



## EXTRACTION OF *CROSSOPTERYX FEBRIFUGA* STEM BARK AND ACUTE TOXICITY STUDIES {LD<sub>50</sub>} ON ALBINO RATS

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### ABSTRACT

The research was undertaken to determine extraction of *Crossopteryx febrifuga* stem bark and acute toxicity studies on albino rats. The stem bark of *C. febrifuga* plant collected from Sebare farm, Mayo belwa, Adamawa state was identified, shade-dried at room temperature, grinded into fine powder using pestle and mortar. Fifty-four (54) healthy albino rats were divided into three groups with 18 rats per group to determine the median lethal dose (LD<sub>50</sub>) of each extract (ethanol, ethyl acetate and aqueous). The result of LD<sub>50</sub> calculated were 5336.67 mg/kg body weight, 5890.0 mg/kg body weight, and 5776.67 mg/kg body weight for ethanol, ethyl acetate, and aqueous extracts, respectively, indicating a wide margin of safety and are safe to use in animals. In conclusion, the extracts contain phytochemicals and hormones of veterinary importance and are safe to use in animals. Further studies on Pharmacological activities of the plant should be investigated in animals.

**Keywords:** *Crossopteryx*, *Febrifuga*, Phytochemistry, Chromatography, Spectrometry

### INTRODUCTION

Extraction of medicinal plants is a process of separating active plant materials or secondary metabolites such as alkaloids, flavonoids, terpenes, Saponins, Steroids and glycosides from inert or inactive materials using appropriate solvent and standard extraction procedure (Abubakar and Hague, 2020). Several methods are used in the extraction of medicinal plants such as maceration, infusion, decoction, percolation, digestion, and Soxhlet extraction. Median lethal dose (LD<sub>50</sub>) is a toxic unit that measures lethal dose of a given substance (Pillai *et al.*, 2021). The value of LD<sub>50</sub> for a substance is the dose required to kill half the members of a tested population after a specified test duration. LD<sub>50</sub> figures are frequently used as a general indicator of a substance's acute toxicity. A lower LD<sub>50</sub> is indicative of higher toxicity. The "minimal lethal dose" was widely used terminology in the early 1900, to express quantitatively the toxicity of a substance and it interpreted differently among toxicologists. Some interpreted minimal lethal dose as the dose just sufficient to kill only an occasional animal or the dose that kills 50% of animals of the dosed group or the dose that kills all the dosed animals. Hence, this study aimed to determine extraction of *Crossopteryx febrifuga* stem bark and acute toxicity on albino rats.

### MATERIALS AND METHODS

#### Experimental Site

The experiment was carried out at the department of Biochemistry Lab, Modibbo Adama University Yola, located within Girei LGA of Adamawa State. It lies between latitude 9° and 11° north of the Equator and longitude 11° and 14° east of the Greenwich Meridian. The state is in the North-East geopolitical zone of Nigeria, bordered by Borno to the Northwest, Gombe to the west, and Taraba to the Southwest while its eastern border forms part of the national border with Cameroon. The climatic conditions of the area are constant. The temperature of the area is high throughout the year, with the maximum temperature of about 42°C observed with minimum temperature range of between 26.9°C and 27.8°C. The rainfall shows a variable element of tropical climate to which most of the characteristics such as amount, frequency and intensity vary widely with time (season), it usually starts during the month of April to October with an annual range of 1500 – 2000 per annum and this is accompanied by high relative humidity (Alexander, 2015). The relative humidity is very low between January and March about (20-30%) which will starts increasing from April to reach its



peak in August and late September, while the cool dry harmattan starts in November to February (Adebayo, 2010). The study area falls within the North Guinea Savannah vegetation zone and has a tropical wet and dry climate. This zone is characterized by high grassland with shrubs and fewer trees (Adebayo *et al.*, 2020).

#### **Plant Collection and Identification**

The stem bark of *C. febrifuga* plant collected from Sebore farm, Mayobelwa Local Government Area of Adamawa State. The plant identified by the staff of the Department of Forestry and Wildlife of Modibbo Adama University Yola. The stem bark was shade-dried at room temperature. The sample was grinded into fine powder using pestle and mortar. The powdered plant sample was stored in appropriate container until required for use.

#### **Extraction Methods**

Three extraction methods used and these are Solvent extraction method using ethanol, solvent extraction method using ethyl acetate and solvent extraction method using aqueous.

##### ***Solvent Extraction Method Using Ethanol.***

The stem bark collected was shade dried at a room temperature in a clean environment to avoid contamination for 14 days and powdered in a domestic grinder. Extract of the stem bark made by maceration method using 95% Ethanol. After vigorous shaking to ensure thorough mixing, it allowed to settle for 48 hours. The solution was filtered using cheesecloth and allowed to evaporate in an oven at a temperature 50<sup>0</sup> C. A dark green residue obtained after the evaporation process is the ethanol extract.

##### ***Solvent Extraction Method Using Ethyl Acetate***

The stem bark was shade dried at a room temperature in a clean environment to avoid contamination for 14 days and powdered in a domestic grinder. Extract of the plant made by maceration method using ethyl acetate as a solvent. Two hundred (200) grams of shade-dried stem bark powder extracted with ethyl acetate for 72 hours at room temperature. The extract filtered through cheesecloth and through Whatman filter paper No 1. The filtrate was evaporated to 500ml at room temperature and then in a vacuum concentrator. The dried powder obtained was stored in a sterile glass bottle and used for the research as described by Javarappa *et al.* (2016).

##### ***Solvent Extraction Method Using Aqueous***

A standard method used to obtain the aqueous extract by boiling the powder of the stem bark in water at a temperature range of 60 – 80<sup>0</sup> C for approximately 20-30 minutes and the mixture filtered through Whatman filter paper to obtain the aqueous extract used for the research.

#### **Determination of LD<sub>50</sub> in Albino rats/Acute Toxicity Study**

A total of fifty-four (54) healthy albino rats were procured from National Veterinary Research Institute (NVRI) Vom through the Department of Biochemistry of Modibbo Adama University and kept in plastic cages and were allowed to acclimatize for a period of one week before the commencement of the study. The albino rats fed adequately with feeds and water ad libitum throughout the experimental period. The method of Karber used to determine the LD<sub>50</sub> of ethanol extract of *Crossopteryx febrifuga* and extract of same specimen using ethyl acetate and aqueous as solvents. The albino rats subdivided into three groups. The first group of eighteen albino rats were used to determine the LD<sub>50</sub> of *C. febrifuga* extract using ethanol as solvent while the second and third groups were used to determine the LD<sub>50</sub> of *C. febrifuga* extract using ethyl acetate and aqueous as solvents. The Karber's method involves the administration of different doses of test substance to various groups, which has three animals each. The first group of animals administered with the vehicle in which the test substance dissolved or diluted. However, from the second group onward receives different doses of the test substance. The animals in each group receives specific doses, while increment in dose progresses from group to group (starting from group 2). The interval means of a number of mortalities recorded in each group and dose different across the groups are key parameters in this method (Turner, 1965). The volume of each dose calculated using the following;

Vol = weight of rat in kg x dose of extract administered/kg

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Concentration of extract in mg/ml



The animals observed for 24-48 hours for toxicity signs and death after oral administration of the extract. The LD<sub>50</sub> of the extract calculated using the arithmetic method of Karber (Angalabiri-Owel *et al.*, 2014; Elekima *et al.*, 2017).

$$LD_{50} = LD_{100} - \sum \left( \frac{a \times b}{n} \right)$$

Where:

LD<sub>50</sub> = Median lethal dose

LD<sub>100</sub> = Least dose required to kill 100%

a = Dose difference b = Mean mortality n = Group population

## RESULTS

### **Behavioural/Clinical Signs in Rats Administered with Ethanol, Ethyl Acetate and Aqueous Extracts of *C. febrifuga* (Stem Bark)**

Table 1 showed the result of LD<sub>50</sub> after the administration of ethanol extract with the toxicity signs observed on albino rats. The result revealed that rats administered with extract at a dose of 500, 1000, and 2000 mg/kg body weight did not show signs of toxicity such as Restlessness, weakness, ataxia, coma and death, while administration of 4000-5000 mg/kg body weight showed toxicity signs of restlessness, weakness, ataxia and coma. Two (2) rats survived per group while all the remaining rats' in-group six (6) died after the administration of the extract at 6000 mg/kg body weight.

Table 1 also showed the results of LD<sub>50</sub> after the administration of Ethyl acetate extract. No toxicity signs such as restlessness, weakness, ataxia and coma observed on the albino rats after the administration of 500, 1000, 2000, 3000, 4000, 5000 mg/kg body weight while administration of 6000mg/kg body weight showed toxicity signs of restlessness, weakness, ataxia, coma and death of one (1) of the albino in group six (6). The calculated LD<sub>50</sub> showed that the extract is safe.

In the same Table 1, the result of LD<sub>50</sub> after the administration of Aqueous extract showed no toxicity signs such as restlessness, weakness, ataxia and coma observed on the albino rats after the administration of 500, 1000, 2000, 4000, and 5000 mg/kg body weight while the administration of 6000 mg/kg body weight showed the toxicity signs of restlessness, weakness, ataxia, coma and death of two (2) albino rats in group six (6).



**Table 1:** Behavioural/Clinical Signs Observed in Albino Rats Administered with Ethanol, Ethyl Acetate and Aqueous Extracts of *C. febrifuga* (Stem Bark)

Groups	Dose/(mg/kg body weight)	Ethanolic		Ethyl acetate		Aqueous	
		Observation	Mortality	Observation	Mortality	Observation	Mortality
1	500	Normal behaviour	0	Normal behaviour	0	Normal behaviour	0
2	1,000	Normal behaviour	0	Normal behaviour	0	Normal behaviour	0
3	2,000	Normal behaviour	0	Normal behaviour	0	Normal behaviour	0
4	4,000	Restlessness, Weakness, Ataxia, Coma	1	Normal behaviour	0	Normal behaviour	0
5	5,000	Restlessness, Weakness, Ataxia Coma	1	Normal behaviour	0	Normal behaviour	0
6	6,000	Restlessness, Weakness, Ataxia, Coma	3	Restlessness, Weakness, Ataxia Coma	1	Restlessness, Weakness, Ataxia Coma	2

**Table 2:** Calculated LD<sub>50</sub> of Ethanol Extract of *C. febrifuga* (Stem Bark)

Group	Dose(mg/kg)	Ethanol extract			
		Dose difference (a)	Dead	Mean death (b)	Product (a x b)
1	500	500	0	0	0
2	1000	500	0	0	0
3	2000	1000	0	0	0
4	4000	2000	1	0.33	660
5	5000	1000	1	0.33	330
6	6000	1000	3	1	1000

$$LD_{50} = LD_{100} - \sum \left( \frac{a \times b}{n} \right)$$

Where:

$$50 = h$$

$$100 = 100\%$$

a = Dose difference b = Mean mortality n = Group population

$$LD_{50} = LD_{100} - \frac{\text{sum of product}}{\text{number of animals per group}}$$

i. Least dose required to kill all the animals = 6000 mg/kg

ii. Sum of product (a × b) = 1990

iii. Number of animals per group (n) = 3

$$\begin{aligned} \text{Thus } LD_{50} &= 6000 - \frac{1990}{3} \\ &= 6000 - 663.33 \\ &= 5336.67 \text{ mg/kg body weight} \end{aligned}$$

The calculated LD<sub>50</sub> showed that the extract is safe.

**Table 3:** Calculated LD<sub>50</sub> of Ethyl Acetate Extract of *C. febrifuga* (Stem Bark)

Group	Dose(mg/kg)	Ethyl acetate extract			
		Dose difference (a)	Dead	Mean death	Product (a x b)
1	500	500	0	0	0
2	1000	500	0	0	0
3	2000	1000	0	0	0
4	4000	2000	0	0	0
5	5000	1000	0	0	0
6	6000	1000	1	0.33	330

$$LD_{50} = LD_{100} - \sum \left( \frac{a \times b}{n} \right)$$

Where:

$$50 = h$$

$$100 = 100\%$$

a = Dose difference b = Mean mortality n = Group population

$$\text{Thus, } LD_{50} = LD_{100} - \frac{\text{sum of product}}{\text{number of animals per group}}$$

i. Least dose required to kill all the animals = 6000mg/kg body weight

ii. Sum of product (a × b) = 330

iii. Number of animals per group (n) = 3

$$\begin{aligned} \text{Thus, } LD_{50} &= 6000 - \frac{330}{3} \\ &= 6000 - 110 \end{aligned}$$

$LD_{50} = 5890 \text{ mg/kg body weight}$

This showed that the extract has wide margin of safety and is safe to be used in animals.

**Table 4:** Calculated  $LD_{50}$  of Aqueous Extract of *C. febrifuga* (Stem Bark)

Group	Dose(mg/kg)	Dose difference (a)	Dead	Mean death (b)	Product (a x b)
1	500	500	0	0	0
2	1000	500	0	0	0
3	2000	1000	0	0	0
4	4000	2000	0	0	0
5	5000	1000	0	0	0
6	6000	1000	2	0.67	670

$$LD_{50} = LD_{100} - \sum \left( \frac{a \times b}{n} \right)$$

Where:

$$50 = h$$

$$100 = 100\%$$

a = Dose difference b = Mean mortality n = Group population

$$\text{Thus, } LD_{50} = LD_{100} - \frac{\text{sum of product}}{\text{number of animals per group}}$$

i. Least dose required to kill all the animals = 6000 mg/kg body weight

ii. Sum of product (a x b) = 670

iii. Number of Animals per group (n) = 3

$$\text{Hence, } LD_{50} = 6000 - \frac{670}{3}$$

$$= 6000 - 223.33$$

$$= 5776.67 \text{ mg/kg body weight}$$

The calculated  $LD_{50}$  for Aqueous extract also showed that the extract has wide margin of safety and is safe to be used in animals

## DISCUSSION

### Acute Toxicity Study for the Determination of $LD_{50}$ in Rats

Acute toxicity study is one of the initial screening experiments carried out with all compounds in the search for new drugs (Angalabiri and Isirima, 2014). Toxicity test examine toxic effects when a chemical is absorbed into the body, via mouth, skin, lungs. The most common test of acute (short term) toxicity is  $LD_{50}$  test. Many different substances are tested in this way, including all drugs, agricultural chemicals, cleaners, some cosmetics and their ingredient (Deora *et al.*, 2010). Median lethal dose ( $LD_{50}$ ) means if we administer any dose of drug to animal group for experimental purpose for the estimation of therapeutic effectiveness of that drug, and 50% of the animal died then it means that particular dose of drug is lethal dose 50 ( $LD_{50}$ ).

The higher values of  $LD_{50}$  – 5336.67mg/kg body weight, 5890mg/kg body weight, and 5776.67mg/kg body weight for ethanolic, ethyl acetate and aqueous extracts of *Crossopteryx febrifuga* (stem bark) respectively obtained in this study is in agreement with (Clarke and Clarke, 1975) with the suggestion that any substance with intraperitoneal  $LD_{50}$  of above 1000mg/kg body weight should be regarded as safe. This was further corroborated by WHO (1986) and Deora *et al.* (2010) who stated that the higher the  $LD_{50}$  the lower the toxicity. The Opposite is also true that the smaller the  $LD_{50}$  value the higher the toxicity. It can therefore be inferred that the plant *C. febrifuga* under study is of low toxicity and safe.

The observed behavioural/Clinical signs of sedation, ataxia and mortality in rats that received  $\geq 4000 \text{ mg extract/kg body weight}$  suggests the involvement of central nervous system in its toxic effect and this is in agreement with Salawu *et al.* (2009).





Although plant extract can be deduced to be safe some dose dependent toxic manifestations may be observed following intraperitoneal administration. This may be due to the effect of one or more chemical constituents present in the extract for example tannins found in adequate quantity in plant have been implicated in decreased and reduced activity in rats

## CONCLUSION AND RECOMMENDATION

The stem bark extracts of the plants consist of important phytoconstituents. The high median lethal dose (LD<sub>50</sub>) of 533.67mg/kg body weight, 5890.0mg/kg body weight, 5776.67mg/kg body weight for ethanolic, ethyl acetate and aqueous extracts of *C. febrifuga* (stem bark) respectively showed that the extracts have wide margin of safety and are safe to be used in animals. It is therefore recommended that, toxicity testing in rats like Median Lethal Dose (LD<sub>50</sub>) should always serve as a key in the discovery of new drugs or medicinal plants in order to avoid under dosage or over dosage in case of toxicity in animals. Further studies on the pharmacological activities of *C. febrifuga* should be investigated in animals.

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