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# ORAL ACUTE TOXICITY STUDIES ON *HBF-19*, A NOVEL MULTIHERBAL ANTIMALARIAL FORMULA MADE FROM SELECTED AFRICAN MEDICINAL PLANTS

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## ABSTRACT

The continuous increase in the cost of orthodox drugs, amidst their side effects has led to the sustained search in the direction of alternative medicine, mostly from plant products, for the management of various ailments. In the present study, the oral acute toxicity profile of *HBF-19* (a multi herbal formula made from selected African medicinal plants) was evaluated. The study was done in two phases using albino mice as animal models. The mice (n = 28) were randomly distributed into seven groups (n = 4). In the first phase of the study, mice in groups 2, 3 and 4 were administered with *HBF-19* at the dose levels of 10, 100 and 1000mg/kg body weight in a single oral dose fashion while in the second phase, mice in groups 5, 6 and 7 received 1600, 2900 and 5000mg/kg body weight of *HBF-19*. The mice in group 1 served as control and were kept on distilled water. All animals were monitored for toxicity signs for the first critical 24 hours and up to 72 hours. At the end of 72 hours, animals were sacrificed and blood samples taken for the evaluation of haemoglobin(HB), Packed cell volume(PCV), aspartate aminotransferase(AST), and alanine aminotransferase(ALT). Liver samples removed from the sacrificed mice were subjected to histopathological investigations. Results showed that up to the maximum *HBF-19* dose of 5000mg/kg administered, no mortality was recorded among the animals. No significant difference in body weight was recorded ($p<0.05$) as a result of acute toxicity treatment. For all the parameters investigated, it was observed that values obtained with *HBF-19* treated mice were similar to those recorded with the animals in the control group. Histological features of *HBF-19* treated mice were also in line with that of healthy control mice with well-

preserved hepatic cytoarchitecture. It was therefore concluded that the multi-herbal formula is safe without a likelihood of causing toxicity or fatality if the doses are extrapolated to humans.

**KEYWORDS:** Acute toxicity, *HBF-19,* herbal formula, African Medicinal plants.

## INTRODUCTION

The high cost of standard drugs in addition to the undesirable (side) effects experienced with the use of those drugs have led to increased campaign for alternative medicine. Unfortunately, at most times, these alternative medications are applied without much regards to accurate dosing protocols. Drugs and substance abuse ultimately lead to toxicity and injury to vital organs in the body. Toxicity may be acute, sub-acute or chronic depending on duration of exposure to the agent. Acute toxicity or lethal toxicity refers to the ability of a chemical substance to bring about ill effect "relatively soon" after one administration or a 4 - hour exposure of a chemical in the air (Senin 2006, Onwusonye *et al.,* 2014a). the term "Relatively soon" is used to describe a short period of time, say minutes, hours or days but usually not longer than two weeks (Senin, 2006., Onwusonye *et al*., 2014b). Acute toxicity studies make for the availability of information on the potential of a substance to cause acute toxicity in man, safe doses for use as well as target organ for toxicity. (Colerangle 2017). Information from acute toxicity studies are also helpful in the description of the time course of drug induced clinical observation as well as for the selection of appropriate dose ranges for multiple dose toxicity studies. The amount of a substance that can be expected to cause death in half the total number of a group of animals of a particular specie is referred to as the median lethal dose (LD 50). Substances with LD 50 values are thus less toxic than those with smaller values.

Medicinal plants have been defined by World Health Organization as plants whose one or more organs contain substances that are useful for therapeutic purpose, or which can serve as precursors for the synthesis of useful drugs (WHO, 1977). Man's use of plants in the treatment of diseases has been a long time practice (Sofowora, 2006). This herbal medicine involves both plants with very powerful actions as well as those whose actions are mild (Weiss, 2000).

*HBF-19* is a herbal formula prepared by a team of researchers from the School of Industrial and Applied Sciences, Federal Polytechnic Nekede, Owerri in Nigeria under the sponsorship

of Tertiary Education Trust Fund (Nigeria TETFUND Institution based Research). The product was composed of extracts from various organs of four medicinal plants-*Alstonia scholaris, Annona senegalensis, Pteridum aquilinum* and *Caesalpinia bonducella*. Earlier studies by the team has shown that the herbal formula contains appreciable amounts of phytochemicals (bioactive compounds) linked with strong antimalarial and other medicinal properties as well as other important dietary factors capable of contributing significantly to the nutritional needs of man and livestock. The present study is therefore aimed at evaluating the oral acute toxicity profile and the median lethal dose (LD50) value of the product in experimental mice as a way of determining the dose ranges that would be safest for use as a medicament.

## 2. MATERIALS AND METHODS

### 2.1 *HBF-19* (Multi herbal Formula)

Collection of Plant samples, identification, preparation and formulation of *HBF-19* was as reported elsewhere by our team [Onwusonye *et al*. (in press)]. The four plant samples were collected from different locations in the South Eastern part of Nigeria and identified by Dr. Duru, a Taxonomist in the department of Biological Sciences, Federal University of Technology Owerri.

The fresh stem bark samples of *Alstonia scholaris* were sorted out to rid it of unwanted materials. They were cut into pieces and air-dried under shade for two weeks and subsequently milled to fine powder. The fresh leaves of *Annona senegalensis* were air-dried under shade for ten days after which they were milled to a fine powder. The fresh leaves and young shoots of *Pteridium aquilinun* were dried under shade for ten days after which they were milled to fine powder. Fresh leaves of *Caesalpinia bonducella* were air dried under shade for two weeks and subsequently milled to fine powder. Five Hundred grams of each plant powder was measured out in different breakers and were subsequently mixed together and homogenized in an electric blender to give 2kg of mixed herbal powder.

The homogenized herbal powder (2kg) was dispersed in six litres of 95% methanol and allowed to stand on the bench with intermittent stirring. After 72 hours, the soaked herbal material was filtered using Whatmann filter paper (8µm). The filtrate received was concentrated to dryness in a rotary evaporator at 45$^{o}$C.

The names of plants used in the formulation of *HBF-19* and the parts used are shown in table 1a.

### 2.2 Experimental Animals

For the oral acute toxicity study on the herbal formula (*HBF-19*) alone, twenty-eight (28) Swiss albino mice (weighing between 24 – 29g) were used. The mice were procured from an animal breeder in Ihiagwa, Owerri and left to acclimatize in the laboratory for seven days prior to commencement of study. All protocols for animal handling were according to the National Institute for Health guide for the care and use of laboratory animals (NIH publications No. 8023 revised 1978). The body weight of all the animals were measured at the end of acclimatization (Prior to commencement of study) and at the end of study.

### 2.3 Experimental Design

The method for the oral acute toxicity study was that described by Lorke (1983) with very slight modification. The study was conducted in two phases as shown in table 1b (each phase with overnight fasted animals). In phase one, 16 mice were randomly distributed into four groups (groups 1 – 4), each group containing 4 mice.

The animals in group 1 served as control and were maintained on distilled water while those in groups 2, 3 and 4 were treated with *HBF-19* at respective oral doses of 10mg/kg, 100mg/kg and 1000mg/kg body weight. Each treatment was given as a single dose and the animals were closely monitored for toxicity signs up to a 72 hour period. In the second phase of the study, another set of mice (12 in number) were randomly distributed into 3 groups, 4 mice per group and treated with *HBF-19* at respective oral doses of 1600mg/kg, 2900mg/kg and 5000mg/kg body weight. All the animals were equally monitored closely for another 72hours for toxicity symptoms. After 72 hours all surviving animals were reweighed and subsequently sacrificed. The blood samples were taken in sterile bottles for the evaluation of haemoglobin (Hb) Packed Cell Volume (PCV), aspartate aminotransferase (AST) and alanine aminotransferase. Liver samples carefully removed from the sacrificed mice were fixed in 4% formaldehyde for histological examination.

#### 2.3.1 Evaluation of Haemoglobin (Hb) Concentration

The haemoglobin concentration in the blood samples were estimated by the cyanomeathamoglobin method described by Jain (1986). Some 0.02ml of blood sample was added to 5mls of Drabkins solution in a test tube, mixed thoroughly and allowed to stand.

After 10 minutes, absorbance was taken at 540nm wavelength against a reagent blank (only Drabkin's solution). The absorbance of a known standard containing 14.6g Hb per dL of whole blood was also measured as control alongside the samples. The haemoglobin concentration of the sample was read off from a table prepared from the calibration curve.

### 2.3.2 Measurement of Packed Cell Volume (PCV)

Packed cell volume in the blood samples were measured using capillary tubes with blood (Dacie and Lewis, 1991). Well mixed whole blood sample was introduced into the capillary tube until it was filled. The tube was filled at one end with plastacine and spun at 10,000 revolutions per minute in a micro haematocrit centrifuge. The spun tube was subsequently placed in the scale (reader) and the PCV was estimated using the relationship.

$$\text{PCV (\%)} = \frac{\text{Volume of R.B.C in given volume of blood}}{\text{Total volume of blood}} \times \frac{100}{1}$$

RBC= Red blood cells

### 2.3.3 Measurement of Aspartate aminotransferase (AST) Activity

This was done according to the method described by Reitman and Frankel, 1957. Exactly 0.05ml of sample was added to 0.25ml of buffer in a test tube. 0.05ml of distilled water to which 0.25ml of buffer was added in a test tube was used as a blank. Each tube was mixed and incubated for 30 minutes at $37^0$C. 0.25ml of 2, 4-dinitrophenyl hydrazine and allowed to stand for 20 minutes at room temperature. 2.5ml of sodium hydroxide was then added and the mixture further allowed to stand at room temperature for 5 minutes. The absorbance of the test sample was read against the reagent blank at 546nm in a spectrophotometer. The AST activity was estimated by reference to a standard calibrated curve.

### 2.3.4 Measurement of Alanine aminotransferase (ALT) Activity

This was done according to the method described by Reitman and Frankel, 1957.Exactly 0.05ml of sample was added to 0.25ml of buffer in a test tube. 0.05ml of distilled water to which 0.25ml of buffer was added in a test tube was used as a blank. Each tube was mixed and incubated for 30 minutes at $37^0$C. To this mixture was added 0.25ml of 2, 4-dinitrophenylhydrazine and allowed to stand for 20 minutes at room temperature. 2.5ml of sodium hydroxide was then added and the mixture further allowed to stand at room temperature for 5 minutes. The absorbance of the test sample was read against the reagent blank at 546nm in a spectrophotometer. The ALT activity was estimated by reference to a standard calibrated curve.

### 2.3.5 Histopathological Examinations

Histological investigations were done according to the method described by Akparie (2004). The liver samples were sliced and washed in saline solution. The sliced samples were processed and embedded in paraffin wax. Sections were taken and stained with haematoxylin and eosin. After preparations, samples were viewed and photographed using x400 objective of microscope equipped with digital camera.

**Table 1a: Names of plants used in the formulation of *HBF-19* and the parts used.**

| S/N | Botanical Name | Common Name | Family | Parts used |
|---|---|---|---|---|
| 1 | *Alstonia scholaris* | Blackboard tree | *Apocynaceae* | Stem bark |
| 2 | *Annona senegalensis* | Wild custard apple | *Annonaceae* | Leaves |
| 3 | *Pteridium aquilinum* | Braken fern | *Dennstaedtiaceae* | young shoots and Leaves |
| 4 | *Caesalpinia bonducella* | Fever nut | *Ceasalpiniaceae* | Leaves |

**Table 1b: Experimental Design.**

| Group | Doses of *HBF-19* (mg/kg body weight) | No. of mice | |
|---|---|---|---|
| 1 (Control) | 0 | 4 | |
| 2 | 10 | 4 | |
| 3 | 100 | 4 | Phase 1 |
| 4 | 1000 | 4 | |
| 5 | 1600 | 4 | |
| 6 | 2900 | 4 | Phase 2 |
| 7 | 5000 | 4 | |

### 2.4. Statistical Analysis

Data obtained were subjected to analysis of variance (Steel and Tories, 1980) implemented in SPSS statistics 17.0(SPSS Inc. 2008). The means were separated using Duncan Multiple Range Test at the 0.05 level of significance.

## 3.0 RESULTS

The results of the various tests conducted on the acute toxicity of the herbal formula *HBF-19* are shown in tables 2-6. The results of the Histopathological examinations of the liver sections of the mice are shown in plates 1-4.

**Table 2: Mortality Pattern in Phase 1.**

| Group | Doses of *HBF – 19* (mg/kg body weight) | No. of Mice | No. of deaths |
|---|---|---|---|
| 1 (Control) | 0 | 4 | 0 |
| 2 | 10 | 4 | 0 |
| 3 | 100 | 4 | 0 |
| 4 | 1000 | 4 | 0 |

**Table 3: Mortality Pattern in Phase 2.**

| Group | Doses of *HBF – 19* (mg/kg body weight) | No. of Mice | No. of deaths |
|---|---|---|---|
| 5 | 1600 | 4 | 0 |
| 6 | 2900 | 4 | 0 |
| 7 | 5000 | 4 | 0 |

**Table 4: Effect of Acute Toxicity treatment on body weight of mice.**

| Group | Doses of *HBF – 19* (mg/kg body weight) | Weight before treatment (g) | Weight after treatment (g) |
|---|---|---|---|
| 1 (control) | 0 | $25.3 \pm 0.8^{a}$ | $25.7 \pm 1.0^{a}$ |
| 2 | 10 | $27.5 \pm 0.6^{b}$ | $27.6 \pm 0.9^{b}$ |
| 3 | 100 | $26.0 \pm 0.9^{c}$ | $25.9 \pm 0.8^{c}$ |
| 4 | 1000 | $25.8 \pm 1.0^{d}$ | $25.4 \pm 0.5^{d}$ |
| 5 | 1600 | $28.6 \pm 0.9^{e}$ | $27.5 \pm 0.4^{e}$ |
| 6 | 2900 | $28.0 \pm 0.2^{f}$ | $27.6 \pm 0.7^{f}$ |
| 7 | 5000 | $28.5 \pm 1.0^{g}$ | $26.0 \pm 1.0^{g}$ |

Values represent Mean ± SEM (n = 4)

a – g: Values in the same row with the same superscript do not differ significantly (P > 0.05).

**Table 5: Effect of Acute Toxicity Treatment with *HBF-19* on Haemoglobin (HB) and Packed Cell Volume (PCV) of Mice.**

| Groups | Doses of *HBF-19* (mg/kg body weight) | Hb (g/L) | PCV (%) |
|---|---|---|---|
| 1 | 0 | $94.56 \pm 0.8^{a}$ | $32.0 \pm 0.6^{c}$ |
| 2 | 10 | $93.60 \pm 0.6^{a}$ | $31.2 \pm 1.0^{c}$ |
| 3 | 100 | $93.00 \pm 1.2^{a}$ | $31.0 \pm 1.2^{c}$ |
| 4 | 1000 | $94.05 \pm 0.3^{a}$ | $31.5 \pm 0.9^{c}$ |
| 5 | 1600 | $87.50 \pm 0.7^{b}$ | $30.0 \pm 0.4^{c}$ |
| 6 | 2900 | $85.31 \pm 1.0^{b}$ | $27.4 \pm 1.0^{c}$ |
| 7 | 5000 | $75.89 \pm 1.5^{b}$ | $25.0 \pm 0.5^{d}$ |

Values represent Mean ± SEM (n = 4)

a - d: Values in the same column with different superscripts are significantly different (P < 0.05)

**Table 6: Effect of acute toxicity treatment on Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) activities of albino mice.**

| Groups | Doses of *HBF-19* (mg/kg body weight) | AST activity (i.u/L) | ALT activity (i.u/L) |
|---|---|---|---|
| 1 | 0 | $34.7 \pm 2.8^{a}$ | $24.5 \pm 1.5^{b}$ |
| 2 | 10 | $35.2 \pm 1.9^{a}$ | $25.9 \pm 0.8^{b}$ |
| 3 | 100 | $33.9 \pm 1.6^{a}$ | $25.0 \pm 1.2^{b}$ |
| 4 | 1000 | $35.6 \pm 2.0^{a}$ | $25.1 \pm 0.6^{b}$ |
| 5 | 1600 | 36.2 ± 2.1a | $25.9 \pm 0.9^{b}$ |
| 6 | 2900 | $36.9 \pm 1.9^{a}$ | $26.3 \pm 1.^{b}$ |
| 7 | 5000 | $37.1 \pm 2.8^{a}$ | $26.2 \pm 0.7^{b}$ |

Values represent Mean ± SEM (n = 4)

a, b : values in the same column with the same superscript do not differ significantly (P > 0.05).

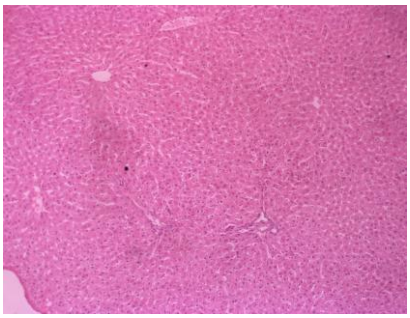

**Plate 1: Photomicrograph of liver of control mice treated with distilled water showing well preserved hepatic cytoarchitecture. (H & E x 400).**

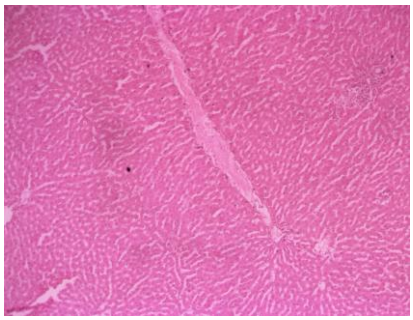

**Plate 2: Photomicrograph of liver of mice treated with *HBF-19* at an acute dose of 1600mg/kg showing showing well preserved hepatic cytoarchitecture (H&Ex400).**

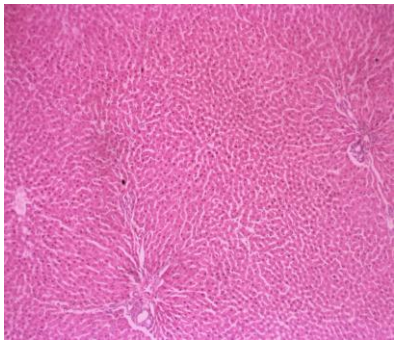

**Plate 3: Photomicrograph of liver of mice treated with *HBF-19* at an acute dose of 2900mg/kg showing showing well preserved hepatic cytoarchitecture (H&Ex400).**

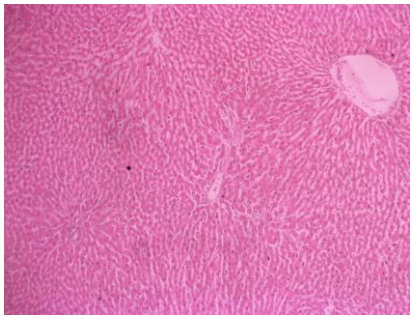

**Plate 4: Photomicrograph of liver of mice treated with *HBF-19* at an acute dose of 5000mg/kg showing well preserved hepatic cytoarchitecture (H&Ex400).**

## 4.0. DISCUSSION

The use of plant products for the management of various disease conditions have remained a sustained practice in many communities especially in various parts of the developing world. The increased use of herbal products in these rural communities requires that experimental screening be done in order to ascertain the safety and efficacy of such herbal products as well as ascertain their active principles (Ogbonna *et al*, 2008). In the present study, no death was recorded among all the mice in both phases of the acute toxicity treatment (tables 2 and 3). This implies that the median lethal dose value of the herbal formula lies above the maximum dose tested (i.e. >5000mg/kg body weight). This observation falls in line with that reported by Mba *et al.* (2020) who worked on extracts from *Napoleonae imperialis.* The herbal formula did not elicit any significant effect on the body weight of the mice, for instance, when the maximum dose was administered (5000mg/kg), body weight of the mice slightly reduced from 28.5 $\pm$ 1.0 to 26.0 $\pm$1.0. The slight change in body weight may be ascribed to slight reduction in feed intake in response to the increased herbal dose. The effect of *HBF-19* on Hb and PCV was slight up to the dose of 1600mg/kg. However, up to the maximum dose, some significant reductions were observed in both parameters (table 5). Up to the maximum

dose of *HBF-19* used, the effect on AST and ALT were not significant ($P < 0.05$). Histopathological examinations showed that up to the highest dose of the formula used, the cytoarchitectural features of the hepatocytes were preserved in line with that of a normal healthy liver. Any substance which is toxic to the liver will normally lead to hepatocellular destruction and necrotic change. The absence of any necrotic change in the liver sections of the herbal treated mice suggests that the formula is safe.

## 5.0 CONCLUSION

The oral median lethal dose (LD 50) value of *HBF-19* in mice was observed to be greater than 5000mg/kg body weight and the effect of the formula on the parameters monitored was moderate. Any substance with LD 50 above 5000mg/kg body weight is considered to be practically harmless (Hodge and Sterner, 2005). It is hereby concluded that the herbal formula is safe and unlikely to cause any fatality in humans.

## Conflict of Interest

The authors hereby declare that no conflict of interest exists pertaining to this work.

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