**Herbal Medicines Journal** **2019; 4(4):163-7**
**DOI: 10.22087/herb%20med%20j.v4i4.778** **ISSN:** 2538-2144

Original Article

# A Preliminary Investigation of the Acute Toxicity of the Ethanolic Extract of *Hoslundia opposita* in *Mus musculus* (Swiss Mice)

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Received: 01.11.2019; Accepted: 28.06.2020

## Abstract

**Background and Aim:** *Hoslundia opposita* has been used traditionally in the management of many health maladies in Southwestern Nigeria. This study investigated the toxicity of the plant for 14 days.

**Materials and Methods:** 20 healthy mice weighing between 13-25 grams were grouped in four cages of 5 mice. They were orally administered extracts of 50, 100, and 200mg $kg^{-1}$ and the control group received distilled water. At the expiration of exposure, animals were weighed and sacrificed. Subsequently, blood was obtained for hematology. Internal organs were harvested, weighed and then the liver was homogenized for biochemical analysis.

**Results:** No noticeable difference between the body and organ weights of the treatment groups and the control group was observed. There was a significant dose-dependent decrease in leucocyte. Hemoglobin and neutrophil increased statistically at 200mg $kg^{-1}$, and 100mg $kg^{-1}$ respectively. Biochemical analytes showed dose-dependent increases in AST, ALT, ALB, Urea, T.Chol, creatinine and DBil against the control, though a significant increase was observed in mice at 200mg $kg^{-1}$ in AST. T.bil increased significantly at 200mg $kg^{-1}$. Insignificant variations in the body and relative organ weight may simply no acute injury and physiologic disturbances attributable to the extract. Insignificant changes in PCV, eosinophils and RBC suggest that the extract does not affect erythropoiesis, osmotic fragility or RBC morphology. The significant leucocyte reduction might imply toxicity on immunity, though clinically irrelevant, for monocytes were insignificant. Moreover, eosinophils at a permissible range might imply the absence of allergy to the extract. Elevated AST and ALT in high doses suggest potential toxicity, but within a permissible range, negating toxicity. Similar values of ALB and T.Prot between the treatment and the control groups suggest that the extract did not alter either the hepatocellular or the secretory mechanism.

**Conclusion:** *H. opposita* is devoid of acute toxicosis at the examined doses.

**Keywords:** *Hoslundia opposite*, Acute toxicity, Body weight, Hematology, Biochemical analytes

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**Please cite this article as:** Oloyede AM, Akindele SK. A Preliminary Investigation of the Acute Toxicity of the Ethanolic Extract of *Hoslundia opposita* in *Mus musculus* (Swiss Mice). Herb. Med. J. 2019; 4(4):163-7.

## Introduction

*Hoslundia opposita* Vahl (Lamiaceae) which is naturally and widely distributed in Nigeria and Africa is commonly known as 'Efrin odan and Oke ota' in Southwestern and Southeastern Nigeria respectively (1). The extract taken from part of the plant or hole-plant extract is used either singly or in combination with other plants for therapeutic purposes in the treatment of certain diseases, including epilepsy, wounds, convulsion, mental disorder, diabetes, and diabetes-induced anaemia (2, 3, 4). The present study was conducted to examine the effect of the extract during consistent daily administration for five consecutive on possible acute toxicity in mice.

## Materials and Methods

**Plant Material**

*H. opposita* leaves were purchased from Oyingbo market, Lagos State, Nigeria, in September, 2019. They were authenticated in Forestry Research Institute (FRIN), Ibadan, with the Voucher Number FHI 108121.

**Preparation of *H. opposita* Extract**

The leaves of *H. opposita* were air-dried under shade and then ground to powder. About 500 grams was macerated and soaked in approximately 1000 ml of 70% Ethanol and was left for 48 hours. Afterwards, it was filtered, and then the filtrate was transferred to an evaporating dish. Subsequently, it was evaporated using a water bath at a constant temperature of 40ºC. The paste-like extract was obtained after evaporation and was freeze-dried. 50, 100 and 200mg $kg^{-1}$ were prepared for the study.

**Animals**

A total of 20 healthy mice weighing averagely 13-25 grams were obtained from the animal house of the Lagos University Teaching Hospital (L.U.T.H.). The mice were divided into four groups of five animals (50, 100 and 200mg $kg^{-1}$and the control) according to their average weight. All the animals were accommodated in well-ventilated cages, fed with standard animal feed and water *ad libitum* and allowed to acclimatize for 7 days. The animals were maintained at the botanical and zoological garden of the Faculty of Science of University of Lagos, Nigeria.

**Treatment**

This acute toxicity was carried out following the guidelines of Organization of Economic Co-operation and Development (OECD) (5), where groups 1, 2 & 3 were orally administered with 50, 100 & 200mg $kg^{-1}$ of the *H. opposita* respectively for 14 days, while the control group received distilled water. After receiving daily treatment for a total of 14 days, the mice were sacrificed via jugular puncture at 24 hours after the last feed. The blood was collected for hematological studies and the livers were harvested for biochemical analysis.

**Hematological Analysis**

Blood samples were collected from the animals in EDTA bottles for conducting several analyses as follows: hemoglobin (Hb), packed cell volume (PCV), white blood cell, neutrophils poly and band, lymphocytes, eosinophils, monocytes, platelet, and red blood cell analyses.

**Biochemical Analysis**

A part of the liver of each mouse was homogenized in 5ml of 0.1mol $L^{-1}$ sodium phosphate buffer (pH 7.4) and taken for analyzing several biochemical parameters as follows: aspartate aminotransferase (AST), urea, creatinine, alanine amino-transferase (ALT), total bilirubin (T bil), total protein (T prot) ,total cholesterol (T Chol), albumin (ALB), and direct bilirubin (D bil).

**Statistical Analysis**

Data were expressed in mean ± SEM, and analyzed using the Student's t-test. Values of $p < 0.05$ were considered significant.

## Results and Discussion

After 14 days, the expiration of the administration of treatment, there were significant increases in body weight in the 100 and 200 mg $kg^{-1}$ groups.

There were no significant changes in the relative weight of the heart, liver, left kidney, right kidney, spleen and lungs.

The biochemical analyses from the liver (Table 3) showed a dose-dependent increase in AST, ALT , ALB, urea, T.Chol, creatinine and DBil against the

**Table 1:** Effects of oral administration of *H. opposita* for 14 days on body weight.

| Treatment | Weight (g) |
|---|---|
| Control | 14.08±1.64 |
| 50mg kg$^{-1}$ | 13.94±1.18 |
| 100mg kg$^{-1}$ | 20.69±1.05* |
| 200mg kg$^{-1}$ | 21.84±1.73* |

Values are Mean±SEM (N=5), * significantly different from the control ($p<0.05$).

After 14 days, the expiration of the administration of treatment, there were significant increases in body weight in the 100 and 200 mg kg**-1** groups.

**Table 2:** Effects of the oral administration of *H. opposita* for 14 days on the relative organ weight.

| Dose | Heart (g) | Liver (g) | Left Kidney(g) | Right Kidney(g) | Spleen (g) | Lungs (g) |
|---|---|---|---|---|---|---|
| Control | 0.09±0.03 | 0.72±0.16 | 0.09±0.02 | 0.1±0.03 | 0.08±0.04 | 0.16±0.05 |
| 50mg kg$^{-1}$ | 0.09±0.01 | 0.69±0.04 | 0.06±0.01 | 0.09±0.01 | 0.08±0.03 | 0.16±0.03 |
| 100mg kg$^{-1}$ | 0.12±0.00 | 0.96±0.07 | 0.09±0.01 | 0.1±0.02 | 0.08±0.03 | 0.22±0.06 |
| 200mg kg$^{-1}$ | 0.1±0.01 | 0.94±0.09 | 0.09±0.01 | 0.11±0.01 | 0.1±0.02 | 0.17±0.02 |

Values are Mean±SEM (N=5)

There were no significant changes in the relative weight of the heart, liver, left kidney, right kidney, spleen and lungs.

**Table 3:** Biochemical analytes from the liver of mice treated with *H. opposita* for 14 days.

| Treatment | AST(u/L) | ALT(u/L) | ALB(u/L) | T.Prot(u/L) | Urea (mg/dl) | T.Chol | Crea | T.Bil(umol/L) | D.Bil(umol/L) |
|---|---|---|---|---|---|---|---|---|---|
| Control | 62.5±47.5 | 367.5±102.5 | 1.84±0.19 | 2.23±0.28 | 18±4.0 | 82.5±28.5 | 0.23±0.0 | 0.16±0.0 | 0.12± 0.0 |
| 50mg kg$^{-1}$ | 190±10.0 | 225±114.3 | 1.03±0.26 | 1.56±0.79 | 39.5±8.5* | 88±5.0 | 0.29±0.0 | 0.2±0.1 | 0.15± 0.0 |
| 100mg kg$^{-1}$ | 268.3±127.8 | 347.5±132.5 | 1.73±0.01 | 2.92±0.21 | 51±15.4** | 98.7±26.7 | 0.46±0.1* | 0.23±0.4 | 0.18± 0.4 |
| 200mg kg$^{-1}$ | 385±90.0* | 485±0.0 | 1.92±0.32 | 1.97±1.02 | 56±31.0** | 198.5±64.5 | 0.5±0.3* | 0.43±0.1* | 0.35± 0.1 |

Values are Mean±SEM (N=5), * significantly different from the control $p<0.05$.

**Table 4:** Haematologic values of mice treated with *H. opposita* extract for 14 days.

| Treatment | Hb (g/dl) | PCV (%) | WBC (ul) | N Poly (%) | Nband(%) | L (%) | E (%) | M (%) | Platelet | RBC(x10$^6$) |
|---|---|---|---|---|---|---|---|---|---|---|
| Control | 11.3±0.3 | 37.9±2.7 | 1800±5900 | 4.5±1.5 | 0±0 | 94±1.0 | 1.0±0.0 | 0.5±0.5 | 424000±37000 | 7.18±0.08 |
| 50mg kg$^{-1}$ | 11.3±0.23 | 38.8±3.4 | 1413±150.5 | 8.25±1.3* | 0±0 | 89.5±0.5* | 1.3±0.5 | 0.5±0.3 | 587750±177853 | 7.56±0.21 |
| 100mg kg$^{-1}$ | 10.9±0.5 | 33±1.5 | 1775±175.0 | 9.5±0.5* | 0±0 | 89.8±3.2* | 0.5±0.5 | 0.5±0.5 | 708500±241500 | 7.85±0.85 |
| 200mg kg$^{-1}$ | 12.1±0.13* | 36.5±0.4 | 3100±437.8* | 6.0±1.5 | 0±0 | 90.3±1.0* | 1.3±0.3 | 1.0±0.3 | 689000±116560 | 7.28±0.06 |

Values are Mean±SEM (N=5), * significantly different from the control $p<0.05$.

control, though a statistically significant increase was observed only in mice treated with 200mg kg$^{-1}$ in AST marker, while T. bil significantly increased in the group administered 200mg kg$^{-1}$ dose.

There were no significant differences in the values of neutrophils, eosinophils, monocytes, platelets and RBC. There was a dose-dependent significant decrease in the number of leucocytes between the control and treated groups. Hemoglobin was statistically different from the control at 200mg kg$^{-1}$, and neutrophil (poly) was statistically different from the control at 50 and 100mg kg$^{-1}$ (Table 4).

*H. opposita* has been used for the management of various health malaises such as chest pain, a sore throat, constipation and jaundice (6). Moreover, Usman *et al*. (7) reported its wide use against mental disturbances as an antimalarial and anticonvulsant plant. They also stated that it could be efficient in the treatment of abdominal pains. Pharmacognostic investigations revealed the presence of phytochemicals

such as 5, 7-dimethyl-6-methylflavone, hoslundiol, euscaphic acid (8), abietane-type esters (9) monoterpenes, 1, 8-cineole, terpineol, sabinene, thymol, car-3-ene, camphor, linalool and limonene (7)

In this investigation, no significant change in organ weight was observed. The significance of organ weight in toxicity studies includes sensitivity to enzymes induction, acute injury and physiologic disturbances. In toxicity studies, body and internal organ weights have been implored as relatively simple and sensitive indicators or indexes of toxicity following the exposure to toxic substances and consequent significant changes in organs affected (10). The absence of any clinical signs of toxicity in the lungs, kidneys, liver and heart was analyzed. It might indicate that the extract has no obvious physical toxicity in the organs weight during the treatment (11).

The level of the detrimental effect of the extract on animal blood could be evaluated using the hematologic profile (11). The absence of any significant change on PCV, Nband, eosinophils and RBC might indicate that the extract does not affect erythropoiesis, osmotic fragility or morphology of the RBC. However, the significant reduction in leucocytes might imply toxicity on the immunity of the animal as leucocytes are regarded as the first line of defense, responding to tissue injury, infectious agents or inflammation (11). Nevertheless, it might not be of clinical relevance, for monocytosis and basophilia were not observed in the mice. Furthermore, eosinophils were maintained within a permissible range implying the absence of allergic response to *H. opposita* (12).

Biochemical assays have been used to investigate possible alterations in the liver and kidney functions effected by drugs and extracts (13). The liver has been established to be the major organ in the metabolism of drugs. Hence, the elevations of ALP, AST and ALT have been implicated in hepatotoxicity (14). Among the biochemical analytes, AST is normally observed in the cytoplasm and mitochondria of numerous cells, very obvious in hepatocytes, skeletal and cardiac muscles (15; 16). ALT often serves as the biomarker of liver function and as the indicator of liver dysfunction or toxicity (16), increased levels of transaminase enzymes AST and ALT, as observed in high doses that might indicate the potential of causing toxicity. However, the range observed is within the physiologically permissible level thereby suggesting the absence of toxicity. Decreased ALB might imply the existence of a potential sign of impaired hepatocellular function (13), but similar values of ALB and T. Prot between the treatment and the control showed that *H. opposita* extract did not cause alteration in hepatocellular or secretory mechanism. Moreover, the kidney plays a pivotal role in drug metabolism. Akindele *et al*. (17) reported that the concurrent evaluation of urea, electrolytes and creatinine could be used to assess renal dysfunction. The dose-dependent significant increase in urea might portend renal injury. However, it is within a permissible range, thereby suggesting the absence of toxicity.

## Conclusion

In general, the mild effects and changes observed in haematologic and biochemical analytes in this study suggest that serious caution should be applied in the use of *H. opposita* extract, particularly at high doses. Moreover, due to the pharmacokinetic differences in biotransformation, the absorption and excretion between humans and laboratory animals leading to different pharmacokinetic parameters such as half-life, clearance and bioavailability, the resultant toxicosis might manifest differently in various species.

## Acknowledgment

I hereby appreciate all the authors that took part in the investigation.

## Conflict of Interest

The authors declare that they have no conflict of interest.

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