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Research Paper

Study to Determine Toxicity of methanolic *Termialia Catappa* Linn. (Almond Fruit) Leaf Extract on Adult Wistar Mice Using Dietrich Lorke Method

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ABSRACT
Purpose*: Toxicity test to determine the potential toxic effect(s) of methanolic Terminalia catappa (Almond fruit) leaf extracts that may occur either immediately or shortly after the administration of the leaf extract on Wistar (albino) mice within a 24 hour period.*
Method: *A total of 23 Wistar mice sourced from the Toxicology Department of Nnamdi Azikiwe University Awka, Anambra state Nigeria, were used for the study. The crushed red and green leaves of Terminalia catappa was extracted using 95% ethanol, filtered, and evaporated (Tabassam method). The dried T. catappa extract was used to identify phytochemical content qualitatively. The dried extracts were also dissolved in sterile dilution. The test was done following the method of Dietrich Lorke. The first phase of study required nine animals, divided into three groups of three animals each. Each group of mice was administered different doses (10mg/kg, 100mg/kg and 1000 mg/kg) of methanolic Terminalia leaf extract introduced via oral route into the three groups of three mice each .The animals were placed under observation for one hour to monitor their behavior as well as if mortality will occur.* ***Result****: shows no mortality. In the phase two experiment, T. catappa leaf extract in doses of 2000mg/kg, 3000mg/kg, 4000mg/kg and 5000mg/kg respectively, was introduced also by oral route into 4 groups of one mouse each and observed for duration of twenty four hours checking mortality. Result also showed there was no mortality.*
Conclusion*: methanolic extract of Terminalia catappa leaf possesses concentration dependent non-toxicity that can be allowed for general human consumption*
Keywords
Terminalia catappa, Onchocerca volvulus, Wistar mice,Toxicity

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I. Introduction

The assessment of the lethal dose, the dose that kills 50% of test animals population (LD_{50}), is used as a major parameter in measuring acute toxicity and usually the first procedure for screening chemical and pharmacological agents for toxicity[4]. Acute toxicity test gives information about LD_{50}, therapeutic index and the degree of safety of a pharmacological agent [14]. The *Onchocerca volvulus* nematode, a causative agent of Onchocerciasis is a severe public health concern [2]. About 99% of cases are found in Africa where 85million people live in endemic areas [1]. Nigeria has the highest number of persons with onchocerciasis, accounting for over one-third of the global prevalence [15]. According to the records of the African Program on Onchocerciasis Control (APOC), 508 of 779 Local Government Areas in Nigeria have indicators of serious onchocerciasis infection that about 35,210 communities are endemic with onchocerciasis and on the whole, more than 25 million Nigerians are at risk of infection [5]. The Nigerian Federal Ministry of Health surveyed in 1993 - 1994 to determine the level of endemicity nationwide; Imo State in the southeastern region of Nigerian was found to be highly endemic with over 1.1 million persons at risk of getting infected with onchocerciasis[8]. *Terminalia catappa* (Linn) has been investigated in various pharmaceutical studies as it contains a variety of chemical components [9]. *The* plant extracts exhibit anthelmintic as well as biological activities, including antioxidant (punicalagin, punicalin, terfluvina A and B, chebulic acid, benzoic acid, cumaric, and its derivatives) [10]. It also contains anti-inflammatory (triterpenic acids, especially ursolicacid and its derivatives) [11], antimicrobial (flavones and flavanols) [12] and hepato-protective activities (punicalagin, punicalin),[13] .In India, a plaster of *T. catappa* leaves is used to treat scabies, leprosy wounds and other skin diseases [15]. Its traditional uses especially

in India, the Philippines and Malaysia include the treatment of diarrhoea and fever [7]. There have been studies done previously that suggested that the most polar fractions gotten from *T. catappa* leaves are effective against bacteria [6] and fungi [3].

II. MATERIALS AND METHODS

Collection of leaves

Terminalia catappa leaves were collected from the plants naturally grown in Umudurunna Abba, Nwangele LGA of Imo State Nigeria. The specimens were collected during the evening period, at the time when the leaves were freely falling off the tree. The leaves were packaged in a bag and taken to the Nnamdi Azikiwe University Agulu campus, Anambra state Nigeria, the next morning for identification at the department of Biological Sciences, of the University. Afterwards, the *Terminalia catappa* leaves were washed thoroughly in distilled water and a known quantity (850gm) were dried at room temperature for 1 week. The red and green leaves were separately ground into fine powder form using clean laboratory motor and pestle. The pulverized plant sample was macerated in 2.5L of methanol for 48 hours with intermittent shaking. It was filtered using a muslin cloth and further filtered through cellulose filter paper (Whatman No.1) filter paper. The filtrate was concentrated using rotary evaporator (Buchi Labortechnik) under reduced pressure at 40^{o}C.

Phytochemical Screening

The dried leaf filtrate was used for the phytochemical test. All extract of the plants were analyzed for the qualitative and quantitative phytochemicals analysis using standard methods, as described by Sofoworo (1993), Trease and Evans (1989) and Harborne (1973). These plants were explored for its phytochemical profile to identify the active constituents of the *Terminalia catappa* leaf extract. The phytochemical analysis included; test for Tannins, test for Saponin, test for flavonoids, test for steroids, test for Cardiac glycosides (Keller-killing test), Alkaloid determination, and Saponin determination.

Purchase of Mice

The Wistar mice were sourced from the Zoology department of NnamdiAzikiwe University, Agulu campus, Anambra state Nigeria.

Acute Toxicity Test

The toxicity study was done using the method employed by Dietrich Lorke (1983). This method is of phases 1 and 2 respectively. The experimentation as to the toxic effect of Methanolic *Terminalia catappa* leaf extracts on Wistar mice was conducted with the supervision of the laboratory scientist of the pharmaceutical and toxicology department of the Nnamdi Azikiwe University Agulu, Anambra state Nigeria.

111. Results

Phase 1- This phase requires nine animals. The nine animals (mice) are divided into three groups of three animals each. Each group of animals is administered different doses (10mg/kg, 100mg/kg and 1000 mg/kg) of test substance. The mice are placed under observation for one hour to monitor their behavior as well as observe if mortality will occur. No mortality was recorded.

Phase 2 - This phase involves the use of three animals, which are distributed into three groups of one animal each. The animals are administered higher doses (2000mg/kg, 3000mg/kg and 5000 mg/kg) of test substance and then observed for 24 hours for behavior as well as mortality. No mortality was recorded.

TEST TABLE (ACUTE TOXICITY (LD $_{50}$)

Pharse / Duration	Extract dose (PO)	Nos of Mice	Death
1hour	1 0mg/kg	3	0
	100mg/kg	3	0
	1000mg/kg	3	0
24 hours	2000mg/kg	1	0
	3000mg/kg	1	0
	4000mg/kg	1	0
	5000mg/kg	1	0

Table shows the result of the acute toxicity test of methanolic *Terminalia catappa* leaf extract administer orally on the 13 Wistar mice, in the two phases, using the method of Dietrich Lorke (1983). Result shows no mortality.

IV Discussion

The purpose of acute toxicity test was to investigate the adverse effect(s) of *T.catappa* leaf that may occur either immediately or shortly after the administration of the leaf extract, within 24 hours. The adverse effect should be any effect that produces impairments in organs and/or biochemical lesions and which could alter the functioning of the organism in general or individual organs. Adverse effects should occur within 14 days of the administration of the substance. The assessment of the lethal dose (LD_{50}) (the dose that kills 50% of test animals population) is used as a major parameter in measuring acute toxicity and usually the first procedure for screening chemical and pharmacological agents for toxicity [4]. Acute toxicity test gives information about LD_{50}, therapeutic index and the degree of safety of a pharmacological agent (Akhil*a et al* 2007).

Then the LD_{50} is calculated by the formula:
D_0 = Highest dose that gave no mortality; D_{100} = Lowest dose that produced mortality (Akhil*a et al* 2007

V. Conclusion

From the research work, it can be concluded that methanolic *Terminalia catappa* leaf extraxt possess concentration dependent non-toxic effects on Wister mice. It can become a potent key ingredient of anthelmintic herbal drug formulation and a safe, sustainable, environmentally friendly and affordable treatment option. The acute toxicity investigation (Table 1) of methanolic *Terminalia catappa* leaf extract recorded no death and as well, no abnormalities were detected for physiological aspects of the mice. This result indicates that the *Terminalia catappa* leaf is safe and possesses insignificant toxicity. The result is same as study by Sivaranji, *et al* (2015) which reported that the methanolic extract of *T. catappa* leaves demonstrated a significantly

high safety margin for the host of *Carassius auratus* (Goldfish), in a concentration of 400mg/kg of *T. catappa* leaf extract, for 3 hours and without any visible effect. It can be possible that *T. catappa* may have toxicity potential in concentration above 5000mg/kg, but there was no study seen in the course of this research work that reported such a finding.

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Availability of data and materials
The dataset(s) and other materials can be accessed upon request via the authors email handler (please put your email)
Ethics approval and consent to participate
An ethical clearance and approval was obtained from the the Ethics Committee,public health department Imo State Ministry of Health Owerri (Protocol number: 105/2014).

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