

Toxicological Range Finding Test of Aqueous Extract of *Borreria verticillata* Aerial Parts in Exposed African Catfish *Clarias gariepinus* (Burchell 1822) Juveniles

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Received date: April 04, 2019; Accepted date: July 23, 2019; Published date: August 03, 2019

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Abstract

The toxic level of aqueous extracts of *Borreria verticillata* aerial parts on *Clarias gariepinus* species was investigated in a 24 hrs static nonrenewal bioassay by using range finding test. The *Clarias gariepinus* (N=80, mean weight and length 21.48 ± 3.32 g and 11.37 ± 1.23 cm) were randomly tested in various concentrations of aqueous extract of *Borreria verticillata*. Five (5) fish were exposed to different concentrations ranging from 1 mg/l to 1200 mg/l in a 24 hours exposure time without feeding 24 hours before and during the study. The fish exposed to the different concentrations showed absence of, loss of balance, skin discoloration, air gulping, erratic movement, hemorrhage and mortality during the 24 hours period of exposure for each of the four range finding test trials. Aqueous extract of *Borreria verticillata* was established to be nontoxic to *Clarias gariepinus* juveniles using behavioral display and mortality as end points in acute exposure. The results of this study will assist toxicologists, toxicopathologists and researchers in determining the toxic/safe concentrations of aqueous extract of *Borreria verticillata* in exposed fish during research/use of the plant extract.

Keywords: Toxicity; *Borreria verticillata*; *Clarias gariepinus*; Nontoxic

Introduction

Borreria verticillata is a plant which is perennial shrub and belongs to the family of Rubiaceae. Commonly referred to as shrubby false button weed or shrubby false button wood, found in places such as tropical and subtropical America, Africa, Asia, as well as Europe [1]. It was first noticed in South and Central America [2]. Used medicinally for various conditions by the traditional practitioners in South America, Asia, Africa, and West Indies, the extract of the flower is administered as antipyretic, analgesic, emetic antidiarrheal, against erysipelas and in the case of hemorrhoids [3,4]. Chopra et al., 1956, reported the used of the plant in the treatment and cure eczema (*Tinea versicolor*), ring worm (*Tinea capitis*), scabies and various skin related lesions such as infectious dermatitis, toothache, headache, and dyspepsia. The extract obtained from aerial part of the plant is used for treating skin related infections and a lotion to relieve skin itches, treatment of diabetes and dysmenorhea and can be used when combined with *Cuscuta Zebrina schnizlein* [5,6]. In Senegal it is used for treating bacterial skin infections and leprosy [7].

Nigeria is a sub Saharan African country where these plants are found, also found in abundance in Sudan savanna, wastelands, non-cultivated fields or within the vicinity of ponds, mostly in the rainy season. In the northern part of Nigeria it is referred to as *Karya garma* by the Hausas, *Irawo-ile* by the south west yorubas, and *Abia-ikana* by the south south ibibio people of Nigeria. The leaves of the plant have been reported to be used by some local people within Nigeria and West African Countries for its diverse biological activities, which includes analgesic, anti-inflammatory, antitumor, antimicrobial, larvicidal,

antioxidant, gastrointestinal, anti-ulcer, and hepatoprotective properties, with compounds like alkaloids and iridoids as the major active principles [8-10]. The roots and leaves have been reported for treatment of malaria, ophthalmic, inflammation in eyes and gums, blindness, pyrexia, spleen complications, injuries, bleeding, dysentery and diarrhea, and also in the treatment of liver ailment, kidney malfunction and it is abortifacient [11].

Phytochemical Constituents of *Borreria verticillata*

Researchers have documented the presence of terpenoid, indole and alkaloids in plant *Borreria verticillata* [12], and the chemical structure of an iridoid known as borrirogenin extracted from a Methanol extract of the flowers of *B. verticillata* [5]. The phytochemistry evaluation of *B. verticillata*, led to the reporting of two new simple indole alkaloids, 6-methoxy-4-(3-methylbut-2-en-1-yl)-1H-indole, named verticillatine A1), and 1-(1H-indol-6-yl)-3-methylbutan-1-one, named verticillatine B1), and also a new iridoid, named 6'-O-(2-glyceryl) scandoside methyl ester1), from the methanol extract of *B. verticillata* roots, isolated asides two other known iridoids, asperuloside and scandoside methyl ester, a mixture of aliphatic acids, a mixture of tri-O-acylglycerols, sucrose, and a mixture of glucose and sucrose were isolated after preparation of the corresponding acetyl derivatives (Moreria et al.,2010). There is currently no literature on the toxicity of *Borreria verticillata* in fish. This study aims to determine the toxic effect of aqueous extract of *Borreria verticillata* aerial parts in exposed *Clarias gariepinus* species by conducting a range finding analysis to establish the need to proceed to a definitive test in order to determine the Median Lethal Concentration (LC50) or otherwise of the plant in exposed *Clarias gariepinus* in an acute bioassay.

Methods

Plant collection

Fresh sample of the aerial parts of *Borreria verticillata* comprising of the stem, leaf and flower were harvested from neighboring Bassawa area within the Zaria metropolis in Kaduna State of Nigeria during the November – December season, in 2016, and was authenticated from the Herbarium unit of Department of Biological Sciences, Ahmadu Bello University, Zaria, where a specimen of the plant was submitted and a voucher number 0670 was assigned.

Phytochemical determination

The harvested fresh sample of the aerial parts of *Borreria verticillata* were shade-dried and grounded into fine powder. The fine powder was screened for the presence of alkaloid, tannins, flavonoids, saponins and phenol using standard methods [13].

Plant preparation

The fresh aerial parts of plant *Borreria verticillata* consisting of the stem, leaves and flowers were shade-dried and grounded into fine powder. The powder was weighed and 3 kg was soaked in 2 L of distilled water and shaken gently for about ten minutes with the help of a shaker to make a homogenous mixture. The mixture was left for 48 hours and filtered [14]. The filtrate was oven dried and used later for the study.

Experimental animals

The Ahmadu Bello University Committee on Animal Use and Care (ABUCAUC) gave ethical clearance with approval number ABUCAUC/2017/014 for this study.

Range finding test

Live juveniles of the African catfish, *C. gariepinus* (N=80, average weight of 21.48 ± 3.32 g and having length of 11.37 ± 1.23 cm respectively were procured from a commercial catfish farm having a reputable standing and authenticated at the Fishery Section, Department of Biological Sciences, A. B.U., Zaria, Nigeria. Fish acclimatization was done for 21 days under the natural day and night photo-periods (12/12-h) pond water was changed once in every three days in a 1000 litres capacity concrete tank. The fish were fed to their satisfaction (ad libitum) twice daily with 2 mm Coppens® fish feed for aquaculture (Coppens® International by Helmond, Holland). After the acclimatization experimental fishes were randomly selected and kept in a static system. The feeding was stopped one day prior to their exposure to aqueous extract obtained from *Borreria verticillata* aerial parts and after that fishes were not fed throughout the test. A range finding test was done to determine the five extract concentrations as described by Fafioye (2005) using a 50 L glass aquarium tank for each concentration. The physicochemical characteristics like Temperature, pH, TDS and electrical conductivity of fish culture water were ascertained using a Hanna “Combo portable hand instrument (Hi

98129, Hanna Instrument, Mauritius) whereas the dissolved oxygen contents was similarly established using the modified Winkler-Azide method [15,16].

Data analyses

Data were expressed as mean (\pm SEM) and were subjected to ANOVA significance at $P < 0.05$ using SPSS version 20.

Results

The physicochemical parameters values of the water used during the study is listed in Table 1. The aqueous extraction carried out on *Borreria verticillata* with dry weight of 3 kg yielded 304.11 g and had an extractive yield of 10.13 w/w. The Phytochemical screening done shows that the aerial parts of *Borreria verticillata* showed the presence of some of the metabolites presented in Table 2. The behavioral and clinical signs exhibited by the exposed fish in the range finding test for the different concentrations of aqueous extract *Borreria verticillata* is given in Table 3. A range limiting test conducted using five (5) fish per concentration showed no mortality at varying concentrations from 0-1200 mg/l (Table 3).

Parameters	Values
PH	7.04 ± 0.01
Temp (OC)	26.01 ± 0.01
Dissolved oxygen (DO) (mg/l)	5.87 ± 0.01
Total dissolved solid (mg/l)	3.27 ± 0.01
Conductivity (μ s/cm)	33.02 ± 0.12
Data are presented as means \pm SEM	

Table 1: Physicochemical properties of the water.

Constituents	Qualitative
Anthraquinones	-
Glycosides	+
Saponins	+
Steroids	+
Tanins	+
Flavonoids	+
Alkaloids	+
Phenols	+
Keys: +=present, -=absent	

Table 2: Phytochemical constituents of aqueous extract of *Borreria verticillata* aerial parts.

	Range Finding Test 1st Trial	Range Finding Test 2nd Trial
Exposure time (HR)	24 hours exposure	24 hours exposure

Concentration (mg/l)	0	1	5	10	15	20	25	50
Vertical/Angular positions	-	-	-	-	-	-	-	-
Loss of balance	-	-	-	-	-	-	-	-
Skin discoloration	-	-	-	-	-	-	-	-
Air gulping	-	-	-	-	-	-	-	-
Erratic swimming	-	-	-	-	-	-	-	-
Hemorrhage	-	-	-	-	-	-	-	-
Mortality	0	0	0	0	0	0	0	0
	Range Finding Test 3rd Trial				Range Finding Test 4th Trial			
Exposure time	24 hours exposure				24 hours exposure			
(HR)								
Concentration (mg/l)	50	100	150	200	400	600	800	1200
Vertical/Angular positions	-	-	-	-	-	-	-	-
Loss of balance	-	-	-	-	-	-	-	-
Skin discoloration	-	-	-	-	-	-	-	-
Air gulping	-	-	-	-	-	-	-	-
Erratic swimming	-	-	-	-	-	-	-	-
Hemorrhage	-	-	-	-	-	-	-	-
Mortality	0	0	0	0	0	0	0	0

Table 3: Behavioral display and clinical signs observed in exposed *Clarias gariepinus* juveniles *Borreria verticillata* in Range Finding Test.

Discussion

The physicochemical values of the water used in this study were within the normal values [17]. The preliminary phytochemical screening of the aqueous *Borreria verticillata* extract showed the presence of various compounds like flavonoids, alkaloids, saponins, sterols, tannins, phenols and glycosides, while anthraquinone and cardiac glycoside were absent. This finding is in agreement with previous reports of Abdullahi et al. [10] in ethanol and aqueous extract of *Borreria verticillata* aerial parts respectively. These compounds are commonly known to exhibit analgesic and anti-inflammatory activities including hepatocurative effect [18]. Various numbers of flavonoids as well as tannins isolated from different plants have been discovered to have significant analgesic and/or anti-inflammatory effects [19]. The inhibitory effect of flavonoids on eicosanoids synthesis has been reported [20,21]. Flavonoids inhibit a wide array of enzymes such as protein kinase C, protein tyrosine kinases, phospholipase A2, phosphodiesterases among others have been documented [22]. Alkaloids and saponins possess analgesic and antispasmodic effects, while the healing of wounds and inflamed mucus membranes are hastened by tannins [23]. Flavonoids are potent water soluble antioxidants which prevent oxidative cell damage [23-25]. Thus, the presence of these constituents may be contributory to the analgesic and anti-inflammatory effects of the plant [10,26]. The range finding test showed that the aqueous extract of *Borreria verticillata* aerial parts was nontoxic to the juvenile *Clarias gariepinus*, exhibited by absence of

mortality or signs of distress at and beyond 1000 mg/l, according to GESAMP classification for acute exposure in aquatic environment any substance that induce no mortality at >1000 mg/l is classified as nontoxic in acute aquatic exposure. This finding agreed with Abdullahi et al. [10], who reported in a different animal model that *Borreria verticillata* extract was non-toxic to rats and mice when given orally or intraperitoneally (LD50>5000 mg/l), which was based on the toxicity classification proposed by Loomis and Hayes (1996) which states that substances with an LD50 values between 500 and 5000 mg/kg and 5000 mg to 15,000 mg/kg body weight are regarded as slightly toxic and practically nontoxic, respectively [27-29]. Due to the nontoxic property of the extract in the range finding test with no mortality at extract concentration greater than 1000 mg/l, this inferred that a very high concentration of the plant extract will be needed to induce mortality which may compromise the physicochemical property of the water making it impossible to attribute mortality to the effect of the plant extract introduced in the range finding test [30,31].

Conclusion

The absence of behavioural signs of toxicity and mortality in the *Clarias gariepinus* species during the range finding test established the nontoxic status of the aqueous extract of *Borreria verticillata* aerial parts in acute exposure to the fish at a concentration <1000 mg/l of plant extract, which led to the conclusion that the extract is nontoxic in acute exposure to the fish, therefore was no need proceeding to a

definitive test to determine the median lethal concentration (LC50) of the plant extract.

Acknowledgement

The authors would like to acknowledge the contribution of Professor Alpha Raj Mekapogu of the Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science, S.V. Veterinary University, Proddatur, Indian, who provided the GESAMP literature that guided the experimental design of this study and also assisted to draw conclusion on the nontoxic property of aqueous extract of *Borreria verticillata* in acute exposed *Clarias gariepinus* in this study.

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