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Research Article

Comparison of Weibull and Probit Analysis in Toxicity Testing of *Hunteria umbellata* K Schum (Apocynaceae) Extract in Mice

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Abstract

Purpose; *Hunteria umbellata has been found to have therapeutic potentials in the treatments of diseases such as yaws, peptic ulcers, diabetes, piles and infertility in Nigeria; hence, the statistical analysis on the determination of acute toxicity of Hunteria umbellata was carried out in mice.*
Methods; *Data on the acute toxicity studies of the seed extract of Hunteria umbellata administered via the intraperitoneal route was analyzed using the two-parameter Weibull model.*
Results; *The median lethal dose (LD_{50}) was 1.61 g/kg of body weight. This result falls in the neighbourhood of the median lethal dose earlier obtained in previous reports.*
Conclusion; *The results show that Hunteria umbellata may be slightly toxic when administered intraperitoneally.*

Keywords*: Hunteria umbellata, Weibull model, Acute toxicity, Median lethal dose (LD_{50}).*

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INTRODUCTION

Many plants have been used as substitutes to orthodox medicines in Africa due to the ease of obtaining them in bushes and forests. These herbal medicines may be sources of substances with better therapeutic potentials than some currently used orthodox medicines [1]. The environment we live in is filled with abundant resources and chemicals endowed to humans by nature for beneficial uses. To explore the benefits of these resources, there is need to make contact with them via any route of exposure depending on the physical and chemical properties of the substance(s) of interest. This could be achieved through inhalation, skin absorption, ingestion or injection.

Hunteria umbellata K. Schum is a small tree of about 15–22 m in height with a dense evergreen crown [2] of great medicinal benefits and is found in West and Central Africa. In Nigeria, it is found in the rain forest zone of the southern part of the country with the local names, osu (Edo), erin (Yoruba) and nkpokiri (Ibo) [3]. The plant is also used for the treatment of yaws, peptic ulcers, diabetes, piles, dysmenorohea, fevers and infertility [4,5,6,7] and inflammation [8,9]. It has been used in the treatment of various ailments in Nigeria and Ghana, especially the leaves, roots and bark [10]. The statistical study of the toxicity of this plant is necessary for gaining knowledge of its toxic effect in relation to its consumption by humans.
This present study was undertaken to statistically determine the acute toxicity of *Hunteria umbellata* extract administered to mice through intraperitoneal route with the aid of Weibull model and compare with probit analysis.

Various mathematical models have been used in the analyses of dose-response relationships to assess the toxic effects of chemical substances. These models range from very simple models to extremely complicated models for which the eventual functional forms cannot be easily expressed as single equations. Specifically, these models are (i) tolerance distribution models: log-probit, probit, Weibull, Mantel-Bryan models [11], (ii) mechanistic models: Hit and multistage models [12], (iii) time-to-tumor models: Lognormal, Weibull, Hatley – Sulken and multistage models [12,13], (iv) physiologically-based pharmacokinetic (PBPK) models [14,15], and (v) biologically-Based Models: Moolgavkar-Venzon-Kundson (MVK) model [16] and Ellwein and Cohen model [17].

To determine the toxic effect of *Hunteria Umbellata* plant, the median lethal dose (LD_{50}) of the two-parameter Weibull model is employed. The Weibull model as a tolerance-distribution model has been used extensively to predict time-to-failure of electrical and mechanical systems and it is more widely applied to dose-response relationships. It is capable of representing threshold and concave curves and sensitive to the shape of the dose-response curve. It also has the advantage of being able to incorporate a time-to-tumor function [13]

EXPERIMENTAL

Plant material and extraction

The ripe fruits of *Hunteria umbellata* were collected from Egor Local Government Area in Benin City, Nigeria. It was first identified by Professor Macdonald Idu of Department of Botany, Faculty of Life Sciences, University of Benin, Benin City, Nigeria and later authenticated by Forest Research Institute of Nigeria, Ibadan, where a sample with number FHI107618 was deposited.

The fresh ripe fruits of *Hunteria umbellata* were opened and the pulp removed. The seeds were squeezed out of the pulp. The pulp was dried in the sun for a week and turned to powder with the aid of a grinder. The powdered material (400 g) was boiled with 1,500 ml of distilled water for 30 min to obtain the aqueous extract. The extract was filtered, concentrated under pressure in a

rotar vapor at 68 ^{o}C and dried in an oven set at 40 ^{o}C for 48 h (yield: 21 %). The dried aqueous extract was preserved in clean glass containers at 4 ^{o}C in a refrigerator until use.

Acute toxicity study using probit analysis

Overnight-fasted Swiss albino mice (17-23 g) of either sex obtained from the Laboratory Animal Centre, College of Medicine, University of Lagos, Nigeria were used for the study. The animals were divided into five groups of five animals each. Groups A to D received 1.4, 1.6, 1.8 and 2.0 g/kg of the extract, respectively, while group E received distilled water intraperitoneally. The number of deaths that occurred in each group was determined, and using probit analysis, the LD_{50} was determined by hand calculation.

Method of analysis of acute toxicity using the Weibull model

Suppose X is a response data with data points x, x_2, …, x_n, then the two parameter Weibull distribution is defined as in Eq 1

$$f(x) = \left(\frac{\alpha}{\beta}\right)\left(\frac{x}{\beta}\right)^{\alpha-1} \exp -\left(\frac{x}{\beta}\right)^{\alpha} \ , \ \alpha\ \beta > 0$$

where $x = \log_e$ (dose) and dose is the total amount of a substance administered or taken up by test subject(s). α *and* β are shape and scale parameters respectively.

In estimating the shape and scale parameters of the Weibull model given as in Eq 2.

$$p(d) = 1 - \exp\left(-\beta d^{\alpha}\right)$$

It is convenient to estimate them from Eq 1. Thus, we employed the maximum likelihood estimation (MLE) method for β and the least square estimation for α respectively.

The likelihood function of (1) with respect to β is

$$L(\beta) = \prod_{i=1}^{n}\left[\left(\frac{\alpha}{\beta}\right)\left(\frac{x_i}{\beta}\right)^{\alpha-1} \exp -\left(\frac{x_i}{\beta}\right)^{\alpha}\right]$$

$$= \left(\frac{\alpha}{\beta}\right)^{n}\left[\sum_{i=1}^{n}\left(\frac{x_i}{\beta}\right)^{\alpha-1} \exp -\left(\frac{x_i}{\beta}\right)^{\alpha}\right]$$

The log-likelihood of (3) is

$$\Delta = \log_e L(\beta) = n\log_e \alpha - n\log_e \beta + \sum_{i=1}^{n}\left[\left(\frac{x_i}{\beta}\right)^{\alpha-1} \exp -\left(\frac{x_i}{\beta}\right)^{\alpha}\right]$$

Taking partial derivatives of Eq 4 with respect to α *and* β and equating the resulting derivatives to zero, we obtain and

$$\hat{\beta} = \left[\frac{1}{n}\sum_{i=1}^{n} x_i^{\hat{\alpha}}\right]^{1/\hat{\alpha}}$$

$$\hat{\alpha} = \frac{\log_e\left[\frac{1}{n}\sum_{i=1}^{n} x_i^{\hat{\alpha}}\right] - 1}{\left[\frac{1}{n}\sum_{i=1}^{n} x_i - \sum_{i=1}^{n}(\log_e x_i) - n\log_e\left[\frac{1}{n}\sum_{i=1}^{n} x_i^{\hat{\alpha}}\right]^{1/\hat{\alpha}}\right.}$$

From Eq 6, the estimate of α would be difficult to obtain. Hence, the necessity for estimation of α via the least squares estimation (LSE) method. This is done by the consideration of the cdf of the two-parameter Weibull distribution.

The cdf of the two-parameter Weibull distribution is given as:

$$F(x) = 1 - \exp - \left(x\beta^{-1}\right)^{\alpha} \ , \ \alpha, \beta \geq 0$$

where $x = \log_e (dose)$

Taking natural logarithm of Eq 7 gives Eq 8.

$$\log_e\left\{-\log_e\left[1 - F(x)\right]\right\} = -\alpha\log_e \beta + \alpha \log_e x$$

This gives a linear equation of the form $y = a + bx$

From Eq 8, the estimate of α is

$$\hat{\alpha} = \frac{\sum_{i=1}^{n}(\log_e x_i)(\log_e\{-\log_e[-F(x_i)]\}) + \frac{1}{n}\left[\sum_{i=1}^{n}(\log_e x_i)\sum_{i=1}^{n}\log_e\{-(\log_e[1-F(x_i)])\}\right]}{\sum_{i=1}^{n}(\log_e x_i)^2 - \frac{1}{n}\left(\sum_{i=1}^{n}\log_e x_i\right)^2} \quad (9)$$

where $F(x_i)$ is estimated as $\frac{rank(x_i)}{n+1}$.

Equations 5 and 9 give the explicit mathematical formulae for the values of the estimates of α and β [18] from which the LD_{50} of the Weibull model is calculated.

Also, the median lethal dose for the Weibull model is given as in Eq 10.

$$\hat{x}_{50} = \hat{\beta}(\log_e 2)^{1/\hat{\alpha}} \quad (10)$$

For the purpose of comparison, Relative Error is employed and defined as:

$$Err_{\mathrm{Re}l.} = \left|\frac{LD_{50}(weibull) - LD_{50}(probit)}{LD_{50}(probit)}\right|,$$

where $ER_{\mathrm{Re}l}$ lies bwteeen 0 and 1.

For the purpose of the study, we set a benchmark at 0.5 and made use of the following criteria for comparison:

Criterion I: If $Err_{Rel.} < 0.5$, the Weibull model should be used to obtain the acute toxicity ,Criterion II: If $Err_{Rel.} = 0.5$, any of Weibull or probit model can be used,
Criterion III: If $0.5 < Err_{Rel.} < 1$, the probit model should be used.

RESULTS

In the determination of LD_{50}, Table 1 shows the various doses used in the acute toxicity studies and their corresponding log doses, mortality ratio of the animals and their corresponding probit values (*n* = 5 per group).

It was observed that the lethal dose at 50 % (LD_{50}) gave 1.66 g/kg (1660 mg/kg) using probit analysis. The estimates of shape and scale parameters of Weibull model from (5) and (9) are obtained as

$$\hat{\alpha} = 2.6946 \quad and \quad \hat{\beta} = 0.5491$$

Also,

$$\hat{x}_{50} = \log_e (\hat{LD}_{50}) = 0.4793$$

It follows that

$$\hat{LD}_{50} = 1.6149 g/kg\,(1614.9 mg/kg)$$

Also, from Eq (11), $Err_{Rel.} = 0.02717$

Table 1: Acute toxicity data for *H. umbellata* seed extract in mice

Dose (g/kg)	Log dose	Mortality ratio	% Mortality	Probit
1.4	0.146	0/5	0	3.040
1.6	0.204	3/5	60	5.253
1.8	0.255	4/5	80	5.841
2.0	0.301	5/5	100	6.960

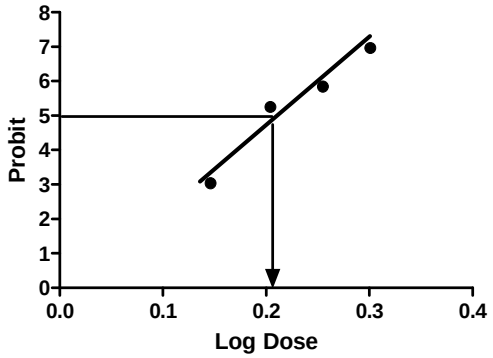

Fig 1: Intraperitoneal acute toxicity of *H. umbellata* seed extract indicating the line of regression. Median lethal dose (LD_{50}) using probit analysis is 1.66 g/kg.

DISCUSSION

Toxic effects in the biological system are not produced by chemical agents unless that chemical agent or its metabolic breakdown (biotransformation) reaches appropriate site in the body at a concentration and a length of time sufficient enough to produce a toxic manifestation. Two major factors that influence toxicity as it relates to exposure situation for a specific chemical are the routes of exposure, duration and frequency of exposure. Toxic agents generally produce the greatest effect and rapid response when

given intravenously. An approximately descending order of effectiveness for other routes would be inhalation, intraperitoneal, subcutaneous, intramuscular, intradermal, oral and dermal. Earlier reports have shown that aqueous fruit pulp extract of *Hunteria umbellata* is not toxic orally [9] hence the need to further determine its acute toxicity profile via another route of administration. Acute toxicity study using the Weibull model gave a median lethal dose of 1.6149 g/kg as compared to the probit analysis median lethal dose of 1.66 g/kg. This indicates that LD_{50} obtained by the weibull model was comparable to that of conventional methods like probit analysis. The results obtained from these two models indicate that *Hunteria umbellata* plant is slightly toxic [19]. In addition, the American Society for Testing and Materials [20], stated that any chemical substance with LD_{50} value less than 2 g/kg but greater than 1 g/kg could be considered to be slightly toxic. The result obtained in the determination of the acute toxicity of *Hunteria umbellata* suggests it is slightly toxic on acute exposure intraperitoneally.

CONCLUSION

The Weibull LD_{50} value for *Hunteria umbellata* was 1.6149 g/kg with relative error of 0.02717. Since the relative error lies in Criterion I, it implies that the Weibull model gives a better result in obtaining LD_{50} than probit model in this study.

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