

EFFECTS OF STEM-BARK EXTRACT OF *OKOUBAKA AUBREVILLIE* ON SOME VISCERAL
ORGANS OF WISTAR RATS

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Article History

Received: May. 23, 2017

Revised Received: Jan. 11, 2018

Accepted: Jan. 12, 2018

Published Online: May. 31, 2018

Abstract

Background: Over the past two decades, there has been a tremendous increase in the use of herbal medicine; however, these herbs have not been properly evaluated to ascertain their effect on the body organs.

Materials and Methods: Effects of stem bark extract of *Okoubaka aubrevillie* on some visceral organs were investigated in Wistar rats. For acute toxicity testing, Wistar rats (n=16), grouped into 4, (A-D) orally received graded doses of *Okoubaka aubrevillie* extract and deaths recorded within 24 hours. For sub-acute study, Wistar rats (n=20) grouped into 5, (A-E) orally received graded doses of *Okoubaka aubrevillie* extract for 31 days. Blood samples were collected from each rat through retro-orbital puncture for biochemical analysis. The liver, kidney and stomach were excised and processed for light microscopy. For toxin inhibition studies, Wistar rats (n=24) grouped into 6 (A-F), were used. Groups A-C and D-F orally received graded doses of Dichlorvos. Groups A-C further received *Okoubaka aubrevillie* extract while D-F received water and death records observed.

Results: For acute toxicity testing, lethal dose (LD₅₀) of 7500 mg/kg body weight was obtained from the inverse of the log-dose. Sub-acute studies revealed significantly elevated mean body weight in group A (210 ± 4.5 gram) compared to control (178 ± 5.0 gram), (p<0.05). Liver of rats in group A revealed some areas of moderate peri-portal lymphatic inflammation. Treated groups revealed intact architecture of liver, kidney and stomach.

Conclusion: *Okoubaka aubrevillie* extract was found to be relatively safe for consumption and is capable of inhibiting toxins.

Key word: Acute toxicity; Graded doses; Mortality; Sub-acute; Toxin inhibition.

Introduction

Since ancient times, herbs/plants have played a key role in the development of human civilization. Herbal/plant extracts have been used in traditional medicine for treating various metabolic and infectious diseases. Today, herbal/plant extracts are used extensively for preparing drugs, bioactive compounds, pharmacological tools and herbal remedies for various medicinal applications (Fabricant & Farnsworth, 2001; Verpoorte, 2000).

Okoubaka aubrevillie is a mysterious plant used by herbalist in West Africa against all kinds of poisoning. Part of the plant is also used symbolically to ward off evil (Blusokava et al., 1995). The tree is acclaimed to be semi-parasitic, this explain why no other tree appear to grow proximally to it and also provides an explanation as to why among other things people believe the tree possess magical power (Veenendal, et al., 1996; Burkill, 2000). It is widely used as medicine for skin problem including those caused by syphilis and leprosy. Bark macerate is drunk to cure tachycardia and is taken as a vapour bath or nose drops to cure oedema. In Western world the bark is used in phyto-therapeutic medicine. It is also used to treat stomach upset caused by poisoning. The aqueous bark extract is consumed as drug for protection against toxins and evil forces with no knowledge of concomitant adverse effect of the substance on different organs following consumption (Adzet et al., 1987). Pharmacologically, it possesses anti-bacterial and phagocytic properties (Cunningham, 1993).

Okoubaka aubrevillie is a wild spread, uncommon hemi-parasitic West African rain forest tree found in Ivory Coast, Ghana, Sierra Leone, Liberia and Nigeria with common names like; *Anyi Okoubaka*, *Aha asante odii*, *Konoyoua*, *Mano yai yili* and *Akoelisi* respectively (Burkill, 2000). In Nigeria, the Ibos called it *Anunuebe*. It consists of two species and variety. One of the species which is common among West and Central Africa is known as *Okoubaka aubrevillei* Pellegr. & Normand while *Okoubaka michelsonii* J. Léonard & Troupin is predominantly found in Central Africa. Another variety of *Okoubaka* found in the Democratic Republic of Congo is called *Glabrescentifolia* J. Leonard (Halle, et al., 1987). The tree attains a height of up to 40 meters tall, cylindrical, straight, width up to 80 cm in diameter. The bark surface is coarse, greyish brown to reddish, branches are horizontal with branch-lets slightly grooved densely hairy.

In South East Nigeria, most information concerning the mystical origin of this tree/plant/herb was passed from generation to generation through folklore. However, there are similarities in description of its features, the ability to kill other trees which try to grow near it, the use of the extract against all kinds of poisons, intestinal infections and allergic problems (Hawthorne and Jongkind 2006).

The antitoxin activities of bark extract of *Okoubaka aubrevillie* have not been proven scientifically in our locality hence the present study. This study was designed to investigate the toxic and poisonous effects of *Okoubaka aubrevillie* on some visceral organs of Wistar rats, the lethal dose of the aqueous bark extract of the plant (LD₅₀), histomorphological effect of aqueous bark extract consumption on the liver, kidney and stomach of Wistar rats, relative effect of the bark extract on liver marker enzyme and to ascertain whether the extract can inhibit toxin.

Materials and Methods

Plant Sample: The *Okoubaka aubrevillie* bark was obtained from traditional healers and herbalists in Enugu, Nigeria. The bark was taken to the herbarium for authentication at the Department of Plant Science and Biotechnology, University of Nigeria Nsukka. A specimen was deposited in the herbarium for future reference with a voucher number of UNH 387a.

Animals: Wistar rats (n=60), aged 2 to 3 months, weighing 120-160 grams were obtained from the animal house, Department of Veterinary Medicine University of Nigeria Nsukka. The rats were kept under standard condition of temperature $27 \pm 2^\circ\text{C}$ with 24 hours light or dark periodicity. They were housed in clean gauzed cage, fed on standard pellet and clean tap water. The rats were allowed for two weeks acclimatization and experiments were conducted with due care and diligence according to the institution guidelines for experiment involving the use of animals.

Extraction: Large quantity of *Okoubaka aubrevillie* bark was cut into small pieces and ground into fine powder using a dry grinder. The grounded samples were sieved to get uniform particle size, then kept in air-tight container and stored for extraction. Water extraction was done according to the method of Mahanta and Murkherjee, 2001 with little modification. The extraction process was carried out by soaking about 100g of dried powder in 1000ml of water for 24 hours. Crude extract was obtained by first filtering through muslin cloth and further clarified by filtration through filter paper (whatman No.1). The extract was concentrated using evaporator set at 50°C to get rid of water completely. The extracts were kept in refrigerator and different doses were administered.

Phytochemical Analysis: The dried ground bark was subjected to preliminary phytochemical analysis for the chemical constituents as described by Ioan, 1984. The presence or absence of chemical compound; carbohydrates, reducing sugar, proteins, tannins, steroids, alkaloids and others were established.

Lethality (acute oral toxicity testing): This was performed according to the procedure described by Lorke, 1983. Wistar rats (n=16), divided into 4 groups (A-D) were used in the determination of lethal dose of the extract. Graded doses of the extract were orally administered to the groups (A = 6000 mg/kg body weight, B = 7000 mg/kg body weight, C = 8000 mg/kg body weight, D = 9000 mg/kg body weight) and the number of deaths in each group were recorded within 24 hours. Log dose and percentage response of each group was plotted on the graph.

Sub-acute dose study: For sub-acute studies, Wistar rats (n=20) grouped into 5 (A-E) orally received graded doses of extract (A = 1500 mg/kg body weight, B = 1000 mg/kg body weight, C = 500 mg/kg body weight, D = 300 mg/kg body weight, E = 0 mg/kg body weight) for 31 days. Blood samples were collected from each rat through retro-orbital puncture for biochemical analysis. The liver, kidney and stomach were excised and processed for light microscopy.

Toxin inhibition/Neutralization: In inhibition study, Wistar rats (n=24), divided into 6 groups (A-F), each with 4 rats. The doses of (60, 40, and 20) mg/kg body weight were obtained from determination of dose-mortality relationship in Dichlorvos in Wistar rats as described by Gajewski and Katkiewicz 1981. These doses of Dichlorvos (60, 40, and 20) mg/kg body weight were given to groups A to C and also to groups D to F respectively. Groups A to C further received *Okoubaka aubrevillie* aqueous extract of 1500, 1000 and 500 mg/kg bodyweight while D to F received water. Death records were observed on the different groups (Vejayan, et al, 2007).

Liver Enzyme Estimation: The enzymatic activities of various liver enzymes in each group were estimated. Alkaline phosphate transaminase (ALP), were analyzed using method of Roy (1970) provided by Teco diagnostics, USA. Aspartate transaminase (AST) and Alanine transaminase (ALT) were established by using the endpoint technique of Reitman and Frankel (1957) provided by Randox Laboratory, United Kingdom.

Tissue Processing for Histology: Five (5) to 8 mm thickness of dissected organs was fixed in fixatives and processed according to histological techniques. About 5 microns cut from the block, each section fixed and stained with Haematoxylin and Eosin (H&E) methods (Baker and Silvertown, 1985).

Statistical analysis: The Statistical Package for Social Science (SPSS) computer software version 17 was used for data analysis. The results of the tests were analyzed using analysis of variance (ANOVA) and student's t-test at 95% confidence interval with p value of ≤ 0.05 been considered as significant.

Ethics: The procedures followed in this study were in accordance with the ethical standards of ethics committee on animal experimentation of University of Nigeria (NHREC/05/01/2008B-FWA00002448-IRB00002323).

Results

The phytochemical analysis results of the stem bark extract of *Okoubaka aubrevillie* revealed the following compounds; steroids, flavonoids, proteins, alkaloids, saponins, resins, tannins and carbohydrates (Table 1).

Table 1: Phytochemical Analysis Results of *Okoubaka aubrevillie* bark Extract

Constituent	Inference
Flavonoids	+++
Anteaquinone Glycosides	-
Anthracene Glycoside	-
Alkaloids	++
Saponins	+++
Tanins	+
Resins	+
Proteins	++
Carbohydrate	++
Reducing Sugars	-
Hydrolysis Test for Glycoside	-
Cyano-Genetic Glycoside	-
Fat & Oil	-
Steroids	++
Terpenoids	-
Acidic Compounds	Neutral
Cardiac Glycoside	-

Key: - Absent, +Present, ++ Moderately Present, +++ Absolutely Present

A lethal dose (LD₅₀) of 7500 mg/kg body weight was obtained from the inverse of the log-dose (Figure 1 & Table 2).

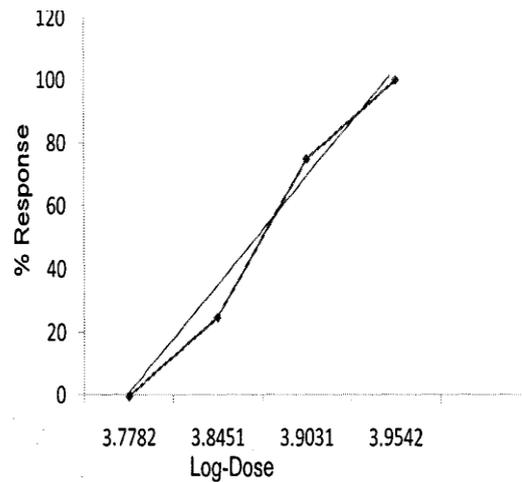


Figure 1: A graph of the percentage response against Log-Dose.

Table 2: Determination of Dose-Mortality of *Okoubaka aubrevillie* in Wistar Rats

Group	No. of Rats	Doses of Drug mg.kg	Log Dose	Results	No. of Death/Total	% Mortality Response
A	4	6000	3.7782	++++	0/4	0
B	4	7000	3.8451	+++	1/4	25
C	4	8000	3.9031	+	3/4	75
D	4	9000	3.9542	-	4/4	100

Key: + Survived - Death

The sub-acute study revealed significantly elevated mean body weight in the treated group A (210.6 ± 4.5) compared to control (E) (178 ± 5.0) ($p < 0.05$) (Table 3). The mean serum levels of liver marker enzymes of the accumulated doses of *Okoubaka aubrevillie* extract (group A) did not differ compared to control (Table 4). In the histological assessment of liver, kidney and stomach tissue of treated rats in sub-acute dose study (figures 2-4), the liver of animals in group A (animals that received 1500 mg/kg body weight of the extract) revealed some areas of moderate periportal lymphatic inflammation (figure 3), while all other treated rats revealed intact liver architecture (figure 2). Examination of kidney and stomach revealed intact architectures (figure 4). The toxin inhibition study revealed no mortality in groups A – C (administered with different doses of Dichlorvos and *Okoubaka aubrevillie*) while there is mortality in groups D – F (administered with different doses of Dichlorvos only).

Table 3: Mean body weight of the drug treated groups of rats and the controls (E)

Groups	No. of Rats	Doses mg/kg	Bodyweight (g) before Drug Administration	Bodyweight (g) after Drug Administration
A	4	1500	157.1 ± 5.6	210.6 ± 4.5
B	4	1000	145.6 ± 4.0	200.3 ± 7.75
C	4	500	137.4 ± 0.25	190.0 ± 6.4
D	4	300	133.0 ± 5.6	180.0 ± 10.3
E (Control)	4	-	152.0 ± 5.5	178.0 ± 5.0

Table 4: Mean liver marker enzyme of the treated groups of rats and the control (E)

Groups	ALT (IU)	AST(IU)	ALP(IU)
A	48.05 ± 2.05	70.25 ± 1.25	54.7 ± 1.7
B	50.00 ± 1.80	90.00 ± 5.00	47.00 ± 7.0
C	53.8 ± 6.60	121.9 ± 23.15	58.7 ± 2.3
D	45.2 ± 2.20	65.3 ± 3.30	75.25 ± 9.1
E (Control)	59.25 ± 5.25	51.35 ± 3.30	60.50 ± 13.65

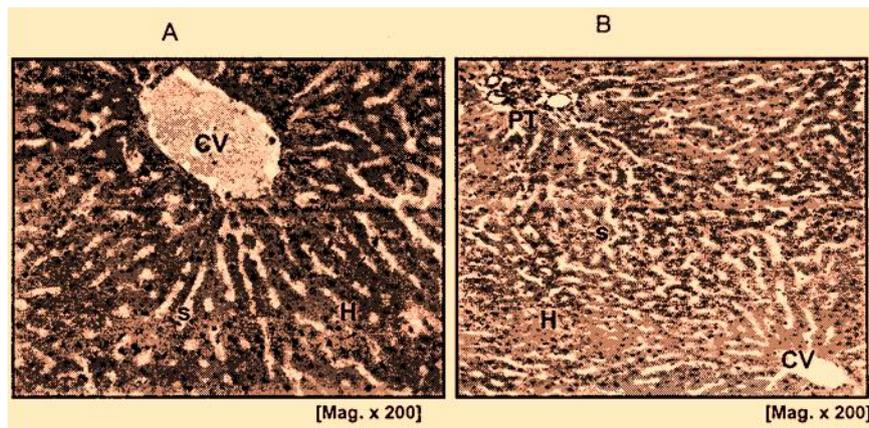


Figure 2: Photomicrograph of Liver of animal that received no plant/herb extract showing normal histoarchitecture of the hepatic tissue. Features observed: Normal Central Vein (CV), Normal hepatocytes (H), Normal sinusoids (s), Portal tracts (PT)

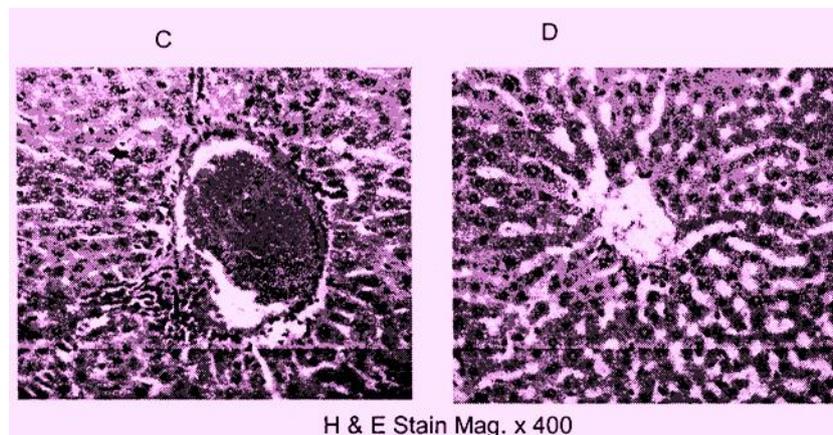


Figure 3: Photomicrograph of Liver of animal that received plant/herb extract showing moderate periportal lymphocytic inflammation.

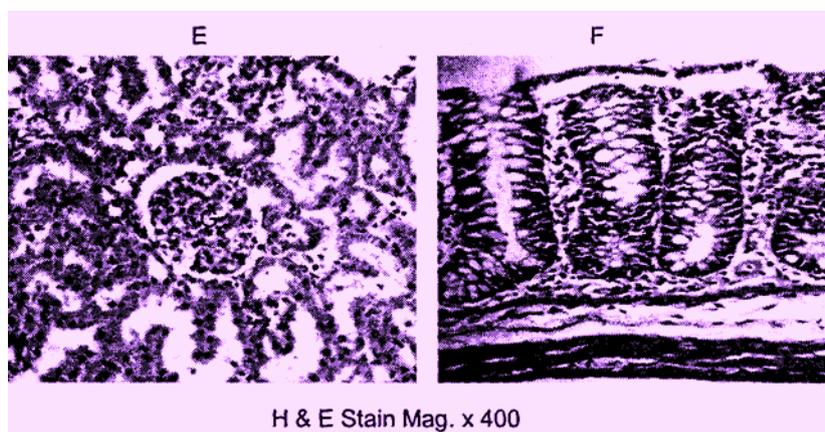


Figure 4: Photomicrograph of Kidney (E) and Stomach (F) of animal that received plant/herb extract showing normal glomeruli and renal tubules and normal colonic mucosa respectively.

Discussion

The bark of *Okoubaka aubrevillie* is a very popular traditional medicine in West Africa and is also used in Western medicine. It is widely sold for medicinal use in local markets throughout coastal countries of West Africa, and is also traded internationally. It is particularly employed in the treatment of skin disorders and poisoning. A macerate of the bark is used in the treatment of tachycardia. The bark is used in phytotherapeutic medicine in the Western world. Its main applications are for stomach upsets caused by poisoning. Skin problems, including those caused by syphilis and leprosy are treated by bathing with a macerate of the bark in water. External application of the bark preparations is also practised to counteract poisoning. A bark macerate is taken as a vapour bath or as nose drops to cure oedema.

The phytochemical analysis of *Okoubaka aubrevillie* bark extract revealed flavonoids, alkaloids, saponins, tannins, resins, proteins, carbohydrates, and steroids. These phytochemical constituents have attracted attention and played significant role in giving solutions to systemic problem. Some of these constituents may have contributed to the observed effects in this study.

The mean body weights of treated rats were significantly increased compared to control. This increase in body weight of treated rats may be due to the phytochemical constituents of the extract. The protein content of this extract may have contributed to the observed increase in the bodyweight of the treated rats since protein is a body builder. The LD₅₀ of *Okoubaka aubrevillie* extract in this study is approximately 7500 mg/kg body weight. The LD₅₀ calculated is expected to cause death of 50 percent of the entire defined experimental animal population. The outcome of this study shows that *Okoubaka* extract is non-toxic. The antioxidant activities of flavonoids may have contributed to the observed non-toxic effect of the extract.

The flavonoids have aroused considerable interest recently because of their potential beneficial effects on human health. They have been reported to have antiviral, anti-allergic, anti-platelet, anti-inflammatory, anti-tumor and antioxidant activities. Natural flavonoids and polyphenolic compounds have been reported to exhibit protective and strengthening activity on the major organs (Adzet, *et al*, 1987).

In the histological assessment of the liver, kidney and stomach tissue of treated rats, group A showed areas of moderate peri-portal lymphatic inflammation in the liver. This may be attributed to the tannins content of the extract. On examination of kidney and stomach, the treated and control group showed intact architecture. Other treated groups showed intact architectures. This might be due to flavonoid content of the extract. Flavonoid is a strong antioxidant which can preserve the architecture of the organs.

The mean serum levels of liver marker enzyme of treated groups did not differ significantly compared to control. These findings showed that the extract may be safe and non-toxic to the liver at the level tested. This may also be attributed to antioxidant activities of flavonoids in the extract.

In conclusion, since this study have successfully evaluated the effects of stem bark extract of *Okoubaka aubrevillie* on some visceral organs and found it to be relatively safe for consumption and capable of inhibiting toxins, it has presented the world with an affordable natural remedy for preventing and treating diseases.

Conflict of interest: We have no conflict of interest to disclose.

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