Assessment of Oral Acute Toxicity (LD_{50}) of Aqueous, Ethanol, and Acetone Crude Extracts of *Plectranthus Neochilus* Leaves on Wistar Albino Rats

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ABSTRACT

The *Plectranthus* genus, comprising more than 300 species distributed through tropical and subtropical areas of Africa, Asia, Oceania and South America, belongs to the Lamiaceae family, which includes some well-established medicinal genus such as mint (Mentha), sage (Salvia) and thyme (Thymus). Toxicology may be defined as the study of harmful, poisonous and adverse effects of drugs and other chemical constituents found in plants, which may increase the chances of mortality or weakness in the general health, physically as well as mentally. Thus, this work was aimed at assessing the oral acute toxicity (LD_{50}) of aqueous, ethanol, and acetone crude extracts of *Plectranthus Neochilus* Leaves and to establish its safety on wistar albino rats (160-200g) as per OECD guidelines. Lorke's method (1983) was used for this study. The results of phase one and phase two of the study showed no acute toxicity or mortality in any of the groups of rats in 24 hours, 72 hours and up to two weeks after single oral administration of 10, 100, 1000, 1500, 2250, 3500 and 5000 mg/kg body weight of the animals. In conclusion, oral administration of the plant extracts did not produce any toxic effect in rats. Oral intake of extracts at dose ≤5000 mg/kg body weight is safe, which implies that the oral LD_{50} is >5000 mg/kg body weight. Hence, the extract can be utilized safely for therapeutic use in pharmaceutical formulations.

Keywords: Acute toxicity, Plectranthus neochilus, OECD, Wistar albino rats, Lethal dose, Lorke's method

INTRODUCTION

During the past few decades, traditional system of medicine has received marvelous attention for in vivo studies [1]. Toxicology is the significant aspect of pharmacology that deals with the adverse effect of bioactive essence or phytocompounds on living organisms prior to the use as drug or chemical in clinical use [2]. Several studies are concentrated on toxicity analysis so as to determine the safeness of medicinal plants and their products. Toxicity analysis is essential, as some herbs consumed might have some toxic effects and many reports have been published for toxicity caused due to long term consumption of herbs. The occurrence of toxicity mechanism could differ depending on the cell membrane and chemical properties of the toxicants in human beings. It might happen within the cell membrane or on the cell surface or tissue underneath as well as at the extracellular matrix. According to the Organization for Economic Cooperation and Development (OECD) guidelines, in order to ascertain the protection and effectiveness of a new drug, toxicological studies are extremely significant in animals like mice, rat, guinea pig, dog, rabbit, monkey etc. Toxicological studies aid to extend decision whether a new drug must be adopted for clinical use or not.

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OECD guidelines such as 401, 423 and 425 do not permit the use of drug clinically without its clinical trial as well as toxicity studies [3]. Depending on the period of drug exposure to animals, toxicological determination could be three types such as acute, sub-acute and chronic toxicological studies. The acute toxicity test in which a single dose is used in each animal on one occasion only for the determination of gross behavior and also LD_{50} or median lethal dose. The chronic tests in which two species, one rodent and one non rodent are dosed daily for complete six months. The sub-acute tests wherein animals (typically rats and dogs) are dosed daily, beginning at around expected therapeutic level and increasing stepwise every two to three days until toxic symptoms are observed [4]. Acute toxicity is the ability of a chemical to cause ill effect "relatively soon" after one oral administration or a 4 – hour exposure of a chemical in air [5]. "Relatively soon," is usually defined as a period of minutes, hours (24) or days (up to about 2 weeks) but rarely longer [5]. LD stands for "Lethal dose." LD_{50} is the amount of material, given all at once, which causes the death of 50% of a group of test animals. The LD_{50} is one way to measure the short – term poisoning potential (acute toxicity) [5].

The *Plectranthus* genus, comprising more than 300 species distributed through tropical and subtropical areas of Africa, Asia, Oceania and South America, belongs to the Lamiaceae family, which includes some well-established medicinal genus such as mint (Mentha), sage (Salvia) and thyme (Thymus). The *Plectranthus* species are commonly used as medicinal plants targeting infectious, dermatologic and gastrointestinal pathologies [6]. A number of work has been done on its dermatological, gastrointestinal, flavoring, antiseptic, repellent, dental effects, carcinogenic effect of its oil, but such information is dearth on its toxicity especially this species- *Plectranthus neochilus* (Schltr.). In order to assess the toxic nature of a bioactive compounds present in the plant extract, acute oral toxicity is the first step to be carried out [7].

MATERIALS AND METHODS

The plant *Plectranthus neochilus* was collected in the month of September, 2019 from No. 1 Museum Street, Jos North, (Coordinates, Latitude 9°54'49.4" N, Longitude 8°53'11.6" E) Jos, Plateau State Nigeria. The authenticity of the plant was confirmed from the deposited specimen at the Herbarium Unit of the Department of Plant Science, University of Jos with Voucher number JUHN21000343.

- **Preparation of the Plant Sample and Extracts**

The fresh leaves of *Plectranthus neochilus* were washed under running tap water, shade dried at room temperature at about 20 to 25 degrees Celsius for 2 weeks and then pulverized to a fine sample by an electric blender and transferred into airtight containers with proper labeling for use.

260g of the plant powder was weighed into 500ml conical flasks and was soaked in ethanol (100%). This was left to stand overnight(s) (72hrs) and shake for 3hrs on a mechanical shaker. The content was filtered using a non-absorbent cotton wool on a Buchner funnel/flask using a vacuum pump. The residue was subjected to several parts of rinsing and filtration with fresh solvent to attain some level of exhaustive maceration (extraction). The collected filtrate was evaporated to dryness using a rotary evaporator and a drying cabinet at a controlled temperature of 60^{o}C. Same procedure was carried out for 260g of the pulverized sample soaked in acetone (100%), the filtrate was concentrated using a rotary evaporator and a drying cabinet at a controlled temperature of 50^{o}C. In like manner, 200g of the powder was soaked in water and exhaustive extraction was carried out, the filtrate was concentrated using a rotary evaporator and a drying cabinet at a controlled temperature of 60^{o}C. The percentage yield of the extracts was determined.

- **Experiment on Animal model**

After getting the ethical clearance, forty-eight (48) white albino rats (male wistar strain) weighing about 160-200g body weight were purchased from the animal house of the University of Jos, Nigeria. Before starting

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the experiment the rats were acclimatized for 7 days. They were maintained at room temperature with 12h/12h light and dark cycle kept in polypropylene cages and were allowed free access to standard pellet feed (purchased from Grand Cereals and Oil Mills Ltd, Bukuru, Jos, Nigeria) and water *ad libitum.* The rats were handled in accordance with the guidelines for the care and use of laboratory animals [8].

- **Experimental Design for Acute Toxicity (LD_{50}) Study of the Extracts.**

The acute toxicity study was conducted in accordance with Lorke's method [9] (1983). The extracts were administered orally using sterile orogastric tubes. The study was conducted in two phases using a total of forty-eight (48) male rats. In the first phase, nine rats were divided into 3 groups of 3 rats each. Groups 1, 2 and 3 animals were given 10, 100 and 1000 mg/kg body weight of the extracts, respectively, to possibly establish the range of doses producing any toxic effect. Each rat was given a single dose after at least 7 days of adaptation. The number of deaths in each group was recorded after 24-hours. In addition, a fourth group of three rats was set up as control group and animals in the group were not given the extract(s). In the second phase, four groups of one rat each were administered (1500, 2250, 3500 and 5000 mg/kg body weight) doses of the extracts (one rat per dose) to further determine the correct LD_{50} value. The extracts were mixed with 2% suspension of tween-80 before administration. The same procedure was carried out on each of the three extracts.

Then the LD_{50} is calculated by the formula

$$LD50 = \sqrt{D0 * D100}$$

D0 = Highest dose that gave no mortality

D100 = Lowest dose that produce mortality

RESULTS AND DISCUSSION

Table 1 shows the result of the phytochemical screening of the crude extracts of. *Plectranthus neochilus S.* The result revealed the extraction potential of the solvents (ethanol, acetone, aqueous) and the degree to which the phytochemical constituents are present.

Table 1. Phytochemical Screening Of Aqueous, Ethanol, And Acetone Extract Of *Plectranthus Neochilus S.*

Constituents	Ethanol	Acetone	Aqueous
Alkaloids	++	–	++
Saponins	+	–	++
Tannins	++	–	+++
Flavonoids	+++	+	+++
Carbohydrates	+++	+	++
Steroids	++	++	+
Terpenes	++	+++	+
Anthraquinones	–	–	–
Cardiac glycosides	++	+++	+

KEY

+++: highly present

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++: moderately present

+: Low,

– : absent

Ref [10] Percentage (*%) yield = (wt of extract)/(wt of crude powder) x 100*

% yield of EtoH extract = 34/260 x 100 = 13.1%

% yield of Acetone extract = 23.6/260 x 100 = 9.1%

% yield of H_20 extract = 56.6/200 x 100=28.3%

The phase I and phase II of the oral acute effect of the extracts are presented in table 2. From the result of acute toxicity study of aqueous, ethanol, and acetone extracts, no mortality was recorded at various doses and in the two phases of the experimental groups in 24hours, 72hours and up to two weeks even after oral administration of 5000mg/kg of each extract(s). According to toxicity classes [11] (2005), any compound with oral LD_{50} (rat) of 5000mg/kg or more should be considered as practically harmless.

Table 2. Mortality Recorded In Oral Lethal Dose (Ld_{50}) Determination Of The Extracts.

Experiment	Dose (mg/kg bodyweight)	Aqueous Extract	Ethanol Extract	Acetone Extract
Phase I	10	0/3	0/3	0/3
	100	0/3	0/3	0/3
	1000	0/3	0/3	0/3
Control	0	0/3	0/3	0/3
Phase II	1500	0/1	0/1	0/1
	2250	0/1	0/1	0/1
	3500	0/1	0/1	0/1
	5000	0/1	0/1	0/1

KEY

0 = Number of Death (After 24hrs, 72hrs and 2 weeks)

3 = Number of *Wistar* albino rats used in the experiment

1 = Number of *Wistar* albino rats used in the experiment

CONCLUSION

The non-toxic nature of the extracts of *plectranthus neochilus* leaves was confirmed by acute oral toxicity test conducted as per the OECD guidelines. The normal behavior of animals during the observation of seven days with no mortality suggests the safety and harmless nature of the extract(s) even up to 5000 mg/kg body weight of animals. Oral intake of extract(s) at dose less than or equal to 5000 mg/kg body weight is safe. Therefore, LD50 of the extract(s) may be considered to be greater than 5000 mg/kg.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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