If inadequate test material, then it should be 1.5 times, and for readily available compound should be 5 MV or more. The minimum final recommendation flow rate permitted is 10 times animal MV [84, 87].

Exposure Conditions: Guideline (OECD Draft guideline 436, 2004) for acute inhalation toxicity testing. This can also be used for longer-term exposures [74].

Exposure conditions	Monitoring
Air flow Nose only:0.5 l/rat/min	Air flow recording: Continuously and recordimg for at least 3 times
Air flow Whole body:12-15 air changes /hr	during exposure
Chamber Tem: 22±3°C RH: 30-70%	T & H: at least every 30 min
Particle size distribution:1-4 µm MMAD	Particle size distribution: at least twice
Chamber oxygen >19%	Concentration: Sample in breathing zone, at least 5 on an hourly basis

RH-Relative humidity, T & H-Temperature & Humidity.

Vehicles for Administration: Vehicle selection is an important consideration in an animal study as many substances are administered in solutions or suspensions [88]. Ideally, vehicles should be biologically inert and compatible, and per se, should not influence the results of the compound under investigation except by offering an optimal exposure [89]. If vehicles have biological effects, the dose can be reduced to minimize or nullify the effects.

Due to solubility or rate of absorption, some substances require a more complex solvent to render them suitable for administration. Many solvents, like distilled water, 0.9% sodium chloride, up to 50% PEG400, no more than 10% Tween 80, up to 2% methylcellulose (MC) / carboxymethyl cellulose (CMC) / hydroxypropyl cellulose(HPC). These are the maximum practicable limits prepared in aqueous component (i.e., distilled water) as quantity sufficient to volume, which has been found suitable for common injectable routes and not greatly affects the activity of interest of the substance under investigations [5, 18].

Saline is preferable over water for injection (WFI) for s.c. and i.v. administration as WFI may cause pain and hemolysis, respectively, upon injection. Corn oil, vegetable oil or peanut oil can be used for an oral and i.m. route only for lipid-soluble substances. Phosphate-buffered saline is also a suitable solvent. The most common cosolvents like dimethy-acetamide (DMAc), ethanol, PEG- 400, propylene glycol (PG) and glycerine are used for increasing the solubility of poorly water-soluble compounds [90, 91].

But mostly, using cosolvents results in drug precipitation upon administration, especially after intravenous bolus administration. Therefore, a slow injection or infusion technique or reduction of the concentration of the drug in the dose, or adding a low percentage of surfactant in the formulation can be used to reduce this effect [89]. In the case of lipophilic formulation involving oil as a vehicle which cannot be given intravenously as such, can be formulated in a 15% oil–water emulsions using lecithin as an emulsifier for successful administration [5].

Some Important Deliberations: For nonaqueous injections, time of absorption should be consider before re-dosing. When a suspension to be administered, the viscosity, pH and osmolality of the material need to be taken into account [92, 93]. Co-solvents system should be used appropriately because they themselves have dose-limiting toxicity. It is desirable to administer the doses with reduced buffer capacity and acceptable pH ranges, because it may deleterious to animal physiologic acid base system *in-vivo* and also may cause irritation or tissue damage, especially, when administering the dose by i.m. or s.c [89].

Formulation Strategies in Preclinical Studies: The studies for proof of concept are aim to validate the target in *in-vivo* pharmacology models. An i.v. or s.c. the route is usually preferred. These studies aim to establish compounds' PK-PD relationships in the early discovery stage, which usually happens before the optimization of compounds for the eventual intended route of delivery in humans(mostly oral) [27]. As there is a need of repeated dosing, hence, continuous delivery options are usually used, which involve the use of osmotic pumps (AlZET) for extended delivery (usually 7-14 d) that are implanted subcutaneously or suspension delivery, in which drugs can be delivered slowly over an extended period of time by the subcutaneous route [94, 95]. Preferably, a continuous delivery option by IV infusion as a first approach and implanted pumps subcutaneously as a second approach could be adopted [94].

For solution formulation development of poorly soluble compounds, common solvents like cosolvents or cyclodextrins have been used [94, 96]. Lead optimization studies involving the molecule structure activity relationships can be tested primarily by pharmacokinetic screening in rodent species, especially rats. This includes i.v. arm and intended route of administration in humans, mostly oral. Only solutions or nanosuspensions can be administered intravenously. Usually, intravenous route is primarily involved in PK studies to obtain clearance and true volume of distribution [97].

This can be achieved through solution formulations. For i.v. arm, Dimethyl sulfoxide (DMSO) solution is used as it can be available in stock form and can be stored as DMSO solution but it is necessary to verify the concentration before dosing. If the DMSO is not acceptable or not practical for any reason [98, 99], then combination approaches are cosolvent plus buffer or cyclodextrin plus buffer may be used effectively [100]. This could be the default formulation for all the series compounds and be modified to accommodate the other series. Whereas for oral dosing of poorly soluble compounds, administration in suspension form is suitable if high doses are administered. A basic combination of 1-2% HPMC as suspending agent and 1% Tween 80/poloxamer as surfactant (or 0.5 % CMC with 0.5% polysorbate 80) are commonly used.

This could be a default approach for formulation. In case of oral solutions, pH adjustment for basic (pka >3) and acidic (pka <8) compounds is used [27]. To improve oral absorption, lipid-based formulation or particle size reduction for highly lipophilic or low soluble compounds can be adopted [27, 101-103]. If required exposure is not achieved for poorly water-soluble compounds, then a complexing agent (15-20% hydroxypropyl β-cyclodextrin or sulfobutyl ether-β-cyclodextrin) can be added to enhance the solubility. If the exposure is acceptable, then the default formulation for the project is identified and may be modified for the compounds in the series. In pharmacology studies to assess efficacy, formulation approaches used in the concept of proof studies are generally accepted if the compounds are administered orally, then, it is desirable to attempt the same formulation approach as taken for the pharmacokinetic studies [27].

An intraperitoneal route of administration is usually chosen in the early drug discovery stage to avoid the intestinal barrier. Although suspension can be dosed, the solution is generally preferable by this route due to the limited fluid supply to the peritoneal cavity to solubilize the particles in the suspension. Sometimes, it is not practicable for every compound to screen in the *in-vivo* assay. Therefore, the generic approach of formulation can be used.

This includes 20% cyclodextrin as a solubilizing agent, 1% HPC as a suspending agent, and 1% as a pluronic F68 as a surfactant. Thus the solubilizing agent may enhance the concentration of the active compound in the solution. But if the concentration

still remains above its solubility, then at least dosable suspension can be formed with the help of the latter two agents. Usually, suspension formulation is useful by subcutaneous route as it bypasses the hepatic metabolism and intestinal barrier; hence, It is oftenly used over the oral route in early drug discovery stages. However, the absorption rate for suspension formulation depends upon the solubilization of particles in the extracellular fluid [104]. Some commonly used formulations in preclinical studies as reported in **Table 2.**

TABLE 2: SOME COMMON FORMULATIONS USED IN PRECLINICAL STUDIES [27, 105]

Oral formulations (q.s. to volume)	Intravenous formulations (q.s. to volume)
Water with pH adjust (2-4 for Base,7-9 for acid)	Up to 20% hydroxypropyl β cyclodextrin w/wo pH adjust(4-8) +q.s.
20% hydroxypropyl β cyclodextrin w/wo pH Adjust +q.s.	10% cremophor EL +10% ethanol+ q.s.
10% cremophor EL + 10% ethanol + q.s.	100% DMSO for single dose only
10% DMAc+ 10% ethanol+ 20% PG+ q.s.	10% DMAc+10% Ethanol+ q.s.
40% PEG-400 + 10% ethanol + q.s.	20% PG +q.s- single dose only
20% PEG-400+ 10% cremophor EL + 10% ethanol + q.s.	20% intralipid emulsion (5-10% soybean oil+1-3% soy or lecithin and buffer) +q.s.
10-50% DMSO + q.s.	Upto 50% PG or PEG-400 + q.s.
25% PG+20 %vitamine E TPGS in PEG-400+q.s.	Up to 40% DMA or N-methyl pyrrolidone or glycerol + q.s.
0.25-2% cellulose derivative (MC/HPMC/HPC/CMC)+ upto 10% tween 80 or poloxamer w/wo pH adjust + q.s.	Nanosuspensions(90% particle <1 µm) with 1-5%HPC-SL and 0.05-0.1% docusate sodium(0.1% Tween 80 or 0.1% SLS can be substituted for docusate sodium) + q.s.
10% DMAc + 20%PG+40%PEG-400 + q.s	
5% labrasol in PEG-400 + q.s.	
100% corn oil or soybean oil	
Upto 30% solutol HS-15(polyethylene glycol/hydroxystearate) in water(w/w) +q.s.	

q.s. (quantity sufficient) to volume with aqueous component. w/wo - with /without. All vehicles for intravenously can also be used intraperitoneally. DMAc- Dimethyl acetamide; PEG-Polyethyle.ne glycol; PG-Propylene glycol; DMSO-Dimethyl sulfoxide; MC-Methyl cellulose, HPMC-Hydroxypropyl methy cellulose; HPC- hydroxypropyl cellulose; CMC- carboxymethyl cellulose, HPC-SL-hydroxypropyl cellulose, grade SL; SLS-Sodium lauryl sulphate; TPGS- α-Tocopheryl-polyethylene glycol-1000 succinate.

In drug discovery stages, toxicology assessment is the final step before nominating a compound as a clinical candidate or to dose in humans. Toxicology studies aim to establish the maximum tolerable dose and the biggest challenge in these studies are with the highest doses, typically 100 x ED50 dose. The FDA-recommended maximum dose can be increased to 2 g/kg [106] if no adverse effect exhibited. The compound's pharmacokinetic properties are well characterized during the stages of drug discovery in the animal species of interest, as well as physicochemical properties. These early studies aim to maximize the exposure and identify key adverse effects that the molecules might potentially elicit. Usually, a preferred formulation in toxicology is 0.5-2% suspending agent (MC, CMC, HPMC) with polysorbate 80 as the wetting agent, but due to non-homogeneity at high doses, which often has physical stability issues, hence, the first approach to enhance the solubility in toxicology formulations is salt formation through pH adjustment, is commonly used. For the oral route, pH 2-9 range for non-buffered vehicles and 4-8 for the buffered vehicle are acceptable. Further, combining the pH adjustment with various other solubilization approaches such as cyclodextrin (sulfobutyl ether β- cyclodextrin (25%) and surfactant (polysorbate 80) may be helpful. Recently, the increased use of amorphous solid dispersions (ASD) in toxicology studies has been observed to enhance the bioavailability of poorly water-soluble compounds. Acceptable polymers such as Hydroxypropyl methyl cellulose acetate succinate (HPMC-AS), Polyvinylpyrrolidone (PVP), and HPMC may be employed in ASD preparation. In preclinical studies, ASD is necessary to be formulated in a suspension vehicle for dosing in rodents *via* the gavage tubing. Hence, ASD should be physically stable not only as is, but also as a formulated suspension, which can be achieved by adding polymers as crystallization inhibitors and increasing the viscosity of the vehicle. For intravenous formulations, slow push injections (30-60 min) or continuous intravenous infusions should be considered when the solubility of the compounds render an acceptable solution

formulation challenging. Additionally, the chemical stability of the compound in a formulation is an important consideration in toxicology formulation development as over the period; the formulation is Spacing is need to be adjusted. Compound physicochemical characterizations such as purity, residual solvents, pKa, and log p, and aqueous solubility profile during lead optimization and toxicological studies, purity, salt content, residual solvents, and impurity profile have been necessitated. Further, formulation physicochemical characterizations at various stages {like proof of concept studies, pharmacokinetic screens, efficacy assessment (EA) and toxicology studies (TS)} during drug discovery are necessary to be conducted such as dose analysis and chemical stability(only for EA, TS) in case of solution formulations whereas homogeneity, particle size analysis, chemical stability(only for EA and TS) and physical stability (only for TS) for dosed and stored formulation in case of suspension formulations [27].

Some commonly used Restraint Technique in Rodents (Mouse and Rat): To perform any experimental procedure, rats and mice should be trained to accept the handling and restraining so that it becomes familiar with the handlers. This is essential to minimize the distress as well as for correct administration. All these procedures should therefore be carried out by the well-versed person known to the animal [107].

Some Common Techniques for Intraperitoneal Injection:

Scruffing Method: This method is performed by double-handed [20, 108]. The tail of mouse/rat can be firmly grasped by the dominant hand and placed over the cage lid, and pulled the tail gently back. Then grasp the scruff of the neck with a non-dominant hand by approaching from the rear. Further, the scruff of the neck can be grasped close to the Base of the skull between the thumb and forefingers and the palm (refer **Fig. 16A**). animal can be restrained by fixing of tail between the palm and little finger of the non-dominant hand as shown in **Fig. 16B** & **C**. This method is recommended in small rats and mice.

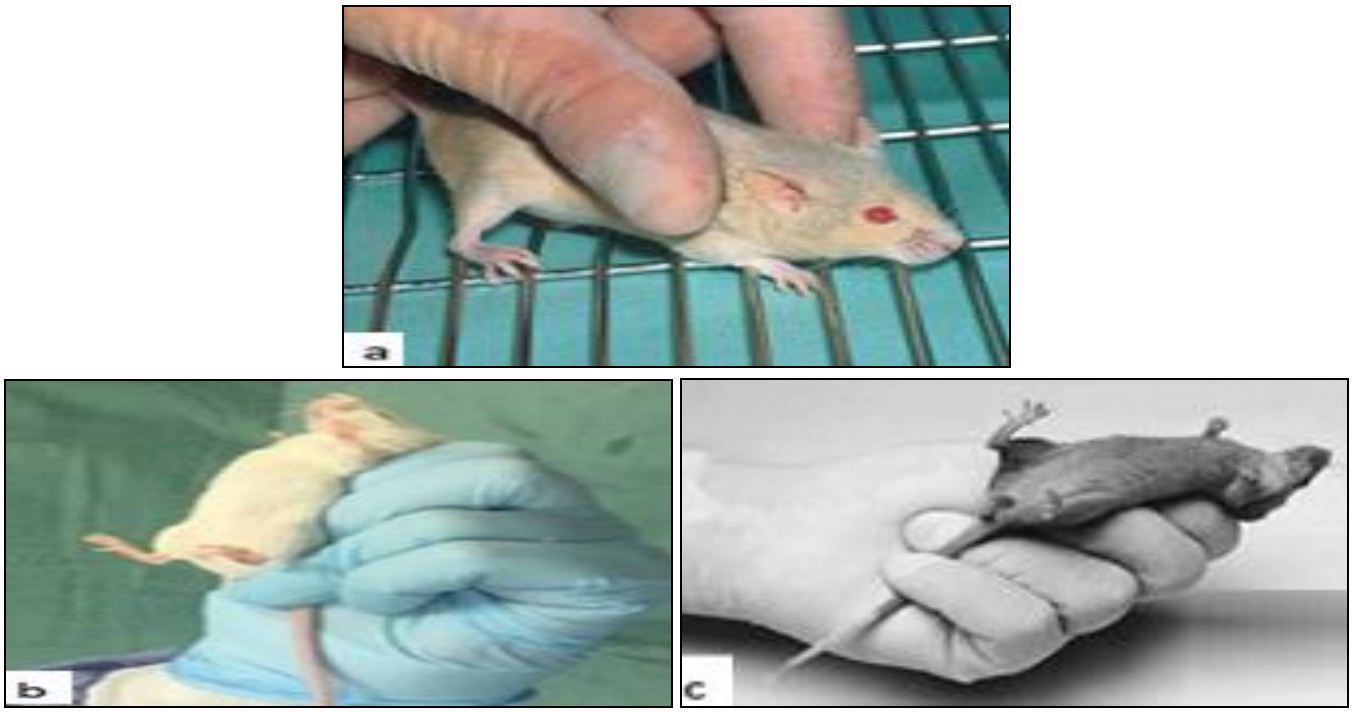

FIG. 16(A, B, C): SCRUFF METHOD MANUAL RESTRAINT OF MOUSE BY DOUBLE HANDED

Over the Shoulder and fore Limb Crisscross Method (refer Fig. 17): A rat's tail can be grasped with the dominant hand over the solid or rough surface and with the non-dominant hand hold the back from the rear. Then rat can be restrained over the shoulder with the index finger on one side of the head and the middle finger on the other side; this restricts the movement of the head. Further

movements of a rat can be prevented by encircling the body behind the forelegs with the third finger, the fourth finger, and the thumb. The lower body can be supported by holding the Base of the tail using the dominant hand. This method is useful in larger rats when access to the head or exposure to ventral surface is needed. Other person can make an i.p. injection by grasping the hind leg above the hock. Fore limb criss cross method (under the shoulder): As mentioned above, a rat can be held. Then rat can be restrained by grasping around the thorax, right under the shoulder, with the thumb and index finger. Further, the rat's forearms should be gently pushed up with the thumb and index finger. This will prevent the rat from moving its head downwards the fingers. Other person can make an i.p. injection by grasping the hind leg.

Some Common Techniques for Injections into the Subcutaneous Space:

Scruffing: For s.c. injection, method described in scruffing can be used for exposure to dorsal surface. Lift the scruff of the rat and allow its hind legs on a solid, flat surface. Create a tent over the neck. Hold the rat in place with weight on the hindquarters. A s.c. the injection can be made into the tent space below the finger.

Animal Hind Limb Holding for Intramuscular Injection and Injection into the Footpad:

Scruffing of Mouse and Small Rat (Under 200 g): Scruffing method, as described above, along with restraint of the hind limb by another person, is used for the intramuscular and footpad injections.

Over the Shoulder Grip or Forelimb Crisscross Method: This involves two persons, one for holding the body as described above and the other for holding the hind limb and to make intramuscular and footpad injections.

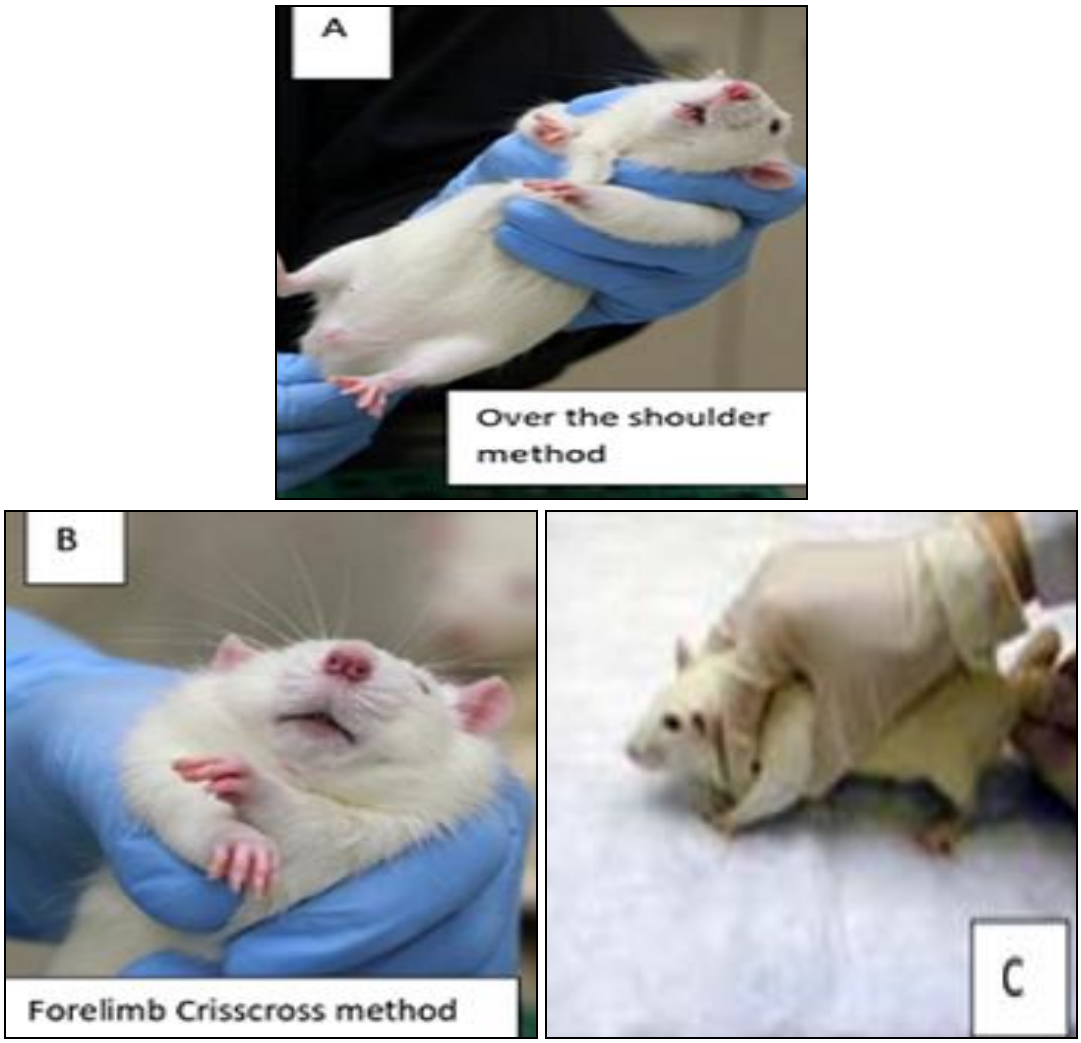

FIG. 17: RAT: A AND C- OVER THE SHOULDER METHO RAT: B- FORELIMB CRISSCROSS METHOD: UNDER THE SHOULDER METHOD

TABLE 3: MAXIMUM VOLUMES: IDEAL DOSE VOLUMES (ABSOLUTE VOLUMES) TO BE USED FOR DOSING OF SPECIES BY ROUTE OF ADMINISTRATION AND NEEDLE GAUGE SIZE [6, 7, 14, 21, 34, 107, 109-111]

Species	Volumes in mL/kg unless otherwise noted							
	Oral	s.c.[c]	i.p.[c]	i.m.[c] ml[a]	i.d. ml[a]	i.v. bolus	i.v. Infusion	i.n. ml[a]
Mouse(M)	10(20)[$]	5 (20)[g]	10 (50)	0.03 (0.05)[#]	0.05 (0.1)	5* (25)[s]	50[d]	0.05
Rat (R)	10(20)[b]	5 (10)[g]	10 (20)	0.1(0.2)[#]	0.05 (0.1)	5*(20)[s]	50[d]	0.1
Needle Gauge (G)Size & Length(L)								
Recommended needle size (M)	18-22G 1-1.5 in.	< 25G 0.5-1in.	< 23G 0.5-1 in.	25-27G 0.5-0.75 in.	<26G 0.5in	< 25G , 0.5-1 in.		
Recommended needle size (R)	16-20G 1.5-3in.[X]	≤ 21G 0.5-1in.	≤ 22G 0.5-1 in.	≤ 22G 0.5-0.75 in.	26G 0.5in.	≤ 22G, 0.5-1 in.		

[$]20/[b]10 mL/kg administered four times/day (a total of 80/40 mL/kg) in a 24 hr period to accommodate a larger volume, [#]dosing is not recommended, *Volume given as an intravenous bolus over approximately 1 min; maximum recommended injection volume for a dosing solution that is given *rapidly* i.v. is 1 mL/kg body weight (b.wt.), [s]slow injection over the course of 5 -10 min, [d]rate -1 mL/min, [a]Values are the total volume in ml per site, Total of 2 sites/day. [g]2-3 sites of administration should be used, when administering large volumes subcutaneously. [c]When administering a solution i.p./s.c./i.m., the viscosity, concentration, tonicity, pH of the solution need to be taken into consideration. i.m. injection: Not preferred in mice due to small muscle mass and possible side effects, such as paresis, paralysis, muscle necrosis, localized sloughing and self-biting of injected areas.[X]18G:-Rat wt.(g):75-100g,L:1-1.5in. & Rat wt.: 100-200g, L: 2-3in.; 16G- Rat wt.-200-300g,L:3-4in; curved-shaped needle for 16-20G. In case of IV continuous infusion : 1(4) mL/kg/h recommended infusion rates in mice and rats and infusion for 4hr/day and continuous infusion for 24 hr period in rats have been reported[112]. Catheter maintenance rate in rats: 0.1-0.5 mL/h. The ideal rate should not exceed 2 mL/kg/hr in embryotoxicity studies in rats.

DISCUSSION AND CONCLUSION: In biomedical or preclinical research, the mouse and rat are the common and frequently used species [1]. Various routes are employed to investigate the biological activity of test substances in solutions/suspensions form. Typically, the preferable principal routes of administration are i.v., s.c., i.p., and oral (gavage) in early animal studies in drug discovery programs, and rat is the leading species [26]. Further, the regulatory influences on the intended route of administration for human is to be investigated. Therefore, to acknowledge the available routes, principal and common routes of administration have been discussed in small animal species like mice and rats. An i.v. or s.c. the route is the primarily explored principal route for establishing PK-PD relationship in early drug discovery programs [13, 27] as the plasma concentrations by this route can be better predicted. An i.v. route provides the more rapid and complete absorption of compounds. A bolus or chronic administration can be given via lateral tail vein or venous (jugular/femoral) cannulation, respectively [31, 42]. In preclinical studies, the other parenteral routes, an i.p. and i.m. are the most frequent and common routes of administration. The deposition route is mostly s.c. administration with slower absorption than i.m. or i.p. usually preferred [28]. An i.m. route provides rapid absorption than s.c. for solutions [5]. The preferable site of s.c. injection is scruff neck region and for i.m. is posterior thigh muscle or bicep femoris muscle[14]. Oily fluid depot is commonly injected by an i.m. route with desired prolonged action[22]. However, in early animal studies, an i.m. route is occasionally used [27] and if necessary, the rat is the preferred species as the muscle mass size in mouse is too small.

An i.p. is the most common route and facilitates rapid absorption. It can also be a justifiable route in drug discovery programs for pharmacological and proof of concept studies that evaluate the target engagement rather than the properties of the substance [113]. Generally, an i.p. injection is preferred in the lower left quadrant of the abdomen due to the risk to viscera [28]. An oral gavage administration is oftenly used to mimic a common dosing route in humans [27] as most discovery programs are aim to design compounds for oral administration in humans; therefore, early

evaluation is necessitated. Most importantly, physicochemical properties impose practical limitations, and also, there are differences in metabolic patterns among different routes; because of these, oral route is preferred in early studies. Appropriate consideration is given to the feeding needle size for administration in mouse and rat. An animal will be assessed for any distress after administration and if necessary, euthanized in failure of gavaging. The other common routes in preclinical studies are i.d., Topical- dermal, intranasal and inhalation routes. An i.d. route is commonly evaluated for immunostimulatory substances [23, 24] and very small volumes (0.05-0.1 mL) are injected [6]. Topical- dermal route is used in testing potential therapeutic compounds for skin disease or the potential to cause skin irritation and is selected when it is the intended route of exposure in humans [8].

An intranasal route of administration can be employed for the local and systemic exposure of substances. This route is used as an alternative over an oral route due to direct dilvery to CNS via the olfactory pathway and systemic circulation [57-60]. An inhalation route in second order regarding absorption rate is commonly used for respiration studies or when rapid absorption is desired [70-73]. In preclinical formulation, vehicles and formulation selection are essential in an animal investigation for the administration of poorly soluble compounds. Further, vehicles should be biologically inert or have minimal adverse effects [111]. The absorption rate is generally arranged in the order, *viz*., i.v. >inhalation> i.p. > i.m. > s.c. > po > i.d. as the rate of absorption depends upon the route of administration (*viz.*, size of absorbing surface, blood flow, and solubility of the substance in the tissue fluid) and substance characteristics (*viz.*, lipid solubility, physicochemical properties, degree of ionization and molecular size) [3, 12, 14].

Therefore, familiarity with various common routes and formulation strategies gives researchers the ability to administer the substance in the best possible way for their research and helps select routes of administration in drug discovery programs or preclinical studies. Eventually, adopting the basic competency in handling and restraint methods can result in less stressed animals and better scientific outcomes [107].

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