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Guidelines on dosage calculation and stock solution preparation in experimental animals' studies

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Abstract
Dosage calculation and stock solution preparation in preclinical studies, involving the use of experimental animals is important in screening and development of new drugs. The present literature provides the basic principles and guidelines on dosage calculations and preparation of stock solution (with reference to crude plant extracts, sylimarin tablets, alloxan monohydrate, gentamicin injection as well as chemiron blood tonic) for experimental animal studies.

Keywords: Dosage calculation, stock solution preparation, crude plant extract, sylimarin and chemiron blood tonic.

1. Introduction

Experimental animals have been of very important tools in the history of non-human research models for scientific purposes in almost every aspect of biomedical, behavioral researches and testing conducted in Universities, Medical schools, Pharmaceutical companies, research institutes, farms and commercial facilities that provide animal-testing services to industry (Festing, 1979). Experiments on animals are necessary in drugs discovery and development as well as to advance medical and biological knowledge (Baker, *et al*, 1979).

Dosage calculation and stock solution preparation based on dosage rationale formula are prerequisites to drug administration in experimental animals. However, drugs dosage calculations and stock solution preparations are not clearly explained in most scientific literatures involving the use of experimental animals, and this is a major challenge to some undergraduate students, post-graduate students and other researchers. Since over 90% of animals used in *in vivo* experiments in medical, physiological, pharmacological, chemical, toxicological, biological, biochemical and genetic studies are rats and mice, this work is aimed to simplify calculation of doses, preparation of stock solution in experimental animal for the benefits of all researchers.

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

A vehicle is any substance that acts as a medium in which a drug is administered. Vehicle, which is an essential consideration in all animal research should be biologically inert, have no toxic effects on the animals and not also influence the results obtained for the compound under investigation. Example of suitable vehicles for animal research include; water, normal saline (0.9% sodium chloride), 50% polyethylene glycol, 5 to 10% Tween 80, 0.25% methylcellulose or carboxymethylcellulose (Karl-Heinz, *et al*., 2001; Nebendahl,2000).

In most researches involving experimental animals, dosages are usually calculated from stock solution of the test drugs dissolved in appropriate volume of solvent (vehicle). According to the OECD's (organization of economic corporation and development's) guidelines, dosage of drug (mg) should be constituted in an appropriate volume not usually exceeding 10 ml/kg (1 ml/100g) body weight of experimental animals (mice and rats) for non-aqueous solvent in oral route of administration. However in the case of aqueous solvents, 20 ml/kg (2 ml/100g) body weight can be considered (OECD, 2000). Large dose volumes (40 ml/kg body weight) can cause unnecessary stress to animals and can also overload the stomach capacity and pass immediately into the small bowel or can result in passive reflux in the stomach, aspiration pneumonia, pharyngeal, esophageal, and gastric irritation or injury with stricture formation, esophageal and gastric rupture and stress (Germann & Ockert, 1994; Hejgaard, *et al*., 1999; Bonnichsen, *et al*., 2005). Lower volume (5 ml/kg) can be considered to dissolve highly soluble solute drugs. Such low volume would ease the administration of drug in solution. However, highly viscous drug solution should be diluted, whenever possible, for ease of administration. However, final dilution volume should not exceed 20 ml/kg.

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

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

NB: 1kg = 1000 g

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

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

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

Table 1: OECD'S guideline on volume selection.

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

3. Dosage calculation and preparation of stock solution of crude plant extract for experimental animals

With reference to table 1 above, stock solutions and doses of a plant extract (With selected doses, 200 mg/kg and 400 mg/kg) for a rat weighing 120 g be calculated as follows;

Step 1: **Dosage calculation**

Body weight of animal =120 g

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

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

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

From the OECD's guidelines,

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

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

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

24 mg = 1.2 ml

40 x 24 mg = 40 x 1.2 ml

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

This examples shows that 1 ml of dissolved plant extract from a given stock solution (960 mg/48 ml = 20 mg/ml) is the required dose (from selected dose of 200 mg/kg) for a rat weighing 100 g. However, 1.2 ml from the same stock solution is the required volume for a rat weighing 120 g (which is meant to receive 24 mg of the plant extract).

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

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

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

The above table shows that animals with higher body weight in the same standard dose category will receive higher doses (mg or ml) of plant extract solution. Also, the stock solution for high dose will be thicker and more concentrated than low dose.

4. Dosage calculation and preparation of stock solution of a reference drug (example: Sylimarin) for experimental animals.

Sylimarin (figure 1) is a reference drug (with dosage formulations of 70 mg and 140 mg per tablet respectively) used in animal model of screening for agents with hepatoprotective, nephroprotective and anti-oxidant properties at standard doses ranging between 25 mg/kg to 200 mg/kg body weight (Claudia, *et al*. 2003; Sohair & Salwa, 2011).

Figure 1: Sylimarin tablet **Source**: https://www.google.com.ng/search?q=Silymarin+tablet,+photos&tbm.

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

Step 1: **Dosage calculation**

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

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

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

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

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

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

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

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

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

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

From the above table, it shows that animals with higher body weight will receive higher doses (mg or ml) of sylimarin solution.

5. Dosage calculation and preparation of stock solution for salt compound (example: Alloxan monohydrate).

Alloxan monohydrate is a diabetogenic agent at a standard dose of 150 mg/kg for rat via intraperitoneal route as reported in several experimental diabetes literatures (Erhirhie, *et al*., 2013; Oluwole, *et al*., 2012). It is in salt form.

The required dose of alloxan monohydrate to induce experimental diabetes intraperitoneally in a rat weighing **200 g** at a standard dose of **150 mg/kg** can be calculated as follows;

Required dose for 200 g rat = $\frac{\textbf{Weight of animal g)}}{\textbf{1000 g}}$ x Standard dose (mg)

$$= \frac{200g}{1000g} \text{ x } 150 \text{ mg} = \mathbf{30\ mg}$$

For intraperitoneal administration, appropriate volume of vehicle ranging between 2 ml/kg to 5 ml/kg in rats is recommended.

Based on 2ml/kg volume selection in this example, **30 mg (0.03 g)** of alloxan monohydrate would be constituted in = $\frac{200\ g}{1000\ g}$ x 2 ml = 0.4 ml of a vehicle (normal saline) corresponding with the volume required for 200 g rat.

Therefore bulk volume of this stock could be prepared for more experimental animals by weighing 300 mg (0.3 g) in = $\frac{0.4\ ml}{30\ mg}$ x 300 mg = 4ml of normal saline. The number of experimental animals determined the quantity of alloxan monohydrate required.

This stock of 300 mg/4 ml would yield a concentration of = 75 mg/ml (table 4).

Table 4: Showing stock solution preparation for alloxan monohydrate and required doses for animals of different body weights.

Standard dose	Stock solution	Animal's body weight	Calculated dose in mg	Equivalent dose in ml
Alloxan monohydrate, 150 mg/kg	300 mg/4 ml = (75 mg/ml) or 600 mg/8ml = (75 mg/ml)	200 g	30.0 mg	0.40 ml
		180 g	27.0 mg	0.36 ml
		160 g	24.0 mg	0.32 ml
		150 g	22.5 mg	0.30 ml

From the above table, animals with higher body weight will receive higher doses (mg or ml) of alloxan monohydrate solution. In this regard, larger volume of the stock solution can be prepared for large number of animals by constituting 600 mg (0.6 g) of alloxan monohydrate in 8 ml of normal saline or 1200 mg (1.2 g) in 16 ml of normal saline.

This approach would be able to conserve and estimate the quantity of alloxan required to induce diabetes in a given population of animals. Analytical weighing balance with higher accuracy and sensitivity should be used for weighing alloxan monohydrate.
However, dissolution volume of 5 ml/kg can be considered for intraperitoneal administration in mice (due to their low body weight compared to rats).

6. Dosage calculation and preparation of stock solution for Ampoules (example: gentamicin injection).

Gentamicin (figure 2) is an aminoglycoside antibiotic used to induce nephrotoxicity in experimental animals (rats and mice) at a standard dose of 80 mg/kg (via intraperitoneal route) as reported in literatures (Chinnapa, *et al.*, 2011; Muhammad, *et al.*, 2011).

Figure 2: Gentamicin injection. S**ource:** www.pharmacygeoff.md400 ×400Search by image.

The required dose of gentamicin to induce experimental nephrotoxicity intraperitoneally in rat weighing **100 g** at a standard dose of **80 mg/kg** can be calculated as follows;
Step 1: **Dosage calculation**

Required dose for 100 g rat = $\frac{Weight\ of\ animal\ (g)}{1000\ g}$ x Standard dose (mg)

$$= \frac{100\ g}{1000\ g} \text{ x } 80 \text{ mg} = 8 \text{ mg}.$$

Step 2: **Required volume calculation for intraperitoneal injection**

From the above calculation, 100 g rat requires 8 mg of gentamicin and this dosage (8 mg) can be withdrawn directly from the stock of gentamicin which is present as 80 mg/2 ml (40 mg/ml) ampoule.

Thus, **40** mg ≡ **2 ml**

100 g rat requires **8 mg** which is equivalent to ≡ $\frac{2\ ml}{40\ mg}$ x 8 mg = **0.2ml**.

From the ampoule of gentamicin (figure 2), required dosages can be administered to animals of varying body weights as illustrated in table 5 below:

Table 5: Showing the required dose (volume) of gentamicin injection required for rats and mice of varying body weights.

STANDARD DOSE	STOCK SOLUTION	Animal's body weight (g)	Calculated dose in mg	Equivalent dose in ml
Gentamicin, 80 mg/kg For rats	80 mg/2ml = (40 mg/ml)	100 g	8 mg	0.20 ml
		120 g	9.6 mg	0.24 ml
		140 g	11.2 mg	0.28 ml
		150 g	12.0 mg	0.30 ml
Gentamicin, 80 mg/kg For mice	80 mg/2ml = (40 mg/ml)	20 g	1.6 mg	0.04 ml
		25 g	2.0 mg	0.05 ml
		30 g	2.4 mg	0.06 ml
		35 g	2.8 mg	0.07 ml

The above table shows that animals with higher body weight will receive higher doses (mg or ml) of gentamicin injection. However, twice serial dilution could be carried out in other to obtain a larger measurable volume for rats and mice.

Twice serial dilution could be done as follows = $\frac{40\ mg}{1\ ml\ of\ gentamicin + 1\ ml\ of\ normal\ saline}$

$= \frac{40\ mg}{2\ ml\ diluted\ gentamicin}$

$= \frac{20\ mg}{ml}$

Therefore, double volume (for example 0.1 ml for 25 g mouse) is required form the diluted stock of gentamicin for experimental animals.

NB: Further serial dilution (3 to 4 times) could be carried out as illustrated above in other to obtain appropriate volume to be administered to smaller animals (especially mice).

7. Direct calculation of animals' dose from human dose

Some experimental situation may arise, when animal doses of compounds of different formulations (syrup, tonic, solution, ampoule injection, suspension and tablet) are calculated from average adult (70 kg) human dose.

We shall consider chemiron blood tonic (figure 3) in this instance.

Figure 3: Chemiron Blood Tonic: Source: www.chemiron.org/chemiron-blood-nourishing-tonic-and-capsules/

For example, if the required dose of chemiron blood tonic for 70 kg human = 10 ml (x3 daily) = 30 ml/70 kg/day.

Dosage for a rat weighing **150 g** can be calculated as follows;

70 kg (70,000 g) adult man requires = **30 ml** of chemiron blood tonic per day,

Therefore, **150 g** rat requires = $\frac{\mathbf{30\ ml}}{\mathbf{70000\ g}}$ x **150 g** = 0.06 ml

Since the volume is very small to be measured with one milliliter syringe, it is recommended to carry out ten times (x10) serial dilution for the tonic by;
Diluting **1 ml** of chemiron blood tonic with **9 ml** of normal saline
or diluting **10 ml** of chemiron blood tonic should be diluted with **90 ml** of normal saline
Required new volume of chemiron tonic for 150 g rat from the diluted chemiron will be = 0.06 ml x10 = 0.6 ml.
Subsequent volume of chemiron can be calculated for rats whose body weights are different from 150 g.
However, further serial dilutions (x15 to x 20) is recommended for mice (which weigh far less than rats) in this scenario in order to obtain a measurable volume.

8. Restraining and administration of substances to laboratory animals by oral and intraperitoneal routes

Having explained the basis of dosage calculation and stock solution preparation of drugs for experimental animals, researchers should be conversant with animals restraining techniques in other to be able to deliver the appropriate drug volume with the aid of gavag attached to 1ml, 2 ml or 5 ml syringes (oral route, figure 4) and appropriate needle size attached to 1 ml syringe (for intraperitoneal route, figure 5).

Figure 4: Oral administration in rat.

Figure 5: Intraperitoneal injection in the rat.

For oral route (figure 4), rats and mice should be restrained gently and firmly by gathering the loosed skin of their neck region upward and slightly backward so that the esophagus is as straight as possible. The tube is then carefully inserted between the tongue and the roof of the mouth until a free space is observed for the drug to be delivered into the stomach (Ahatty, 2012).
Intraperitonial injection (figure 4) involves administration of the material into the space surrounding the abdominal cavity, avoiding direct injection into the viscera organs.Volume of administration also influences the absorption of substances given intraperitoneally, and larger volumes can result in pain and distress (Ahatty, 2012; Barrett, 1991). Research personnel should have training to familiarize themselves to animals restraining techniques in other to eliminate errors associated with experimental animals' techniques.

9. Conclusion

Proper dosage calculation, stock solution preparation and substance delivery to experimental animals via an appropriate route are indispensable parts in many scientific researches involving rodent (rats and mice). Therefore, this work would aid researchers to ensure that studies involving experimental administration of substances to animals are planned and conducted appropriately by following illustration given in the graphical summary (figure 6).

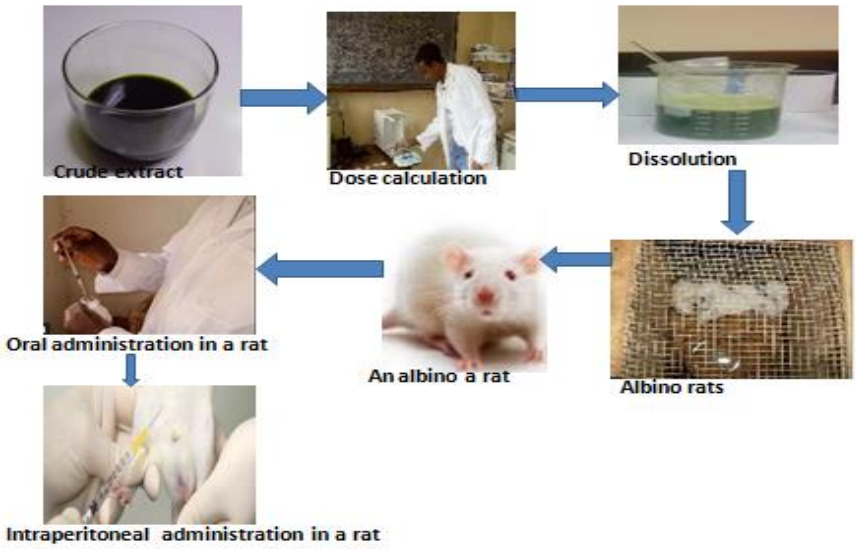

Figure 6: Graphical summary.

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