Futo Journal Series (FUTOJNLS)
e-ISSN : 2476-8456 p-ISSN : 2467-8325
Volume-2, Issue-2, pp- 299 - 308
www.futojnls.org

Research Paper December 2016

Phytochemistry and LD_{50} of Aqueous and Ethanol Extracts of *Strophanthus Hispidus*

*Iheanacho, K.M.E[1]., Ibegbulem, C.O[1]., Emejulu, A.A[1]., Okwu, G.N[1] and Igbo, N.M[2]

[1]*Department of Biochemistry, Federal University of Technology, Owerri, Nigeria*.
[2]*Department of Medical Laboratory Science, College of Medicine, Abia State University,*
Uturu, Nigeria.
**Corresponding Author E-mail: iheanachokizito@gmail.com*

Abstract

The phytochemistry and acute toxicity studies of aqueous and ethanol extracts of *Strophanthus hispidus* was carried out using standard methods.`Probit method was used in determining the lethal dose (LD_{50}) of aqueous and ethanol extracts of *Strophanthus hispidus* while the phytochemical screening was carried out on the aqueous and ethanol extracts of *Strophanthus hispidus*.The phytochemical screening showed the presence of alkaloids, flavonoids, saponins, tannins, proteins, carbohydrate, reducing sugars, glycosides and cardiac glycosides in both aqueous and ethanol extracts. Saponins were predominant in aqueous extract while tannins and cardiac glycosides were predominant in ethanol extract. Anthracene glycosides and cyanogenic glycosides were only present in ethanol extract. The acute toxicity studies, LD_{50} of aqueous and ethanol extracts of *Strophanthus hispidus* were 4,020mg/kg body weight and 4,290mg/kg body weight respectively. Based on the findings, some of the chemical constituents identified have been implicated in the treatment of diseases and the values of LD_{50} shows that the extracts are slightly toxic.

Keywords: *Albino rats, aqueous, ethanol, phytochemistry, toxicity*

1.0 Introduction

Strophanthus hispidus belongs to the order apocynate and family apocynaceae. The name *Strophanthus* is derived from the Greek words Strophos (a twisted cord or rope) and anthos (a flower). The main feature of Strophanthus is the limb of the corolla which is divided into five long tail-like segments. The seeds are lance-ovoid, flattened and obtusely–edge from 7-20mm, 4mm in breath and 2mm in thickness (Grieve, 1973). The seeds of all species of *Strophanthus* possess hairs that have characteristic thickened

base (Grieve, 1973). There are eight species of Strophanthus, these are *Strophanthus courmonti*, *Strophanthus emini*, *Strophanthus glabra, Strophanthus gratus, Strophanthus hispidus, Strophanthus kombe, Strophanthus nicholsoni* and *Strophanthus tholloni* (Grieve, 1973). The seeds of all Strophanthus species are used in arrow poison and fish poison (Dalziel, 1937). In Igbo tribe of Eastern Nigeria, *Strophanthus hispidus* is known as 'Osisi Kaguru'.

Agyare *et al*., (2013) reported that methanol leaf, stem, bark and root extracts of *Strophanthus hispidus* have antimicrobial, antioxidant and wound healing properties. Aqueous root extract of *Strophanthus hispidus* exhibited anti-inflammatory activity which compared favourably with the positive control (Agbaje & Fageyinbo, 2011; Ishola *et al*, (2013). Gundamaraju *et al*, (2014) reported that ethanol extract of *Strophanthus hispidus* showed significant cardiac protective and anti-hypertensive activity in rats. Ojiako and Igwe, (2009) reported that phytochemical screening of *Strophanthus hispidus* showed the presence of alkaloids, flavonoids, saponins, cardiac glycosides and cyanogenic glycosides. They further reported that a time dependent study on the hypoglycaemic activity of chloroform and ethanol extracts of *Strophanthus hispidus* administered to rats showed better efficacy with 50% of the extracts than when administered 20% of the extracts.

Williams *et al*, (2000) described dosages as either lethal doses (LD_{50}) which means that the response measured is mortality; toxic doses (TD) which implies that the response measured is a serious adverse effect other than lethality and sentinel doses (SD) which implies that the response measured is a non or minimal adverse effect. William *et al*., (2000) reported that the difference in dose between the toxicity curve and sentinel effect represents the margin of safety. They further stated that the margin of safety is calculated by dividing toxic dose (TD_{50}) by sentinel dose (SD_{50}). Also, the higher the margin of safety the safer the use of the chemical. The relative ranking of acute toxicity of the substance (William *et al*, 2000): practically nontoxic dose (>15,000 mg/kg body weight); slightly toxic dose (5000 to 15,000 mg/kg body weight); moderately toxic (500 to 5,000 mg/kg body weight); very toxic (50 to 500 mg/kg body weight); extremely toxic (5 to 50 mg/kg body weight); and super toxic (<5 mg/kg body weight).

The aim of this study was to identify the chemical constituents in aqueous and ethanol extracts of *Strophanthus hispidus*. Also to estimate the level of safety by determining the lethal dose that will kill fifty (50%) percent of the animals.

2.0 Materials and Methods

2.1 Materials

2.1.1 Plant Sample

The roots of *S. hispidus* was collected from a herbalist at Relief Market Owerri, Imo State, Nigeria and authenticated by a taxonomist at the Department of Plant Science and Biotechnology, Imo state University, Owerri, Nigeria. A specimen of the plant sample was deposited at the University herbarium.

2.1.2 Preparation of Aqueous Extract

The root of *Strophanthus hispidus* was cut into small chips and 250 g of the chips was continuosly extracted with 500cm^3 of water using soxhlet apparatus for 48 hrs at a temperature of 30 to 40^{o}C.

2.1.3 Preparation of ethanol extract

The root of *Strophanthus hispidus* was cut into small chips and 250 g of the chips was continuously extracted with 500 cm^3 of 99% absolute ethanol using soxhlet apparatus.

2.1.4 Experimental animals

Albino rats of both sexes weighing between 75 – 200 g and 8 – 9 weeks old were obtained from the animal house of Department of Biochemistry, University of Port Harcourt, Nigeria. The animals were fed growers mesh and water was given to them *ad libitum*.

2.2 Method

2.2.1 Phytochemical screening

Phytochemical investigation of the powdered sample was carried out using standard procedures of Harborne , (1973); Soforowa, (1993) and Trease and Evans, (1983). The powdered sample was gotten after oven drying the aqueous and ethanol extracts at a temperature of 50 to 60^{o}C for about 3 to 4 hrs.

2.2.2 Lethal dose estimation (LD_{50}) of crude aqueous extract

Lethal dose estimation of crude aqueous extract of *Strophanthus hispidus* was carried out using the probit method (Wardlaw , 1987; Woolf , 1968). The rats were put into six

groups of five rats per group. Rats in Group 1 (control) were orally administered normal saline, while those in Groups 2 to 6 were administered 1000, 2000, 3000, 4000mand 5000 mg/kg body weight of crude aqueous extract of *Strophanthus hispidus* respectively. After 48 hrs, the number of deaths recorded in each group was noted.

2.2.3 Lethal dose estimation (LD_{50}) of crude ethanol extract

Lethal dose estimation of crude ethanol extract of *Strophanthus hispidus* was carried out using the Probit method (Wardlaw , 1987;Woolf, 1968). The rats were put into six groups of five rats per group. Rats in Group 1 (control) were orally administered normal saline while those in Groups 2 to 6 were administered 1000, 2000, 3000, 4000 and 5000 mg/kg body weight of crude ethanol extract of *Strophanthus hispidus* respectively. After 48 hrs, the number of deaths recorded in each group was noted.

3.0 Results

The phytochemical screening of aqueous and ethanol extracts are shown in Table 1. The phytochemical screening of aqueous and ethanol extracts of *Strophanthus hispidus* revealed the presence of alkaloids, flavonoids, saponins, tannins, proteins, carbohydrates, reducing sugars, glycosides and cardiac glycosides. Anthracene and cyanogenic glycosides were present in ethanol extract but absent in aqueous extract. Saponins were predominant in aqueous extract than ethanol extract while tannins, cardiac glycosides and anthracene glycosides were predominant in ethanol extract than aqueous extract.

The results of the computation of LD_{50} of rats administered aqueous extract of *Strophanthus hispidus* and LD_{50} of rats administered ethanol extract of *Strophanthus hispidus* are shown in Tables 2 and 3.

The plots graphs of LD_{50} estimate of rats administered aqueous extract of *Strophanthus hispidus* and LD_{50} estimate of rats administered ethanol extract of *Strophanthus hispidus* are shown in Fig.1 and 2.

Table 1: Phytochemical screening of aqueous and ethanol extracts of *Strophanthus Hispidus*

Chemical constituent	Aqueous extract	Ethanol extract
Alkaloid	+	+
Flavonoids	+	+
Saponins	++	+
Tannins	+	++
Proteins	+	+
Carbohydrate	+	+
Reducing sugar	+	+
Glycosides	+	+
Cardiac glycosides	+	++
Anthracene glycosides	-	++
Cyanogenic glycoside	-	+

Legend

++ = very present

+ = present

– = absent

Table 2: LD_{50} of rats administered aqueous extract of *Strophanthus hispidus*

Concentration (mg/kg)	Log dose	Number of rats	Number dead	Proportion of death	Pecentage death(%)	Probit value
1000	3.000	5	0	0	0	0
2000	3.3010	5	1	0.2	20	4.16
3000	3.4771	5	1	0.2	20	4.16
4000	3.6021	5	2	0.4	40	4.75
5000	3.699	5	3	0.6	60	5.25

Table 3: LD_{50} of rats administered ethanol extract of *Strophanthus hispidus*

Concentration (mg/kg)	Log dose	Number of rats	Number dead	Proportion of death	Pecentage death (%)	Probit value
1000	3.000	5	0	0	0	0
2000	3.3010	5	1	0.2	20	4.16
3000	3.4771	5	1	0.2	20	4.16
4000	3.6021	5	1	0.2	20	4.75
5000	3.699	5	3	0.6	60	5.25

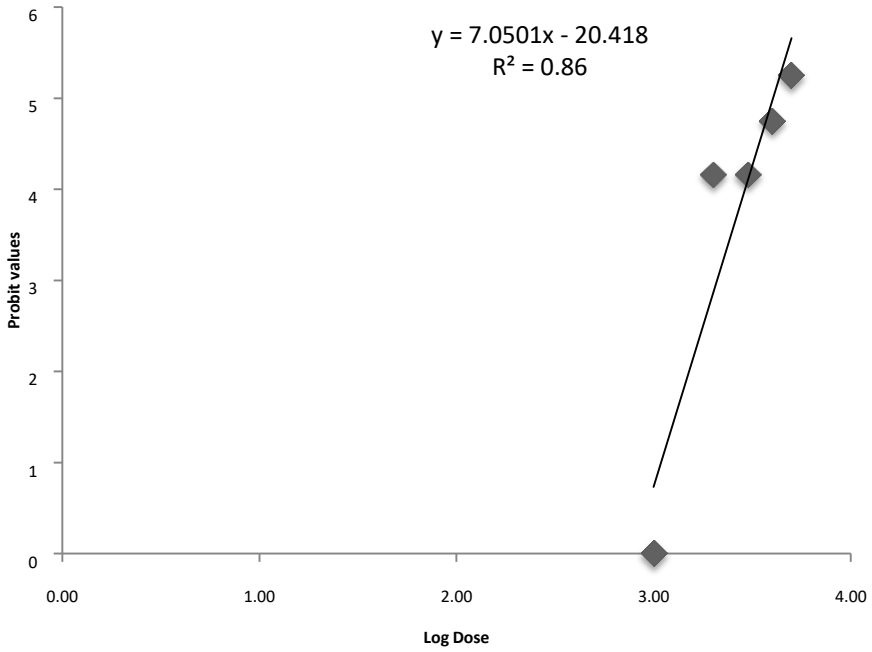

Fig. 1: LD_{50} estimate of rats administered aqueous extract of *Strophanthus hispidus*.

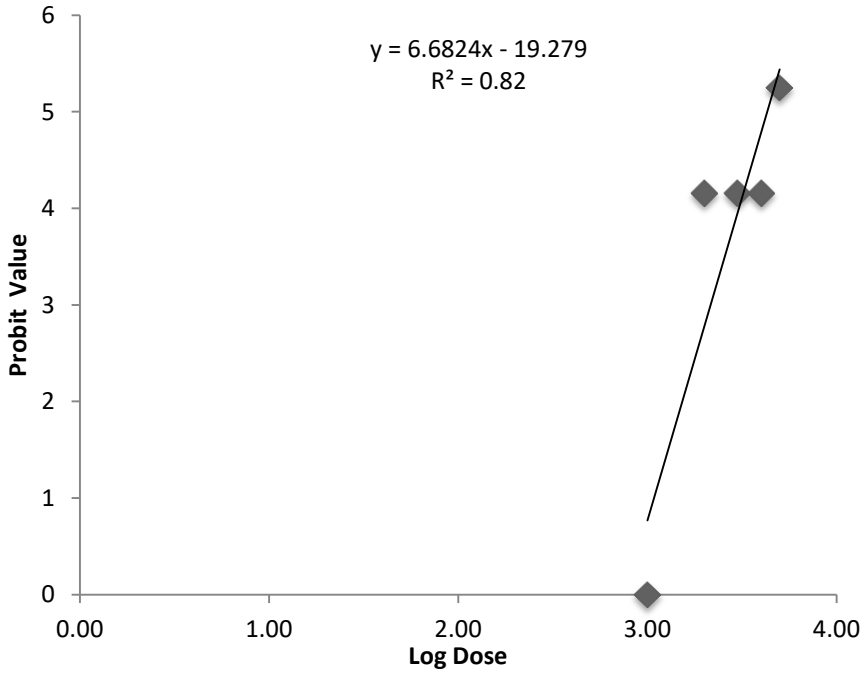

Fig. 2: LD_{50} estimate of rats administered ethanol extract of *Strophanthus hispidus*.

4.0 Discussion

Have been identified by Bnouham et al., (2006), Marles and Farnsworth (1994), Oliver and Zahnd (1979). Some of the chemical constituents or combinations of constituents present in *Strophanthus hispidus* that are responsible for the hypoglycaemic activity in experimental animals. The chemical constituents identified in aqueous and ethanol extracts of *Strophanthus hispidus* corroborates the findings of Onyeze *et al*, (2006) on the chemical constituents of ground root *Strophanthus hispidus*.

The LD_{50} estimate of rats administered aqueous extract of *Strophanthus hispidus* was 4020 mg/kg while the LD_{50} estimate of rats administered ethanol extract of *Strophanthus hispidus* was 4290 mg/kg. The toxicity rating as reported by Clarke and Clarke, (1975), Hodgon, (2004), Lorke, (1983), Wardlaw, (1987) and Woolf, (1968) classified 50 - 5,000 mg/kg LD_{50} values as moderately toxic. Based on the above classification the aqueous extract of *Strophanthus hispidus* with LD_{50} value of 4,020 mg/kg is moderately toxic and the ethanol extract of *Strophanthus hispidus* with LD_{50} value of 4,290 mg/kg is moderately toxic.

5.0 Conclusion

The LD_{50} values 4020 and 4290 mg/kg of aqueous and ethanol extract of *Strophanthus hispidus* indicates that the extracts are moderately toxic and care should be taken in the administration of aqueous and ethanol extracts of the roots of *Strophanthus hispidus* to human beings.

References

Agbaje, E.O., & Fageyinbo, M.S.(2011). Evaluating anti-inflammatory activity of aqueous root extract of *Strophanthus hispidus* DC. (Apocynaceae). *International Journal of Applied Research in Natural Products*, 4(4), 7-14.

Agyare, C., Dwobeng, A.S., Agyepong, N., Boakye, Y.D., Mensah, K.B., Ayande, P.G., & Adarkwa-Yiadom, M.(2013). Antimicrobial, antioxidant and wound healing properties of *Kigelia Africana* (Lam) Beneth and *Strophanthus hispidus* DC. *Advances in Pharmacological Sciences*, 1-10

Bnouham, M., Abderrahim, Z., Hassane, M., Abdelhafid, T & Abdelkhaleq, L (2006). Medicinal plants with potential antidiabetic activity. A review of ten years of herbal medicine research (1990-2000). *Int. J. Diabetes and Metabolism*, 14, 1-25.

Clarke, E.G.C & Clarke, M. L. (1975). *Veterinary Toxicology*. New York: Macmillian Publishers Ltd 10-11.

Dalziel , J . M (1937). *The useful plants of West tropical Africa*. London: Crown Agents 612.

Evans, W.C. (2002). *Trease and Evans Pharmacognosy*. (15th ed.) New York: Harcourt Publishers Limited 135 – 149.

Grieve , M.(1973). *A modern herbal*. (3rd ed.) London: Tigers Books International 777-778

Gundamaraju, R., Vemuri, R.C., Singla, R.K., Manikam, R., Rao, A.R., & Sekaran, S.D.(2014). *Strophanthus hispidus* attenuates the Ischemia-Reperfusion induced myocardial infarction and reduces mean arterial pressure in renal artery occlusion. *Pharmacognosy Mag*, 10 ,557-62.

Harborne, J.B (1973). *Phytochemical methods. A guide to modern techniques of plant analysis*. London: Chapman and Hall Publishers, 1-32.

Harborne, J.B (1973). Phytochemical methods. A guide to modern techniques of plant analysis. New York: John Wiley and Sons. Inc. 33-80.

Ishola, I. O., Awodele, O., Oreagba, I.A., Murtala, A.A., & Chijioke, M.C. (2013). Antinociceptive, anti-inflammatory and antiulcerogenic activities of ethanol root extract of *Strophanthus hispidus* DC(apocynaceae). *Journal of Basic and Clinical Physiology and Pharmacology*, 24(4), 277-286.

Lorke, D. (1983). A new approach to practical acute toxicity testing. *Archives of Toxicology*, 54 , 275- 287.

Marles, R. J & Farnsworth, N.R (1994). Plants as sources of antidiabetic agents. *Economic and Medicinal Plant Research*, 6,149-187.

Ojiako, O.A., & Igwe, C.U. (2009). A time-trend hypoglycaemic study of ethanol and chloroform extracts of *Strophanthus hispidus*. *Journal of Herbs, spices and Medicinal Plants*, 15(1), 1-8.

Oliver, B & Zahnd, G.R (1979). Plants with oral hypoglycaemic action. *Quart. J. Crude Drug Res*, 17(3-4), 139-196.

Onyeze, G.O .C., Ukoha, A.I & Iheanacho, K.M.E.(2006). Preliminary chemical analyses of the ethanol extract of *strophanthus hispidus* used in the management of diabetes. Plant Product Research Journal,10, 46-49.

Soforowa, (1993). *Medicinal plants and traditional medicinal plants in Africa.* (2nd ed.) Ibadan: Spectrum Books Ltd. 134-156.

Trease , G.E & Evans , W.C (1983). *Pharmacognosy*. (12th ed.) Bailliere Tindall London,343-383.

Wardlaw, A.C (1987). *Practical Statistics for Experimental Biologists*. New York: John Wiley and Sons Publishers 104-111.

Williams, P.L., James, C.R & Roberts, M.S (2000). *Principles of toxicology*. (2nd ed.) New York: John Wiley and Sons. Inc. 3 – 34.

Woolf, C.M (1968). *Principles of Biometry*. New York: D. Van Nostrand Publishers Company Ltd, 289-297.