CHAPTER 7

EVALUATION OF TOXICOLOGICAL PROPERTIES OF STEM BARK OF *PHYLLANTUS ACIDUS* (L.)SKEELS AND ROOT BARK OF *CROTON CAUDATUS* GEISELER

7.1: Introduction

Traditional and alternative medicine is extensively practiced in the prevention, diagnosis, and treatment of various illnesses. It has attracted increasing public attention over the past 20 years as this type of medicine is easily accessible in some regions (***Humber, 2002***)**.** The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins; flavonoids and phenolic compounds ***(Hill, 1952*)**. They contribute great importance in daily life by providing wide range of nutrients, vitamins and other compounds which widen the therapeutic value. They are sometimes added to foods meant for pregnant and nursing mothers for medicinal purposes ***(Okwu, 1999, 2001).*** In general, natural products play a dominant role in the development of novel drug leads for the treatment and prevention of diseases (***Newman et al., 2003***)**.**

Toxicity indicates the state of adverse effects led by the interaction between toxicants and cells. This interaction may vary due to the chemical properties of the toxicants and the cell membrane, as it may occur on the cell surface, within the cell body, or in the tissues beneath as well as at the extracellular matrix. The toxic effects may

take place prior to the binding of the toxicants to the vital organs such as liver, brain and kidneys. Hence, evaluation of toxic propertiesof a substance is crucial when considering for public health protection because exposure to chemicals can be hazardous and lead to adverse effects on human being. In practice, the evaluation typically includes acute, sub-chronic, chronic, carcinogenic and reproductive effects ***(Asante-Duah, 2002).***

In general *in vivo* toxicity study is the toxicological analysis of many medicinal plants and its potency to evaluate qualitatively and quantitatively by histopathology and oral acute toxicity studies. Oral acute toxicity testing in mice could be used to evaluate natural remedies for different pharmacological activities, taking into account the basic premise that pharmacology is simply toxicology at a lower dose ***(Sasidharan et al., 2008)***. A toxic substance might elicit interesting pharmacological effects at a lower non-toxic dose. Toxicity results from animals will be crucial in definitively judging the safety of medicinal plants if they are found to have sufficient potential for development into pharmacological products ***(Moshi, 2007)***. As use of medicinal plants increases, experimental screening of the toxicity of these plants is crucial to assure the safety and effectiveness of those natural sources.

So, the present aim of the study was to investigate the toxicity potential of the stem bark of *Phyllantus acidus* (L.)Skeels and root bark of *Croton caudatus* Geiseler on liver, brain and kidney of Swiss albino mice.

7.2: Materials and Methods

7.2.1 Preparation of extract:

The root bark of *Croton caudatus* Geiseler and stem bark of *Phyllantus acidus* (L.)Skeels were clearly washed with water, shade dried (air dried) and powdered. The powdered plant materials (300gm) were defatted with petroleum ether. After washing with petroleum ether the residue were extracted exhaustively with 200ml distilled methanol by using soxhlet apparatus. The extract was filtered through cotton followed by vaccum suction. The solvnt was then removed by distilling at 20-30^{0}C to give a reddish brown sticky smi-solid mass and blakish brown semi-solid extract.

7.2.2 Experimental design:

Animals:

Swiss albino mice weighing between 25-30g, purchased from Pasteur Institute, Shillong were used for the study. The mice were divided into four groups (I, II, III, and IV) of five animals each. The animals were maintained in closely inbred colony under conventional laboratory conditions at room temperature and in 12hr.light/dark cycle. Mice were provided with standard food pallet and water *ad libitium*. All experimental procedures followed animal research guidelines.

The present study aims to determine the toxicity of methanolic extract of stem bark of *Phyllantus acidus* (L.)Skeels and root bark of *Croton caudatus* Geiseler using an acute oral toxicity test **(*Joshi et al., 2007*)** in animal models**.** The acute oral toxicity testing was carried out on Swiss albino mice following the guidance of the Organization for Economic Cooperation and Development **(*OECD, 2001*).**

7.2.3 Statistical analysis: Results are presented as Mean ± SEM (using MS Excel v 2003).

7.2.4 Acute toxicity study (determination of LD_{50}) of stem bark of *Phyllantus acidus* (L.)Skeels:

Four groups of five animals were used in the study. Group I serve as control group and the other groups II, III and IV were tested groups, the dose of extracts being 1000, 2000 and 3000mg/kg respectively. The number of mice that died within 24hrs was noted and their LD_{50} of the extract was calculated using the arithmetic method of ***Karber*** as modified by ***Aliu and Nwude (1982)*** as shown below:

LD_{50} = Least dose that killed all the animals – Sum of (Dose difference X Mean dose)/No. of animals.

Or

LD_{50} = Maximum dose that killed all the animals – Sum of (Dose difference X Mean dose)/No. of animals.

7.2.5 Sub-acute toxicity study of stem bark of *Phyllantus acidus* (L.) Skeels:

None of the animals in the oral toxicity study died. Therefore, the extract was administered to the animals for seven days. The animals used in this study received their respective doses with free access to food and water. Signs of toxicity and mortality were observed daily for seven days and were monitored daily for changes in body weight. On 8^{th} day, the mice were sacrificed by decapitation and the vital organs namely the liver, kidney and brain of each mice were carefully removed and were fixed in 10% formal saline for further histopathological investigations.

7.2.6 Absolute and relative organ weight of mice treated with extract of stem bark of *Phyllantus acidus* (L.)Skeels:

The alterations of body weight gain and internal organ weights of mice would reflect the toxicity after exposure to the toxic substances (***Carol, 1995)*.** The body weight changes are indicators of adverse effects of drugs and chemicals and it will be significant if the body weight loss occurred is more than10% from the initial weight (***Raza et al., 2002, Teo et al., 2002*).** The relative organ weight is fundamental to diagnose whether the organ was exposed to the injury or not and also an important index of physiological and pathological status in animals. The liver, brain and kidney are the primary organs affected by metabolic reaction caused by toxicant (***Dybing et al., 2002*).** So, organs such as liver, brain and kidney were collected and weight of all the organs was taken. Relative organ weight were measured and compared with the control group.

7.2.7 Acute Toxicity Study (LD_{50} determination) of root bark of *Croton caudatus* Geiseler:

The acute oral toxicity testing was carried out on Swiss albino mice following the guideline of the Organization for Economic Cooperation and Development (***OECD, 2001***). Different doses of the extract were administered orally to four groups of mice consisting of 7 mice in each group. The animals in group I served as control and received distilled water. The animals in groups II, III and IV received 1ml aquous solution of 500, 1000 and 1500mg of extracts per kg body weight respectively through oral administration with a graduated syringe. They were all placed under observation for 24 h after which the numbers of dead rats were recorded, and LD_{50} was determined by

oral route according to the method of ***Karber*** adapted by ***Aliu and Nwude (1982).***

7.2.8 Sub-acute toxicity study of root bark of *Croton caudatus* Geiseler:

None of the animals in the oral toxicity study died. Therefore, the extract was administered to the animals for seven days. The animals used in this study received their respective doses with free access to food and water. On 8^{th} day, the rats were sacrificed by decapitation and the vital organs namely the liver, kidney and brain of each mice were carefully removed and were fixed in 10% formal saline for further histopathological investigations.

7.3: Results and Discussion

7.3.1 Acute toxicity study of stem bark of *Phyllantus acidus* (L.)Skeels:

No mortality was observed after the administration of metanolic extract of stem bark of *Phyllantus acidus* (L.)Skeels at the doses of 1000, 2000 and 3000mg/kg body weight. The calculated LD_{50} was presented in Table 7.4.1.

Table 7.3.1. LD_{50} of stem bark of *Phyllantus acidus* (L.)Skeels calculated by arithmetic method of Karber Groups.

Groups	Dose(mg/kg)	No of mice	No of death	Dose difference	Mean death(Md)	Dose differenceX Md
I	Control (1ml dis.H_20)	5	0	0	0	0
II	1000	5	0	1000	0	0
III	2000	5	0	1000	0	0
IV	3000	5	0	1000	0	0

LD_{50}= Maximum dose–Sum of (Dose difference X mean death)/No of animals

=3000 mg/kg.

7.3.2 Absolute and relative organ weight of mice treated with extract of stem bark of *Phyllantus acidus* (L.)Skeels:

The liver, brain and kidney are the primary organs affected by metabolic reaction caused by toxicants ***(Dybing et al., 2002*)**. Administration of methanolic extract of *Phyllantus acidus* (L.)Skeels did not show any effect on the absolute and relative organ weight and was almost same in control and treated groups as shown in theTable and Fig.7.3.2(a).

Table 7.3.2(a). Absolute organ weight (g) and Relative organ weight volume (g/100g b.wt.) of acute toxicity study of *Phyllantus acidus* (L.)Skeels extract in Swiss albino mice.

	Control		PAE-1000		PAE-2000		PAE-3000	
Name of organs	A.O.W	R.O.W	A.O.W	R.O.W	A.O.W	R.O.W	A.O.W	R.O.W
Liver	1.66±0.05	6.24±0.21	1.70±0.10	6.26±0.25	1.71±0.10	6.22±0.18	1.79±0.06	6.45±0.21
Brain	0.55±0.01	2.10±0.05	0.58±0.02	2.15±0.06	0.60±0.03	2.18±0.05	0.64±0.03	2.31±0.12
Kidney	0.36±0.02	1.34±0.04	0.39±0.02	1.43±0.10	0.40±0.03	1.47±0.12	0.46±0.02	1.67 ±0.17
Body weight	26.6 ±1.21		27.2±1.28		27.5±1.34		27.9±1.24	

Key note; PAE-*Phyllanthus acidus* extract, A.O.W= Absolute organ weight, R.O.W= Relative organ weight.

The body weight changes in albino mice treated with stem bark extract of *Phyllantus acidus* (L.)Skeels is shown in the Fig.**7.3.2(a)**.

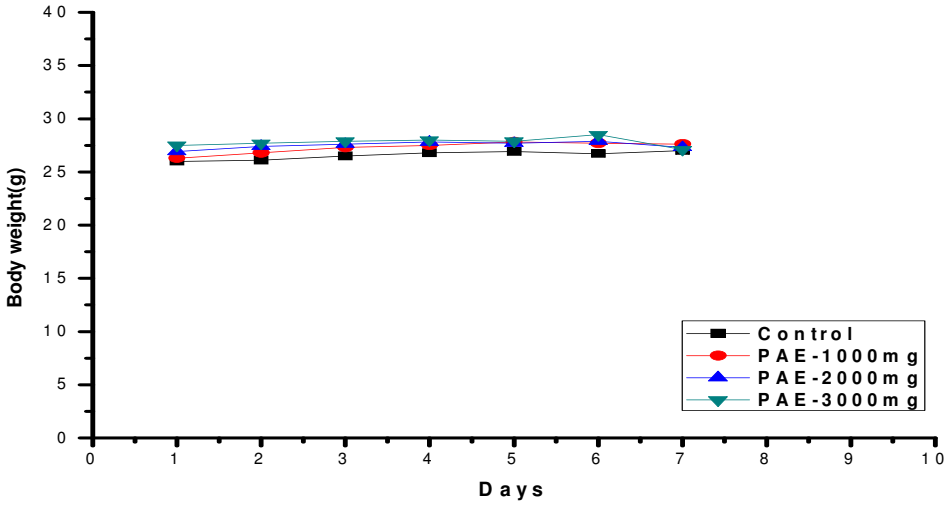


Fig.7.3.2(a): Effect of *Phyllantus acidus* (L.)Skeels extract on body weight changes in albino mice.

The histopathological changes observed in various organs are shown below the Table **7.3.2(b)**.

Table 7.3.2(b): Histopathological changes observed in various organs of mice treated with different doses of stem bark extract of *Phyllantus acidus* (L.)Skeels**.**

Dose (mg/kg)	Observed changes		
	Liver	Brain	Kidney
Control(1ml dis.H_20).	--	--	--
1000	--	--	--
2000	Mild congestion	--	--
3000	Mild congestion	Mild congestion	Mild congestion

The absence of histopathological lesions in the liver, brain and kidney except some mild congestion [**Table 7.3.2(b)]** indicates that the stem barks extract of *Phyllantus acidus* (L.)Skeels is of low toxicity.

7.3.3 Acute Toxicity Study (LD_{50} determination) of root bark of *Croton caudatus* Geiseler:

In the acute oral toxicity study, no death was recorded even at the higher dose of 1500mg/kg body weight (**Table 7.3.3**) which is an indication that the LD_{50} of the plant is higher than 1500mg/kg.

Table 7.3.3: LD_{50} of root bark of *Croton caudatus* Geiseler calculated by arithmetic method of Karber Groups.

Groups	Dose(mg/kg)	No of mice	No of death	Dose difference	Mean death(Md)	Dose differenceX Md
I	Control (1ml dis.H_20)	7	0	--	--	--
II	500	7	0	500	--	--
III	1000	7	0	500	--	--
IV	1500	7	0	500	--	--

LD_{50}= Maximum dose–Sum of (Dose difference X mean death)/No of animals.

=1500mg/kg.

7.3.4 Histological studies of root bark of *Croton caudatus* Geiseler:

The livers, brain and kidneys were dissected out and immediately fixed in 10% formalin solution. Using a standard tissue processor, the tissues were then dehydrated in ascending grades of alcohol: 70, 95% and absolute alcohol in 2 changes each. After which clearing was done with xylene/absolute alcohol [50:50v/v ratio].This was followed by infiltration in molten paraffin wax at 60°C in 2 changes. They were further processed for staining with haematoxylin and eosin (H&E) as described by ***John et al., 1990.*** Photomicrographs of the slides were taken for histological examination.

7.3.5. Histopathology of Liver

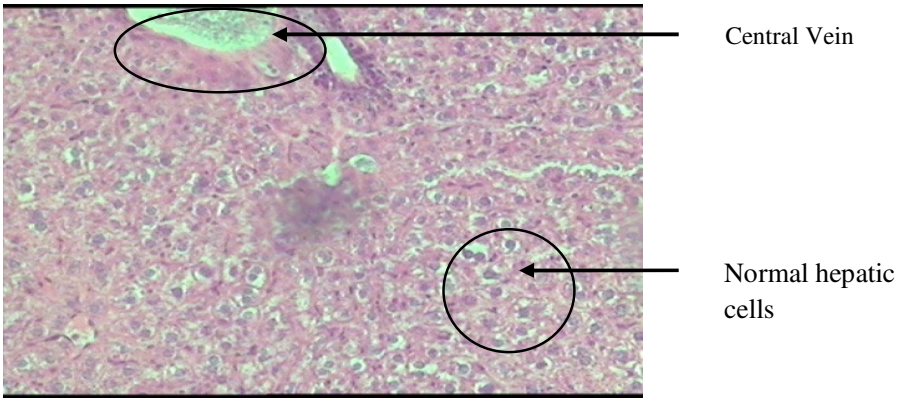


Fig.7.3.5(a): Transverse section of the liver of control mice which receives1ml/kg, distilled water.

It showed normal hepatic cells with well preserved cytoplasm, prominent nucleus and nucleolus and central vein.

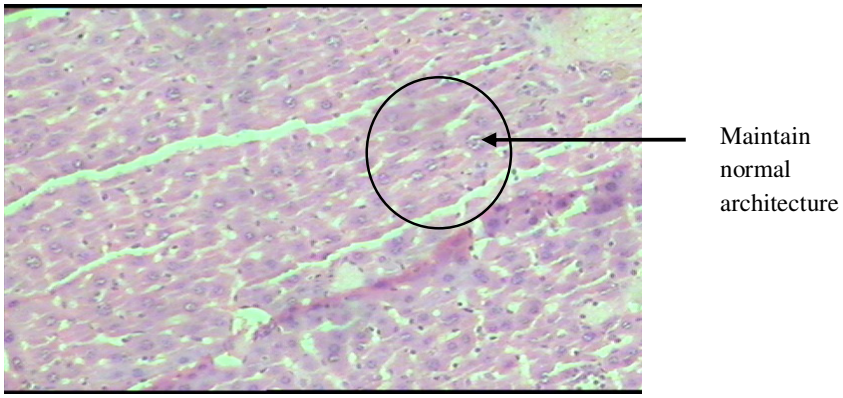


Fig.7.3.5 (b): Transverse section of the liver from mice which receives (1500mg/ kg) of root bark extract of *Croton caudatus* Geiseler.

Histology of liver of treated group showing that the cells are generally maintained normal architecture after repeated treatment of root bark extract of *Croton caudatus* Geiseler of higner dose(1500mg/kg body weight) as compared to the normal.

7.3.6. Histopathology of Kidney

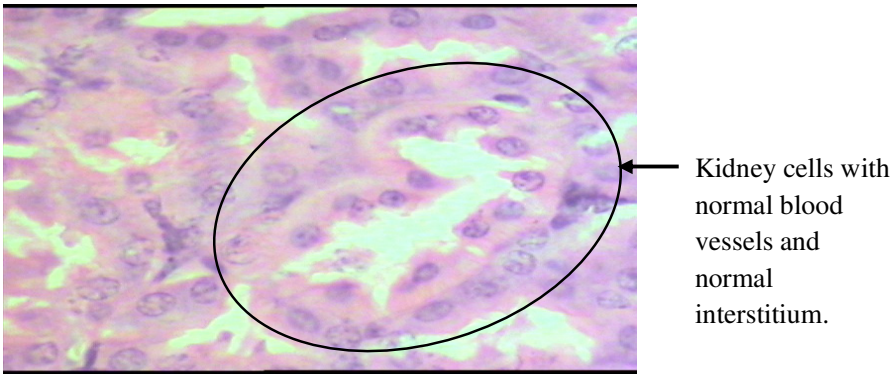


Fig.7.3.6 (a): Transverse section of the kidney of control mice which receives 1ml/kg, distilled water.

In the kidney of control treated mice, it was observed that the glomerular tufts, the blood vessels and the interstitium were quite normal.

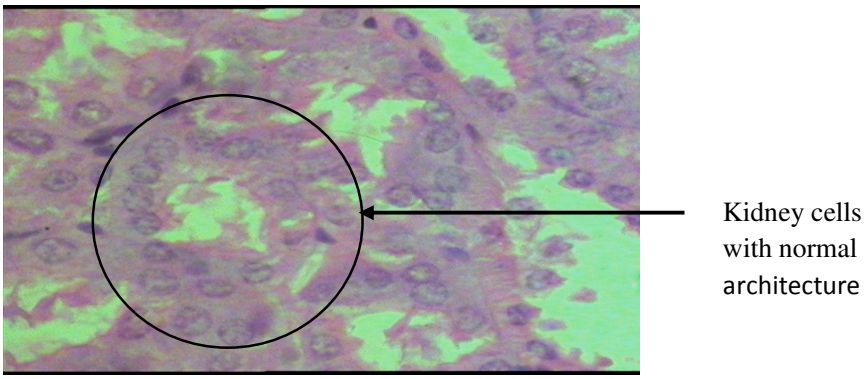
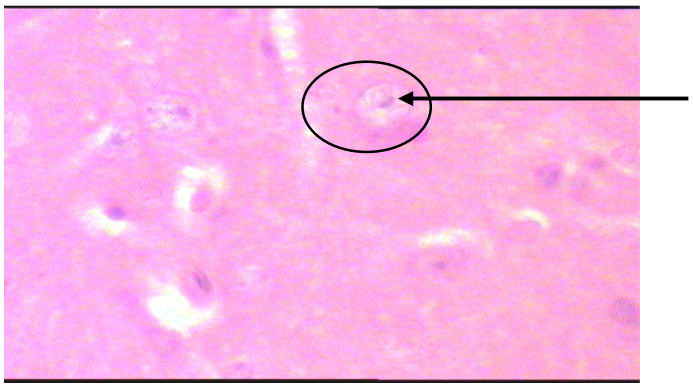


Fig.7.3.6(b): Transverse section of the kidney from mice which receives (1500mg/kg) of root bark extract of *Croton caudatus* Geiseler.

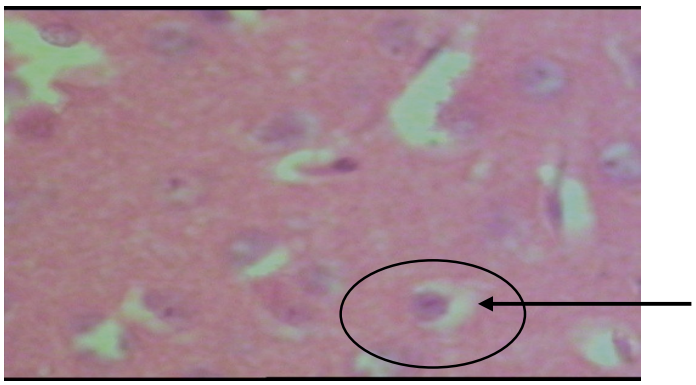
No appreciative architecture changes takes place in kidney in 1500mg/kg body weight of treated mice group. The kidney tubules remain same as normal with bland nucleus and abundant cytoplasm.

7.3.7. Histopathology of brain



(H & E X 40)

Fig.7.3.7(a): Transverse section of normal brain which receives 1ml/kg, distill water.



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(H & E X 40)

Fig.7.3.7(b): Transverse section of the brain from mice which receives (1500mg/ kg) of root bark extract of *Croton caudatus* Geiseler.

The brain was found to be normal as there are some enlarge nucleus of the brain cell but no appreciative architectural changes in 1500mg/kg body weight of treated mice as compared to the normal one.

From the above oral acute toxicity study, the calculated LD_{50} of the extract of stem bark of *Phyllantus acidus* (L.)Skeels is found to be 3000mg/kg body weight and that of *Croton caudatus* Geiseler is

1500mg/kg body weight, indicating that both the extracts is of low toxicity.

The absence of histopathological lesions in the liver, brain and kidney except some mild congestion [Table 7.3.2(b)] also indicates that the stem barks extract of *Phyllantus acidus* (L.)Skeels is of low toxicity.

Under the dose (500, 1000 and 1500mg/kg) treatment of the methanol extract of *Croton caudatus* Geiseler, the body weight of the experimental groups (albino mice) remains same as compared to the control groups.

The liver of the mice which receives 1500mg/ kg of root bark extract of *Croton caudatus* Geiseler did not produce any potential toxicity on as there was no necrosis, inflammation or fibrosis [Fig.7.3.5(b)]. The limiting plate was found to be well preserved and the central veins were normal.

The kidney tubules also remain same as normal with bland nucleus and abundant cytoplasm in 1500mg/kg treated mice group.The blood vessels and the interstium were essentially normal and there was no inflammation or fibrosis [Fig.7.3.6(b)] as compared to the normal.

The brain was found to be normal with some enlarge in the nucleus of the brain cell but no appreciative architectural changes in 1500mg/kg body weight of treated mice group [Fig.7.3.7(b)].

According to the toxicity scale of Hodge and Sterner, any compound with an oral LD_{50} of between 500 – 2000 mg/kg should be considered practically non toxic which agrees with the findings of ***Allain (2000)*** who ranks all plants whose LD_{50} figures are less than 25 mg/kg in "very toxic" group, between 25 and 200 mg/kg in "toxic "group, and from 200 to 2000 mg/kg in "no toxic" group. Presently, the

chemical labeling and classification of acute systemic toxicity based on oral LD_{50} recommended by the Organization for Economic Co-operation and Development (***OECD, Paris, France, 1998***) (***Walum, 1998***) are as follows: very toxic, <5 mg/kg; toxic, >5<50 mg/kg; harmful, >50<500mg/kg; and no label, >500<2000 mg/kg.

7.4: Conclusion

The LD_{50} obtained from oral administration of the extracts, lack of mortality when orally administered and histopathological studies in mice, both the methanol extract of stem bark of *Phyllantus acidus* (L.)Skeels and root bark of *Croton caudatus* Geiseler were found to be non toxic. Both the extract could be used with some degree of safety especially when consumed by oral route.

7.5: Bibliograpy

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