TOXICITY STUDY OF DESEEDED OIL PALM FRUIT HEAD (DOPFH) FRACTION B IN ALBINO MICE

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ABSTRACT

Deseeded oil palm fruit head (DOPFH) is commonly used to prepare food delicacies native to the Southeast part of Nigeria. Three fractions: DOPFH-A, B and C were derived from the filtrate of DFOP ash by fractional crystallization. DOPFH-B is an alkaline substance with a pH of 10.9. To determine the lethal dose (LD_{50}) of DOPFH-B in mice, Lorke's method was compared with the up-and-down procedure (UDP). The tests were carried out in male and female mice which involved subcutaneous and oral administration. In Lorke's method, female and male mice were divided into three groups (n = 3) for subcutaneous and oral administration. Varying doses; 10, 100 and 1000 mg/kg were given. In the second phase, two groups (n = 3), were administered 2000 and 5000 mg/kg subcutaneously and orally. For UDP, 5000 mg/kg was administered subcutaneously (SC) to each male and female mouse in the limit test. In the main test, 7 and 12 male mice; 7 and 8 female mice were treated subcutaneously and orally with UDP doses of 550, 1750, 2000 and 5000 mg/kg. Using Lorke's method, LD_{50} of 3250 mg/kg was obtained for both female and male mice by both routes. UDP estimated LD_{50} of 2100 mg/kg for females and LD_{50} is greater than 2100 mg/kg for male mice administered subcutaneously. LD_{50} for the orally administered female and male mice was given 5000 mg/kg. UDP and Lorke's method did not highlight differential sensitivities of male and female mice to DOPFH-B.

Keywords: Ashed deseeded oil palm fruit head, Ash toxicity, Lorke's method, Up-and-down procedure, Albino mice

INTRODUCTION

The oil palm tree - *Elaeis guineensis* Jacq (Arecales: Arecaceae) is a tropical plant originating from West and Southwest Africa and naturalized in Madagascar, Sri Lanka, Malaysia, Indonesia, Central America, Cambodia, West Indies, and several islands in the Indian and Pacific Oceans (Udoetok, 2012). It is an essential and valuable commercial plant that is central to the lives of many West African traditional societies including Nigeria. The different parts of African oil palm are used in several ways including their use as a medicinal plant. The leaves are used for wound healing (Sasidharan *et al.,* 2010; 2011). It also serves as a food source; the sap from the tree

stem is drunk as palm wine. The mesocarp of the de-seeded palm fruit is a major source of edible palm oil. The palm kernel is likewise edible and serves as a source of oil for human and industrial use, including cosmetic production and the resulting palm kernel cake (PKC) is used in the formulation of animal feeds (Keng *et al.*, 2009; Martinez *et al.*, 2017).

The by-products left after oil production from the mill consist mostly of palm shells and empty palm fruit heads, which serve as biomass for renewable and sustainable energy. The ash by-product does not go to waste; it is used locally as a scouring powder and crop fertilizer (Israel and Akpan, 2016). It is also used in the production of African black soap used locally to treat acne and eczema (Ogunbiyi and Enechukwu, 2012; Ogunsuyi and Akinnawo, 2012). The de-seeded fruit head of oil palm is usually incinerated, and the ash filtrate is used as an ingredient for common food dishes such as 'Abacha' (made from cassava). The chemical composition of incinerated ash of deseeded oil palm fruit head includes chloride, sulphate, nitrate, phosphate, zinc, calcium, potassium, magnesium and metals with a pH of 10.9 (Udoetok, 2012). Furthermore, the physicochemical properties, anion and cation composition of DOPFH-B have been elucidated (Udeinya *et al.*, 2021).

A case study has reported on the efficacy of the ash filtrate of de-seeded fruit head of oil palm in managing chronic fatigue syndrome (Igwe and Okoloekwe, 2015). Non-crystalline cellulose (NCC) extracted from oil palm empty fruit bunch is a potential bio-based drug carrier owing to its biodegradability and biocompatibility (Foo *et al.*, 2019).

In humans, acid-base balance is crucial to cell homeostasis. Acidosis is observed in numerous inflammatory processes. Ash filtrate of de-seeded fruit head of oil palm has a pH of 10.9 and can potentially serve as an alkanalizer. No study has established the effect of DOPFH-B in a biological system concerning pH manipulation. The promising potential of DOPFH-B as an alkanalizer informed this toxicological study of the black chemical fraction. The study aimed to assess the LD_{50} of orally and subcutaneously administered DOPFH-B in mice, comparing two different methods.

MATERIALS AND METHODS

Ethics: The Research Ethics Committee of the College of Medicine, University of Nigeria approved this study. The approval number was 065/03/2019.

Animals: Thirty-four (four to six-week-old male and female mice with a mean weight of 30.2 ± 3.4 grams) were selected and used for the study. They were purchased from the Animal House, Department of Pharmacology and Therapeutics, College of Medicine, University of Nigeria Enugu Campus. They were bred and cared for in the study environment to ensure that all the animals formed a homogenous population. The mice were caged in stainless steel metabolic cages housed in a cross-ventilated room and kept under standard environmental conditions of a 12/12-hour light and dark cycle, fed with standard commercial feed (Vital Feed grower mash, 15.22 ± 1.65 g/100 g crude protein, and 2478.40 kcal/kg metabolizable energy, Grand Cereals Limited, Jos, Plateau State, Nigeria), and water *ad libitum* and were allowed to acclimatize for seven days.

Chemical: DOPFH-B is patented and was provided by Professor I. J. Udeinya.

Methods: Lorke's method (Lorke, 1983) and the up-and-down procedure (UDP) described by the OECD Guideline for Testing of Chemicals – OECD 425 (OECD, 2001) were used in this study.

Lorke's method: This method has two phases which are phases 1 and 2, respectively. Phase 1, required nine male and female mice, which were divided into three groups of three mice each. Each group of mice were orally administered different doses (10, 100 and 1000 mg/kg) of DOPFH-B. The mice were regularly observed for 24 hours while monitoring their behaviours and mortality. Phase 2, involved the use of two mice, which were orally administered higher doses (2000 and 5000 mg/kg) of DOPFH-B and observed for 24 hours for behavioural changes and mortality. LD_{50} was calculated using the formula: $LD_{50} = \sqrt{(D_0 \times D_{100})}$, where D_0 = Highest

dose that gave no mortality and D_{100} = Lowest dose that produced mortality.

Up-and-down procedure: This was performed following the method of Bruce (1985) and the Organization for Economic Co-operation and Development (OECD) Test Guidelines 425 (OECD, 2001). The OECD 425 gave the default dosing required to implement the UDP. The program recommends a dosing range of 2000, 550, 175, 55, 17.5, 5.5, 1.75 mg/kg. The single dose is given one at a time per animal of single-sex progressively. If the animal survives the first dose. The next dose to be administered to the next animal is increased by a factor of 3.2 times the original dose. The program indicates when to stop dosing animals, estimates the LD_{50} and confidence interval for the LD_{50} (OECD, 2001)

Male and non-pregnant female albino mice, aged 6 – 8 weeks, were randomly selected. These animals were kept under standard conditions for 5 days. The mice were kept without food for 3 – 4 hours, before drug dosing, and had access to water *ad libitum*. Dose administration to a single mouse was according to the body weight. The limit test for DOPFH-B was performed with 5000 mg/kg p.o. as a single dose.

For the first 30 minutes and the next 4 hours, the mice were closely observed. Food was provided after 1 – 2 hours of DOPFH-B dosing. If the mice died during the limit test, then we proceeded to the main test.

For the main test, additional mice were administered the recommended dose progression in descending order: 2000, 1750, 550, and 100 mg/kg under the same conditions. The same procedure was followed for vehicle treated control group of 6 mice. Both groups were observed closely for any toxic effect of DOPFH-B within the first 6 hours and at regular intervals for a total period of 14 days. The surviving mice were observed to determine the onset of the toxic effects of DOPFH-B.

Limit Test: For both the oral and subcutaneous administration routes, a limit test was performed by administrating 2000 mg/kg to a single female and male mice. Both survived within 6 hours of treatment (short-term outcome). The main test was carried out for both oral and subcutaneous administration routes after the mice survived the limit test.

RESULTS

Lorke's Toxicity of DOPFH-B to Both Female and Male Mice: Using Lorke's method, LD_{50} of 3,250 mg/kg was obtained for both female and male mice by both oral and subcutaneous routes. UDP estimated LD_{50} as 2000 mg/kg for females and >2000 mg/kg for male mice administered subcutaneous DOPFH-B. The LD_{50} for the orally administered female and male mice was 5200 mg/kg.

Up-and-Down Toxicity of DOPFH-B to Both Female and Male Mice

Oral administration: All UDP DOPFH-B dosing regimens were as recommended by OECD 425. For the female mice, dosing was stopped after the 8th mouse was administered. All female mice survived the 550 mg/kg and 1750 mg/kg doses and all survived at the 5000 mg/kg dose. Statistical estimate based on the long-term outcome estimated LD_{50} at 5000 mg/kg (the one dose with partial response). 95% confidence interval was 2011 to 9892 (Table 1).

Similarly, for the male mice, dosing was stopped after the 12th mouse was administered DOPFH-B. All male mice survived the 550 mg/kg and 1750 mg/kg doses while one mouse died at the 5000 mg/kg dose. Statistical estimate based on the long-term outcome estimated LD_{50} at 5000 mg/kg (the one dose with partial response). 95% confidence interval was 2426 to 6660 (Table 2).

Subcutaneous administration: All UDP DOPFH-B dosing regimens were as recommended by OECD 425. For the female mice, dosing was stopped after the 7th mouse was administered. Six female mice survived the 100, 500 and 2000 mg/kg doses while one mouse died at the 2000 mg/kg dose on the 12th day of treatment. Statistical analysis, based on the long-term outcome, estimated LD_{50} at 2000 mg/kg (the only dose with a partial response).

Table 1: Mortality and survival among non-pregnant female mice administered orally various doses of DOPFH-B in the up-and-down study

Order of study	Animal Identity	DOPFH-B Dose Administered (mg/kg)	Outcome after treatment with DOPFH-B	
			Within 6 hours of treatment (Short term outcome)	Between 6 hours and 14 days of treatment (Long term outcome)
1st female mice	BF1	550	Survived	Survived
2nd female mice	BF2	1750	Survived	Survived
3rd female mice	BF3	5000	Survived	Survived
4th female mice	BF4	5000	Survived	Survived
5th female mice	BF5	1750	Survived	Survived
6th female mice	BF6	5000	Survived	Survived
7th female mice	BF7	1750	Survived	Survived
8th female mice	BF8	5000	Survived	Survived

Table 2: Mortality and survival among male mice administered various oral doses of DOPFH-B in the up-and-down study

Order of study	Animal Identity	DOPFH-B Dose Administered (mg/kg)	Outcome after treatment with DOPFH-B	
			Within 6 hours of treatment	Between 6 hours and 14 days of treatment
1st male mice	BM1	550	Survived	Survived
2nd male mice	BM2	1750	Survived	Survived
3rd male mice	BM3	5000	Survived	Survived
4th male mice	BM4	5000	Survived	Died
5th male mice	BM5	5000	Survived	Survived
6th male mice	BM6	1750	Survived	Survived
7th male mice	BM7	5000	Survived	Survived
8th male mice	BM8	5000	Survived	Survived
9th male mice	BM9	1750	Survived	Survived
10thmale mice	BM10	5000	Survived	Survived
11th male mice	BM11	1750	Survived	Survived
12th male mice	BM12	5000	Survived	Survived

The 95% confidence interval was 1198 to greater than 20,000 mg/kg (Table 3).

Similarly, for the male mice, dosing was stopped after the 7th mouse was administered DOPFH-B. Six male mice survived the 100, 300, 500 and 2000 mg/kg doses while one mouse died at the 500 mg/kg dose. Statistical estimate based on the long-term outcome estimated LD_{50} to be greater than 2000 mg/kg (Table 4).

Behavioural Patterns of Mice Treated with DOPFH-B: For doses below 5000 mg/kg observed between 30 minutes to 14 days, the male and female mice did not exhibit mortality, salivation, itching, coma, convulsion, or tremors.

The mucous membrane, the colour of urine, faecal consistency, sleep pattern, fur and skin appearance, eyes, respiration, and somatic motor behavioural pattern remained normal for both female and male mice in the various modes of administration.

In male mice mortality occurred before the 14th day with both administration routes. For both male and female mice, hyperextension of the hind limb, and fast respiration were observed before they died.

DISCUSSION

Acute toxicity tests can provide preliminary information on the toxic nature of a chemical or pharmacological agent. Such information can be used to deduce doses for further toxicity testing (Erhirhie *et al.*, 2018; Strickland *et al.*, 2018). Acute toxicity determination in mammals plays an important role in the evaluation of pollutants and drug design. The acute toxicity of a chemical in mammals can be obtained by determining lethality expressed as LD_{50}, from a single dose of the chemical within 24 hours. Mice are one of the animal species used in such studies (Wang *et al.*, 2015).

Table 3: The outcome of varied doses of DOPFH-B administered to female mice subcutaneously following an up-and-down procedure

Order of study	Animal Identity	DOPFH-B Dose Administered (mg/kg)	Outcome after treatment with DOPFH-B	
			Within 6 hours of treatment (Short term outcome)	Between 6 hours and 14 days of treatment (Long term outcome)
1st female mice	BF1	100	Survived	Survived
2nd female mice	BF2	500	Survived	Survived
3rd female mice	BF3	2000	Survived	Died
4th female mice	BF4	500	Survived	Survived
5th female mice	BF5	2000	Survived	Survived
6th female mice	BF6	2000	Survived	Survived
7th female mice	BF7	2000	Survived	Survived

Table 4: The outcome of varied doses of DOPFH-B administered to male mice subcutaneously following an up-and-down procedure

Order of study	Animal Identity	DOPFH-B Dose Administered (mg/kg)	Outcome after treatment with DOPFH-B	
			Within 6 hours of treatment	Between 6 hours and 14 days of treatment
1st male mice	BM1	100	Survived	Survived
2nd male mice	BM2	500	Died	Died
3rd male mice	BM3	300	Survived	Survived
4th male mice	BM4	500	Survived	Survived
5th male mice	BM5	2000	Survived	Survived
6th male mice	BM6	2000	Survived	Survived
7th male mice	BM7	2000	Survived	Survived

Mice are a preferred model for animal studies concerning pH because of their similarity to humans concerning physiology and genomics (Lampreht Tratar *et al.,* 2018).

In this study, DOPFH-B acute oral and subcutaneous LD_{50} of 3250 mg/kg was obtained for both female and male mice. Biochemical substances with LD_{50} range between 500 and 5000 mg/kg body weight are classified as nontoxic while LD_{50} between 5000 and 15000 mg/kg body weight is classified as fairly toxic (Erhirhie *et al.*, 2018). Using this classification method, DOPFH-B appears to be non-toxic. Another study that investigated lethality toxicity for different routes that is oral administration or injections (intramuscular, intraperitoneal, subcutaneous administration) showed that the regression analysis in injection routes was related to the oral route and suggested that this may have occurred due to the same bioavailability of chemical in the animal species. The study also highlighted that the metabolism of pharmacological agents via oral or injection is different because first–pass metabolism occurs in oral routes but not injection (Wang *et al.*, 2015).

Other studies that investigated some pharmacological agents have shown LD_{50} disparities in gender as well as administration via different routes (Domingo, 2003; Fuentes *et al.,* 2014).

The death of mice administered DOPFH-B subcutaneously may have been caused by the error in the route of administration rather than the dose. Being more fragile these mice were nevertheless handled as carefully as possible. A toxicity study of DOPFH fractions A and C demonstrated that subacute doses of the fractions do not appear to have any deleterious effect on haematological parameters, hepatic/renal functions as well as cardiac and splenic histoarchitecture in the experimental animals (Nubila *et al.*, 2020)

Conclusion: This study can provide information for appropriate dose selection of DHFCOP-B for pH studies. The results of this study suggest that a safe therapeutic regime can be derived for further investigation using DOPFH-B. Further studies should explore the potential of DOPFH-B as an alkanalizer to restore body pH to normal in disease conditions characterized by acidosis.

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