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Acute Toxicity (LD_{50}) of Petroleum Ether, Ethanolic and Aqueous Stem Bark Extracts of A*dansonia digitata* on Albino Wister Rat

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Abstract

Adansonia digitata Linn. Commonly known as "Baobab" is a deciduous tree and belongs to the plant family called Bombacacea. The tree is mostly known for its exceptional height and may live for several hundred years. The bark tends to be smooth, ranging in colour from reddish brown to grey, being rough and wrinkly like elephant skin. Acute toxicity of petroleum ether, ethanolic and aqueous extract of stem bark of *Adansonia digitata* was carried out using albino Wister rat as animal models. This study was conducted to determine the acute toxicity level of the crude extracts using Albino Wister rats. The study was conducted in two phases. In the phase I, three groups of three rats (3 per group) with weight range of 100 – 120g were administered with respective oral doses of 10 mg/kg, 100 mg/kg and 1000 mg/kg body weight of each extract. While the control groups were administered normal saline. These were observed 24 hrs and up to a period of 2 weeks. In second phase II, the dosage of each extract was increased to 1600 mg, 2900 mg and 5000 mg/kg body weight respectively for another three groups of one rat (1 per group). The control groups were administered normal saline. These were equally observed for toxicity signs namely salivation, brushing of the nose on the floor, isolation, weakness, sleeping, coma and possible deaths. The result shows that throughout the 2 weeks period, no mortality was observed in any of the test animal groups up to the highest dose of the extract tested. It is thefore concluded that administration of *Adansonia digitata* stem bark extract in rat may be safe up to a dose of 5000 mg/kg body weight. This may serve as a base line data value for the development of ethnopharmacologically active substances with potention to be use as modern medicine.

Key words: Acute toxicity, lethal dose, *Adansonia digitata*, Extracts.

INTRODUCTION

Adansonia digitata plant is traditionally used to treat diarrhoea and infectious diseases (Kubmarawa *et al.*, 2007). The bark of the trunk is used in the treatment of malaria and also used to bath babies to encourage a smooth skin (Kristensen and Lykke, 2003.The bark is used instead of quinine for curing fever (Shukla *et al.*, 2001) The stem bark is made into a decoction for internal use and functions due to its soluble and insoluble tannins (Yusha'u *et al.*, 2010). The stem bark extract is been used in the treatment of stomach upset, diarrhoea, dysentery, antioxidant, antimaleria, antiinflammation and semi-fluid gum obtained from baobab bark is used to treat sore throat (FAO, 1988). The bark produces strong fibers used in making ropes, mats, bags and hats (Igboeldi *et al.*, 1997).

Acute toxicity is the ability of a chemical to cause ill effect "relatively soon" (minutes, hours (24) or days and up to about 2 weeks) after one oral administration or a 4 hour exposure of a chemical in air (Senin, 2006). The LD_{50} is one way to measure the short term poisoning potential (acute toxicity) (Senin, 2006). The LD_{50} for a particular substance is essentially the amount that can be expected to cause death in half (i.e. 50%) of a group of some particular animal species, usually rats or mice, when entering the animals' body by a particular route (Senin, 2006).

Toxicologists can use many kinds of animals but most often testing is done with rats and mice. It is usually expressed as the amount of chemical administered (e.g. milligrams) per 100grams (for smaller animals) or per kilogram (for bigger subjects) of the body weight of the test animal (Senin, 2006).The LD_{50} can be found for any route of entry or administration, but dermal and oral administration methods are the most common. The LD_{50} value obtained at the end of the experiment is identified as LD_{50} (oral), LD_{50} (skin) e.t.c. as appropriate. The most frequently performed lethality study is the oral LD_{50}. The results of oral studies are important for drugs, food and accidental domestic poisonings. In general, the smaller the LD_{50} value, the more toxic the chemical is. Also, the larger the LD_{50}value, the lower the toxicity (Senin, 2006). LD_{50}value can be compared to other values using a toxicity scale. The two most common scales used are the "Hodge and Sterner scale" and the "Gosselin, Smith and Hodge scale (Senin, 2006)." These tables/scales differ in both the numerical rating given to each class and the terms used to describe each class. It is important to know that the actual LD_{50} value may be different for a given chemical depending on the route of exposure (Oral, dermal, inhalation) (Senin, 2006).

The use of plant extracts in treatment of diseases without any standard dosage accompanied with lack of adequate scientific studies has raised concern on their toxicity (Senin, 2006). The toxicity studies help in assessing the right dosage to be administered without causing health risks in the organisms (Ashafa *et al*, 2012). The lack of precise dosage in traditional medicine is not only unique to traditional medicine as it also occurs in modern medicine and there is likelihood to overdose the patient due to imprecise nature of diagnosis and dosage (Kokwaro, 2009). Toxicity tests are therefore important so as to determine the lethality of drugs and to determine the harmless concentration of drugs for consumption (Hood, 2009).

The toxicity testing using Wister rats is easy and it is an appropriate bioassay of determining the LD_{50} of various extracts (Apu *et al*., 2010).The objective of this work is to determine the acute toxicity level of the crude extract of petroleum ether, ethanolic and aqueous extracts of stem bark of *Adansonia digitata* using Albino Wister rat. This was aim to develop and see the possibility for its production as candidate drug for the pharmacological steps.

MATERIALS AND METHODS

Plant Collection and Identification

The stem bark of *Adansonia digitata* was collected from Bayero University, campus (Old Site) Kano, Nigeria. During dry season by scraping the tree bark using sterile knife. Identification and authentification of the plant material was done by a Taxonomist at the Department of Plant Biology, Bayero University, Kano. Voucher specimen (BUKHAN 0036) was deposited in the Department herbarium.

Preparation of extracts

The stem bark of *Adansonia digitata* was air-dried at room temperature (28±2^{o}C) in the laboratory for two weeks. The dried stem bark was pulverized into powder using clean mortar and pestle. The pulverized *Adansonia digitata* stem bark was store in air tight plastic container (Mukhtar and Okafor 2002). One hundred grams (100g) of the powdered plant was weighed and separately percolated with 500ml of petroleum ether, ethanolic and aqueous for two weeks with shaking at regular intervals.

The percolates were separately filtered through a clean muslin cloth and subsequently with Whatman No.1 filter paper. The filtrates are allowed to evaporate at ambient temperature (28±2^{o}C), and that of aqueous was evaporated using water bath set at 45^{o}C. The crude extracts were refrigerated at 4^{0}C until required for further use (Betoni *et al*., 2006).

Acute Toxicity Study

The acute toxicity study was done according to the method described by Lorke (1983). Forty eight (48) albinos Wister rats (weighing 100-120 g) of both sexes were purchased from the animal house of Biological science, Bayero University Kano. The study was conducted in two phases. In phase I, three groups (1, 2, and 3) of three rats each were orally administered with the petroleum ether, ethanolic and aqueous extracts of *Adansonia digitata* stem bark at doses of 10 mg/kg, 100mg/kg and 1000mg/kg body weight., and then observed for 24 hours up to 2 weeks for signs of toxicity namely salivation, stretching of the body, weakness, brushing of nose on the floor, sleep, coma and death. In phase II, three groups of one rat each were orally administered with the extracts at doses of 1600 mg/kg, 2900 mg/kg and 5000 mg/kg body weight. The control groups were orally administered with normal saline. They were observed for 24 h and up to two weeks for any signs of toxicity or mortality.

Determination of median lethal dose (LD_{50})

The method of Lorke (1983) was used in the LD_{50} determination. In phase I Three groups of three rats each were orally administered with the extracts at doses of 10mg/kg, 100mg/kg and 1000mg/kg body weight and were observed for any signs of toxicity namely stretching of the body, weakness, isolation, brushing of the nose on the floor, salivation, sleeping, coma and death in 24hours. In phase II, three groups of one rat each were administered orally with the extracts at doses of 1600mg/kg, 290mg/kg and 5000mg/kg. They were observed for 24hours and number of death was recorded. While the control groups were administered normal saline.

RESULTS

The Lethal Dose (LD_{50}) of petroleum ether, ethanol and aqueous extracts of *Adansonia digitata* stem bark at varying doses of 10 mg/kg, 100 mg/kg, and 1000mg/kg body weight for each extract, shows no mortality within 24 hours and up to 2 weeks after treatment with the extracts (Table 1). The Lethal Dose (LD_{50}) of petroleum ether, ethanol and aqueous extracts of *Adansonia digitata* stem bark at varying doses of 1600 mg/kg, 2900 mg/kg, and 5000mg/kg body weight for each extract shows no mortality within 24 hours and up to 2 weeks after treatment with the extract (Table 2). And the LD_{50} value was found to be greater than 5000 mg/kg body weight on the three (3) plant extracts tested.

Table 1: Lethal Dose (LD_{50}) for petroleum ether extract, ethanol extract and aqueous extract of *Adansonia digitata* stem bark.

PHASE I

Extract Dose (mg/kg body weight)	PEE	EE	AE
10	0/3	0/3	0/3
100	0/3	0/3	0/3
1000	0/3	0/3	0/3

KEY: PEE= Petroleum ether extract, **EE=** Ethanolic extract, **AE=**Aqueous extract
(0/3)= 0= Number of death, **3=** Number of rats used for the test

Table 2: Lethal Dose (LD_{50}) for petroleum ether extract, ethanolic extract and aqueous extract of *Adansonia digitata* stem bark.

PHASE II

Extract Dose (mg/kg body weight)	PEE	EE	AE
1600	0/1	0/1	0/1
2900	0/1	0/1	0/1
5000	0/1	0/1	0/1

KEY: PEE = Petroleum ether extract**, EE=** Ethanolic extract**, AE=**Aqueous extract
(0/1) =0= Number of death**, 1=** Number of rats used for the test

DISCUSSIONS

This study demonstrate the acute toxicity level of petroleum ether, ethanolic and aqueous extracts of stem bark of *Adansonia digitata* using albino Wister rats, With no mortality recorded in any of the experimental groups in 24hours and up to two weeks after oral administration of 5000mg/kg of each of the extracts. The stem bark is unlikely to cause fatality in humans because any substance with LD_{50} above this observed level is considered to be practically harmless,

A preliminary study has shown that this plant contains alkaloids, Flavonoids and Saponins (Cowan, 1999). In particular the Flavonoids were reported to be responsible for antimicrobial activity associated with some ethno medicinal plants (Singh and Bhat, 2003). Saponins enhance nutrient absorption and aid in animal digestion. Significant toxicity is usually as a result of suicide attempt or inappropriate self administration for therapeutic purposes (Raffi and Mark, 2009).

Also, alkaloids have some pharmacological effects and are used as medications, recreational drugs, or in entheogenic rituals e.g. the local anesthetic and stimulant cocaine, the stimulant caffeine, the analgesic morphine or the antimalarial drug quinine (Tailang and Sharma, 2009). The different plant parts provide food, shelter, clothing and medicine as well as material for hunting and fishing (Venter & Venter (1996) cited in Gebauer *et al.,* 2002). The bark contains the alkaloid 'adansonin', which has a strophanthus-like action (Sidibe & Williams, 2002).

This agreed with the report that, the extracts are non-toxic to brine shrimp larvae (Musila *et al.,* 2013). This supports the results obtained in this study on the nontoxic nature of extracts on albino Wister rat. Non toxicity of *A. digitata* explains why most of the plant parts, seeds, fruit pulps, stem and leaves are consumed by many communities (Kamatou *et al.,* 2011; Nguta *et al*., 2011). According to toxicity classes of Hodge and Sterner (2005), any compound with oral LD_{50} (rat) of 5000mg/kg or more should be considered as practically harmless. Hence, oral administration of "petroleum ether, ethanolic and aqueous extracts" at a dose of less than or equal to 5000mg/kg could be safe.

CONCLUSION

It may be concluded that the administration of crude stem bark extracts of *Adansonia digitata* proved to be practically nontoxic on albino Wister rat. This may explains the scientific basis for the use of crude stem bark extracts of *Adansonia digitata* in the traditional system of medicine for treatment of diseases.

RECOMMENDATIONS

There is a need for further research on chronic toxicity profile of the stem bark extracts to assess the significant effects on th e function of liver, kidney, small intestine an d other vital organs.

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