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Acute toxicity studies of *Lophira lanceolata* leaf extract

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ABSTRACT

Acute toxicity studies were carried out using rats weighing between 100-160g. Five groups of six rats each were used. They were administered (intraperitoreally) with varying doses (500, 1000, 2000, 4000 and 6000 mg/kg) of all the extract (ethanol extract 'A', acetone extract 'B', Chloroform 'C', and Water, 'D'). There was no mortality 24 hours after administration of extract 'E' water. However, in extract C there was mortality at 2000 and 6000 mg/kg. The clinical signs appeared to be dose-dependent Tables 1 to 10 show the calculation of the LD_{50} of the extract A, B, C and D and the dose administered to mortality rate. The result of an acute toxicity on administration of the extract inter peritoneally for 24 hours showed that the LD_{50} calculated for the extract were at close range (3.750 g kg^{-1}- 6.0g kg^{-1}). However the chloroform extract was found to posses a higher LD_{50} value. Although the extracts are a combination of many different compounds, therefore it is possible to get much lesser LD_{50} with isolated pure compounds. The percentage mortality equally shows that the water extract was less toxic on comparison with the other three extracts

Keywords: Acute toxicity; Leaf extract; *Lophira lanceolata*

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INTRODUCTION

Over the past few decades natural products research has undergone a number of significant changes and a variety of natural products have been isolated, characterized, and synthesized, providing treatments for many otherwise incurable human and animal diseases, and agrochemicals for improved food production [3].

Antispasmodic, often called spasmodics or carminatives, are used to ease gripping or colic pains, expels wind (flatus) and relieve dyspepsia or indigestion. The plant kingdom is rich in such compounds; in fact most remedies used in conventional medicine include at least one antispasmodic of plant origin, even if only the ubiquitous peppermint oil [5].

The dried part of the plant is used in Mali for the treatment of toothache when the hot water extract is taken orally by the human adult. The dried stem is used as chewing stick in Mali. The bark is used for fever and gastrointestinal disorders. The root bark is a remedy in southern Nigeria for simple jaundice conditions (yellow fever). The leaves are used in the river Benue as a wash for women in childbirth. An infusion of the young leaf shoots is used as a lotion, used internally for fever, in respiratory complains and to relieve the griping of dysentery. In Liberia, the tree is a great native medicine for leprosy using an infusion of the bark taken orally or the oil, leaves or bark beaten up and rubbed on the body, and the finely grounded dry bark or leaves spread over the ground where the patient lies [1]. An infusion of the young leaves plants is taken orally used internally for treatment of fever [4]. The aim of the present work was to determine the acute toxicity (LD_{50}) of the plant leaf extract. The purpose of the study was to determine the effect of the extract on administration and to show the order of lethality.

EXPERIMENTAL

Plant material & extraction. The plant under screening is a spermatophyte collected from, Sakaru village along Jos road Zaria, in December 2005 and identified at the Herbarium section, Biological Science Department Ahmadu Bello University Zaria as *Lophira lanceolata*_ a voucher coded 4002B was deposited in the Herbarium.

Dried and coarsely powdered leaves of *Lophira lanceolata* (1kg) were maceration with petroleum ether (60-80^{o}C) for 10 hours. The extract was decanted off and fresh quantity of the petroleum ether was added again and macerated to exhaustion. The defatted leaves were completely dried and extracted twice with acetone to exhaustion. The combined acetone extracts (marked 'B') where concentrated on water bath whereby a highly viscous greenish – brown mass was obtained. This was refluxed with petroleum ether (60-80^{o}C), ethanol (marked 'A') and chloroform (marked 'C') successively until the solvent in each case was almost colourless. The residue left behind was then treated with hot water (marked 'D'). The water insoluble portion was dissolved in acetone and dried under reduced pressure. A solid brown residue obtained respond to usual flavonoid colour tests was marked 'E'. The aqueous solution

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was extracted with ethyl acetate. The process was repeated twice. The ethyl acetate extracts were combined and the solvent was recovered under reduced pressure. The semi-solid residue thus obtained was marked 'G' and was tested for the presence of flavonoids.

Experimental animals

The rats weighing between 100-160g were used for this study. The animals where maintain with local animal feed. They were handled humanely according to the international ethical committee on animal handling.

Acute toxicity studies were carried out using rats weighing between 100-160g. Five groups of six mice each were used. They were administered (intra peritoneally) with varying doses (500, 1000, 2000, 4000 and 6000 mg/kg^{-1}) of all the extract (A, B C and D). The Rats were observed for clinical signs of toxicity and death.

RESULTS

The tables 1 to 10 show the calculation of the LD_{50} of the extract A, B, C and D, and the dose administered to mortality rate.

DISCUSSION

The result of the phytochemical analysis of the leaves extracts of *Lophira lanceolata* showed that the extract contains carbohydrates, tannins, saponins, cardiac glycosides, terpeniods, steroids, anthraquinone, and flavonoid. Alkaloid was absent

Table 1: Calculation of the LD_{50} of Extract 'A' Ethanol

Group	Dose of extract (mg/kg)	Dose difference (Dd)	No. of Rats	No Dead	Mean Dead (Md)	Dd x Md
1	500			0		
		500			0	0
2	1000		6	0		
		1000			0	0
3	2000		6	0		
		2000			0.5	1000
4	4000		6	1		
		2000			3.0	6000
5	6000		6	2		

Calculation of LD_{50}

$$LD_{50} = LD_{100} - \frac{Dd \times Md}{n}$$

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= 6000 – 7000/6
= 6000 – 1166.67
= 4.833g/kg n = number of animals per group

Table 2: Calculation of the LD_{50} of Extract B Acetone

Group	Dose of extract (mglkg)	Dose difference Dd	No. of Rats	No of Dead	Mean Dead Md	Dd x Md
1	500		6	0		
		500			0	0
2	1000		6	0		
		1000			0	0
3	2000		6	0		
		2000			1	2000
4	4000		6	2		
		2000			4	8000
5	6000		6	6		

Calculation of LD_{50}

$LD_{50} = LD_{100} - \frac{Dd \times Md}{n}$

= 6000 - 10,000/6
= 6000 – 1666.67

LD_{50} = 4.333 g/kg n = Number of animals per group

Table 3: Calculation of the LD_{50} of Extract 'C' Chloroform

Group	Dose of extract (mgkg)	Dose difference Dd	No. of Rats	No of Dead	Mean Dead Md	Dd x Md
1	500		6	0		
		500			0	0
2	1000		6	0		
		1000			0.5	500
3	2000		6	1		
		2000			2	4000
4	4000		6	3		
		2000			4.5	9000
5	6000		6	6		

Calculation of LD_{50}

$LD_{50} = LD_{100} - \frac{Dd \times Md}{n}$

= 6000 – 13500/6 = 6000- 2250 LD_{50} = 3.750 gkg^{-1} n = animal per group

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Table 4: Calculation of the LD_{50} of extract 'D' (water)

Group	Dose of extract (mg/kg)	Dose difference Dd	No of rats	No of Dead	Mean Dead Md	Dd x Md
1	500		6	0		
		500			0	0
2	1000		6	0		
		1000			0	0
3	2000		6	0		
		2000			0	0
4	4000		6	0		
		2000			0	0
5	6000		6	0		

Calculation of LD_{50}

$LD_{50} = LD_{100} - \frac{\text{Dose difference x mean death}}{\text{Number of rats (n)}}$

$LD_{50}.LD_{100} - \frac{Dd \text{ x } Md}{n}$

$LD_{50} = LD\ 6000 - \frac{Dd \text{ x } Md}{n}$

$= 6000 - 0/6$

$= 6000 - 0 = 6000 = 6.0 mg/kg$ n = Number of animals per group

Table 5: Mortality Rate of extract D, water and extract 'A' Ethanol

The dose of extract water administered and the mortality rate				The dose of extracts A ethanol administered and the mortality rate			
Group	Dose of Extract (mgkg)	No. of deaths	Mortality %	Group	Dose of Extract (mgkg)	No. of deaths	Mortality %
1	500	0	0	1	500	0	0
2	1000	0	0	2	1000	0	0
3	2000	0	0	3	2000	0	0
4	4000	0	0	4	4000	1	17
5	6000	0	0	5	6000	2	33

Table 6: Mortality Rate of extract 'B' Acetone and extract, 'C' Chloroform

The dose of extract B Acetone administered and the mortality rate				The dose of extracts C Chloroform administered and the mortality rate			
Group	Dose of Extract (mgkg)	No. of deaths	Mortality %	Group	Dose of Extract (mgkg)	No. of deaths	Mortality %
1	500	0	0	1	500	0	0
2	1000	0	0	2	1000	0	0
3	2000	0	0	3	2000	1	17
4	4000	2	33.0	4	4000	3	50
5	6000	6	100	5	6000	6	100

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Table 7: Lethal dose (LD_{50}) determinations - First phase

S/No	Group	Weight of mice(g)	Dose(mg/kg)	No. of Death
1	1	25	1000	0
2	1	25	1000	0
3	1	23	1000	0
4	2	24	100	0
5	2	25	100	0
6	2	24	100	0
7	3	24	10	0
8	3	16	10	0
9	3	21	10	0

Table 8: Lethal dose (LD_{50}) determinations - Second phase

S/No	Group	Weight of mice(g)	Dose(mg/kg)	No. of Death
1	I	26	80	0
2	II	28	160	0
3	III	28	320	0
4	IV	25	640	0

Table 9 Extract 50mg/kg body weight

S/No	Weight of mice (g)	Number of abdominal constrictions
1	20	0
2	20	4
3	24	2
4	17	10
5	20	5
6	20	4

Table 10: Extract 100mg/kg body weigt

S/No	Weight of mice (g)	Number of abdominal constrictions
1	23	0
2	17	3
3	17	0
4	19	6
5	24	11
6	25	5

It has been shown from scientific investigation that the potential use of plant extracts for treatment is due to the phytochemicals present in the extract, for example tannins have stringent properties which are important in wound healing. They act by precipitating proteins thereby protecting underlying tissues [7]. Saponin is naturally occurring glycosides that have the common characteristic of foam formation in aqueous solution, bitter taste and ability to heamolyse red blood cells. Highly toxic to cold blooded animals and their toxicity being related to their activities in lowering surface tension [9]. Saponins are known to have expectorant

properties and these have been made use of in the treatment of upper respiratory tract inflammation, [10].

The mechanism of anti-carcinogenic properties of saponins include, antioxidant effects, direct and selective cytotoxicity of cancer cells, immune-modulation, acid and neutral sterol metabolism and regulation of cell proliferation. Saponins are believed to prevent colon cancer. Normally bile acids pour into the stomach to help absorb fat food. Some bacteria in the large intestine turn the bile into a substance that is highly carcinogenic. Research suggests that saponins stop the toxic materials from foaming. Flavonoids are naturally occurring phenolic compounds in plants. The disease fighting potential of flavonoids stems from their ability to reduce inflammation, prevent the release of histamine (which causes allergy symptoms such as congestion) fight free radicals, boost immunity, strengthen blood vessels and increase blood flow.

They are also potent antitoxicants, some even more powerful than vitamin C or E in preventing cell damage caused by unstable oxygen molecules, [6]. Alkaloids are also a class of chemical compounds which have varied medicinal properties e.g alkaloids like reserpine, deserpidine and rescinamine are used in psychiatric cases and as antihypertensive [11].

The result on an acute toxicity on administration of the extract interperitoneally for 24 hours showed that the LD_{50} calculated for the leaves were at close range (3.750 g kg^{-1}- 6.0g kg^{-1}). However the chloroform extract was found to possess a higher LD_{50} value. Although the extracts are a combination of many different compounds, therefore it is possible to get much lesser LD_{50} with isolated pure compounds. The percentage mortality equally shows that the water extract was less toxic on comparison with the other three extracts

CONCLUSION

The LD_{50} of the crude extract was found to be 3.750 g kg^{-1}- 6.0g kg^{-1} body weight i.p in Rat.

REFERENCES

[1] Awachie PIA and Ugwu FO. Int J Pharmacol 1997; pp 338-343
[2] Birk AJ. Aust J Chem 1969; 8: 263.
[3] Gunatilaka AAL. J Natural Prod 2006; 69: 509−506.
[4] Igoli J O, Ogaji OG, Tor-Anyiin TA and Igoli NP. African J Trad CAM 2005; 2 (2): 134 – 152.
[5] Johnson R L. Food Aust 1987; 47:426
[6] Oladele SB, Ayo JO and Adaudi AO West African Jf Pharmacol Drug Res 1995; 11: 134-14.
[7] Pondrimoli S and Grazi E. J of Comp Biochem 1969; 17: 163-189.
[8] Pusztai R, Beladi I, Bakai M, Musci I And Kukan E. Acta Micro Acad Sci Hung 1966; 13: 11 3.
[9] Trease E and Evans WC. Pharmacognosy. Balliere Tindall USA as edited in 15th edition, 2002 , pp 193, 196, 223 – 4, 336 – 7, 544.

[10] Trease GE and Evans WC. A text book of Pharmacognosy, 15th edition, 2002, Elsevier Company, Philadelphia, USA. pp.191-418.
[11] Sofowora EA. Medicinal plants and Traditional Medicine in Africa, University of Ile-Ife Nigeria, 1982, pp. 144-146, 170-173.