Short Communication

Feeding of organic plant extracts to animals during vivisection, extract dosage feeding formula (exdff): a novel technique.

Mirza Muhammad Fahd Qadir¹, Attya Bhatti*¹, Mansur Abdullah Sandhu², Muhammad Usman Ashraf¹, Sidrah Anjum¹, Peter John¹

¹Atta-ur-Rahman School of Applied Biosciences, National University of Sciences and Technology, Islamabad, Pakistan. ²Faculty of Veterinary and Animal Sciences, Pir Mehr Ali Shah Arid Agriculture University Rawalpindi

*Corresponding author email: attyabhatti@gmail.com
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Abstract

The aim of this paper is to propose an equation by which scientists performing herbal extract based vivisection can appropriately feed correct quantities of their desired test dosage. The usage of this formula is not only useful to mice but also can be extended to other animal species that may come under study during ethnobiology studies. In this study, 116 papers were evaluated for their role in extract preparation and feeding with respect to certain notable diseases. Later these papers were screened for those most appropriate in generating the design for the equations projected. The LaTeX online editor software was used in generating a suitable formula and then tested using experimental data. The generated formulae were aggregated to assimilate a singular formula named extract Dosage Feeding Formula (exDFF). This formula will hypothetically simplify the complex series of arithmetic required to determine dosages during experimental feeding.

Keywords: Animal Science; Plant Extracts; Experimental design; Formula.

INTRODUCTION

Numerous scientific groups around the world use plant life as a source of novel chemical identification and discovery. The first step in this process is the evaluation of plant usage with respect to a particular disease. This calls for a possible bio-active compound being present in the plant which may lead to the alleviation of the disease in question. The study of plants and their medicinal properties have hence become a rekindled science, particularly in Southeast Asia, where numerous plants and herbs are still in medical use Mukherjee et al. (2007); Tang et al. (2009). Ethnopharmacology, is hence a derivative specialty from which chemists and drug developers are able to evaluate the potency of multiple components present in the plants under their study (Cordell, 2000).

During the process of study, in vivo trials on rodents, particularly mice, are performed to calculate the LD50 as well as the ED50 of an extract with respect to a disease (Mabona and Van Vuuren, 2013.). With the advent of better separation techniques like liquid-phase extraction or gas-phase extraction, organic substances can now be easily separated and prepared for feeding trials. Scientists have previously mentioned the use of the t-test and chi test to evaluate the lethal dosage of certain
extracts. A graphical representation of log-values to probits is commonly used to evaluate the lethal dosage 50 (LD50) value, of plant extracts (Randhwa, 2009). There is, however a dearth of an effective feeding technique when feeding of an extract is done by the oro-gastric or intra-peritoneal route Zhang et al. (2008). This leads to many issues and difficulties which arise when evaluating the accurate amount of extract to feed an experimental group. Scientists still rely on unitary methods as well as correlation to evaluate the amount of extract required in their feed, as per their pre-planned requirement (Randhawa, 2008). In order to facilitate researchers, the extract Dosage Feeding Formula (exdff) is a simplistic formula which allows researchers to effectively calculate the mass of extract they require which will be equivalent in the animal under study as 1. grams or milligrams per kilogram body weight.

Therefore the purpose of this paper is to report the usage of a tested formula which will allow researchers to easily measure the amount of extract they wish to administer to their animal groups under trial.

**MATERIALS AND METHODS**

**Literature search:**

Initially 116 articles were downloaded with reference to herbal extract feeding in mice. The type of mice chosen in order to maintain uniformity was BALB/c. These articles were then evaluated for their potential in dosage calculation by processing their methodology. The type of manuscripts using organic extracts in the range of 10-1000 mg/Kg body weight (BW), were screened out leaving 29 articles. After screening the remaining 11 articles were chosen on the basis of their similarity and design. These articles were selected for establishing a model on which to develop a uniform formula for extract dosage calculation.

**Theoretical design**

The formula was designed using mathematical models, while incorporating multiple variables. Variables included, desired extract dosage, weight of mice, the number of mice and number of days for which the feed must be prepared. The outcome of this formula was the evaluation of the correct amount of extract required to be fed to an animal test group during a feeding trial. The formula incorporated the requirements that each mouse of the group gets an equal amount of the extract to ensure the appropriate dosage as per body weight calculation. The formula was further extended then to include the number of days for which the extract was required to be fed.

Finally an algebraic expression was designed on the basis of these parameters.

**Formula design**

The formula was generated using the online, freely available equation design software. The software (LaTeX online editor) was used to develop the equations in this article. The editor is powered by the platform Codecogs ver 3.16. The entries were made separately into the generation platform available on http://www.codecogs.com/latex/eqneditor.php. Each entry generated a separate equation which was then saved in Gif format for display in this article. The entries are given below:

1. Dosage per mouse  \( = \frac{Ew \times Mw}{1000} \)
2. Dosage per test group \( = \frac{Ew \times Mw \times Mn}{1000} \)
3. Dosage per test group for duration experiment \( = \frac{Ew \times Mw \times Mn \times Dd}{1000} \)
4. Plant \( = \frac{Pwc}{Ec} \times \frac{Mw}{Mn} \times \frac{Dd}{1000} \)

The resulting formulae for testing were formulated and each equation had its own specific property. Equation 1 gave dosage per mouse. In this equation, the dosage requirement is placed, for example, if the dosage required to be fed is 2g/KgBW of the mouse then the value of Ew is set at 2. The division by 1000 is known as the weight calculation factor, it is used to convert the per Kilogram body weight requirement to per gram body weight requirement. Equation 2 gives dosage per test group. Here the equation is extended to give the number of mice in the test group. Usually the test group consists of 10 mice, however depending on the experiment; the number of animals used per group may vary. It is important to note however that the authors advise that the population size per group (n) is >3, in order to allow for significant results. Equation 3 gives dosage per group for the duration of experiment, as the total days required for the experiment are shown here. Finally equation 4 shows the plant weight per test group. It is important to note that equation 4 gives the overall plant weight used during the entire stretch of the experiment as entered in the 3rd equation. The value Pwc/Ec remains consistent as it is dependent on the extent of extraction. Care should be taken to ensure that the units of weight used for both values are similar. Figure 1, 2, 3, 4.

Hence, if the dosage of extract you wish to use in your experiment is 1 g/KgBW, then Ew is 1, Mw is taken as 22 (assuming the average weight of the mouse in question is 22g). This will give a Dosage per mouse of 0.022g,
Equation 1: \[ \text{Dosage per mouse} = \frac{Ew \times Mw}{1000} \]

Figure 1. Equation 1 is represented here. Shown are: extract weight in grams (Ew), mouse weight in grams (Mw).

Equation 2: \[ \text{Dosage per test group} = \frac{Ew \times Mw \times Mn}{1000} \]

Figure 2. Equation 2 is represented here. Shown is: Number of mice (Mn).

Equation 3: \[ \text{Dosage per test group (Duration of Experiment)} = \frac{Ew \times Mw \times Mn \times Dd}{1000} \]

Figure 3. Equation 3 is represented here. Shown is: Duration in days (Dd).

Equation 4: \[ \text{Plant per test group} = \frac{Pwc}{Ec} \times \frac{Ew \times Mw \times Mn}{1000} \]

Figure 4. Equation 4 is depicted here (exDFF). Shown is: Plant weight content (Pwc) and extract content (Ec).

meaning if 0.022g of extract are fed to a mouse weighing 22g then the dosage would be equivalent to 1 g/KgBW of mouse. This value can be multiplied by the number of mice in a group, assuming it is 10, then that would give a value of 0.22 g. This means that 0.22g of extract is required for 10 mice at a dosage of 1 g/KgBW. Later in the next equation, the number of days are equated, assuming the experiment in question lasts 21 days (3 weeks) then the value resulting will be 4.26g, this shows that 4.26g are required for the duration of the experiment lasting 21 days, for 10 mice. In the final equation it is shown, that a ratio between the mass in grams of raw plant used for extraction and the mass in grams of extract obtained from that raw mass, may be also equated by simple division. This ratio when multiplied by equation 3 allows us to evaluate the equivalent mass of raw plant used as an extract. For example if from 20g of extract we obtain 1.5g of extract the ratio will be 13.33, hence the value for equation 4 is seen as 56.8g. Hence this means that when 4.26g of extract were used for the entire group for the duration of the experiment, it was equivalent to feeding 56.8g of the raw plant, having a dosage of 1 g/KgBW of extract per mouse per day.

DISCUSSION

Many scientists and ethno-botanists use simple arithmetic to evaluate the dosage required to feed their animals during extract feeding. Hence it has been a considerable issue as to the nature of the calculations used, as well as the reliability of the formulations. Extracts have been administered in studies involving *Cinnamomum verum* and its anti-diabetic role Kannappan et al. (2006). The same plant has also been studied in its anti-arthritic role, in Rheumatoid Arthritis (RA) Vetel et al. (2013). The use of such a plant has been highlighted by the authors due to its effective anti-oxidant ability, hence a significant remover of oxidative free radicals, which contribute to the progression of autoimmune disorders, particularly RA Dhuley et al. (1999); Mathew et al. (2006). The antioxidant role of numerous other plants of significance like *Acacia nilotica* have been studied as well Singh et al. (2009). Similarly the role of Vitamin E has been seen to be significant as it is a potent anti-oxidant, its role has been evaluated in broiler birds; another example of why a feed-dosage formula is required Sandhu et al. (2012); Sandhu et al. (2013). The role of
plant extracts being fed to animals with Alzheimer's disease have also been studied, particularly the role of plant extracts in alleviating oxidative changes, brought about by pathophysiological changes discussed by us previously Qadir et al. (2014); Perry et al. (1999). The anti-diabetic effect of many plant extracts has also come under considerable scrutiny over the past few decades. Many researchers have used many plant extracts in order to evaluate the anti-hyperglycemic indices observed in diabetic patients Raza et al. (2000); Ahmed et al. (2001). There is one such example Artemisia herba alba a plant whose extract has been under considerable scrutiny for its anti diabetic role Al-Shamaony et al. (1994). Hence it has been shown that, numerous scientific groups around the world are using animal models, particularly mice in order to test their efficacy in various models for the disease.

**CONCLUSION**

With the advent of better purification techniques and more precise scientific experimental methodology, ethnobiology is a dying field some might argue. However, with the understanding that there is an increased carcinogenic and mutagenic propensity in synthetic treatments, ethno-pharmaceuticals have once again emerged as a new frontier for safer drugs. Determining the correct milieu of plants to be used as medicine has confounded scientists and practitioners alike. Many still oppose the traditional method of treatment to the newer, more ‘efficacious’ methods, but millions of years of evolution have proven time and time again, that plant life has indeed the potential and ability to perform what we only hope to achieve in the field of medicine. This article, hopes to promote the scientific discovery of new bioactive compounds extracted from plants. The formula proposed is a stepping stone in evaluating the correct amount of extract being used, hence a saving time and energy during ethnobiological research. The authors hope that this research will contribute towards a better understanding of ethnobiology.

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**REFERENCES**


