6. Jeney E. and Zsolnai T., Zentr, **Bakteriol, Parasitenk.** Abt. I. Orig, 1956, 167, 55.: **Chem. Abstr**, 1957, 51, 7579.

7. Bito T., **Bull. Nagoya Inst. Technol.**, 1952, 4, 218: **Chem. Abstr.**, 1954, 48, 2991.

8. Vogel A. I, A text book of Practical Organic Chemistry 1971, 909.

9. Gilfillan F.A. and Merritt John R., **J. Am. Pharm. Assoc.**, 1936, 25, 860: **Chem. Abstr**, 1937, 31, 214.

10. D. Sh. Rozina and Snytkovskaya R.G., **Metody Polucheniya Pokhim**, 1962, 4-5, 109. Organic Synthesis collective Volume I, Henry Gilmann, Editor in Chief II'nd Edition John Wiley and Sons, Inc. London, 1941, 511.

In Vivo Antisnake Venom Activity of A Furanoid Diterpene from *Aristolochia albida* Duch (Aristolochiaceae)

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The antisnsake venom activity of a furanoid diterpene lactone isolated from the rhizome of *Aristolochia albida* (Family : Aristolochiaceae) was evaluated. The whole animal (*in vivo*) studies were conducted using the mortality of male Swiss albino mice after intra-peritoneal (i.p.) injection of lethal doses (LD_{100}), 8.75 mg/kg and 4.20 mg/kg of venoms of *Naja nigricollis* (spitting cobra) and *Bitis arietans* (puff-adder) respectively. The deterpene was found to significantly reduce the toxic symptoms and protect the mice against the lethal doses of these two snake species commonly found in Northern Nigeria. However, the compound is more effective aganist the venom of N.nigricollis (ED_{50}=45 mg/kg) than that of B.arietans (ED_{50}=74 mg/kg).

ARISTOLOCHIA ALBIDA is a climbing shrub commonly found in the tropical West Africa[1] and is used in several gastro- intestinal disorders[2]. There are reports that the rhizomes are used in skin diseases and against snake bites[3,4]. The isolation of fargesin (neolignan) was reported earlier[5]. Recently, the isolation of phytosterols and glucose has been reported[6]. No pharmacological work on this medicinal plant has been reported so far to validate the claims of the folk-loric uses except for the molluscidal activity of the plant.[7] Ethnopharmacological reports from northern Nigeria about the use of the rhizomes of this plant against snake bites[8] have prompted us to investigate the plant phytochemically and pharmacologically. This communication reports the antisnake venom activity of a diterpene isolated from **A.albida.**

The plant was collected in mid-July and authenticated by the Ahmadu Bello University herbarium, Zaria. The air dried, powder of the rhizomes was defatted with petroleum ether (b.p.60- 80°)and then extracted with methanol in a Soxhelt apparatus. The column chromatography (silica gel) of the methanolic extract furnished the diterpene which upon crystallisation from chloroform/methanol gave fine colorless needlesefine colourless needles, m.p. 182°.

***For Correspondence**

Table

Group number	Dose of diterpene (mg/Kg) administered	Envenomation with LD_{100} of **N. nigricollis**		Envenomation with LD_{100} of **B. arietans**	
		Number of survival out of 15 (n=15)	% of survival	Number of survival out of 15 (n=15)	% of survival
1	5.0	0	0.0	0	0.0
2	10.0	1	6.7	2	13.3
3	20.0	3	20.0	3	20.0
4	40.0	7	46.7	6	40.0
5	80.0	11	73.3	9	60.0
6	Saline	0	0.0	0	0.0

The venoms of **N.nigricollis** and **B.arietans** were collected by holding the snakes over small beakers covered with thin sheets of polythene. The snakes were made to strike the polythene and penetrate it with their fangs just to realease the venom into the containers (the milking process). The venoms were lyophillised immediately. The LD_{50} and LD_{100} of these venoms were determined by dissolving the venoms separately in physiological saline solution to make a stock solution of 1 mg/ml each. The solutions were further diluted to give (0.1 ml) doses between 1 mg/kg to mg/kg body weight. These different doses were administered (i.p) into six groups of mice consisting of fifteen in each group and the mortality was recorded up to 24 hours as described by Theakston and Reid[9]. The values of the LD_{50} and LD_{100} were found to be 5.25 mg/kg and 8.75 mg/kg respectively for N.nigricollis and 2.58 mg/kg and 4.20 mg/kg respectively for **B.arietans**.

Six groups of healthy well fed male Swiss albino mice (18-22 g) were pretreated with defferent doses of diterpene (i.p.) five minutes before injecting (i.p) lethal dose (LD_{100}) of **N.nigricollis** venom. The survival of the mice were observed within 24 hours. The same procedure was repeated usinng the venom of **B.arietans** and the results are shown in the **table**.

The median effective doses (ED_{50}) of the diterpene aganist the LD_{100} of **N.nigricollis** and **B.arietans** venoms were calculated and found to be 45 mg/kg and 74 mg/kg, respectively, by probit analysis according to Miller and Tainter[10]. It was observed that the mortality of mice was reduced progressively with increasing doses of the compound and significant protection ($p<0.05$) was observed at 80 mg/kg dose. Death from **N.nigricollis** resulted mainly due to neurotoxicity by curare-like action of cobrotoxin on the respiratory muscles causing flaccid paralysis leading to respiratory depression[11]. The diterpene was found to interfere with acetylcholine receptors[12] and therefore, it might mediate its actions by antagonising the actions of the neurotoxic substances in the venom at the acetylcholine receptor sites. This might be the reason for its higher activity against **N.nigricollis** venom when compared to that of **B.arietans** which (the latter)interferes primarily with haemostasis and causes cardiotoxicity. The methanolic extract of the plant was found to be nearly as potent as the diterpene. This observation suggested the possibility for the presence of other active compounds in the plant.

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REFERENCES

1. Irvine, F.R. :Woody Plants of Ghana with Special Reference to Thier Uses. ford University Press, London, 1961,p:878

2. Watt J.M. and Brayer, G.M.: Medicinal and poisonous Plants of Southern and Eastern Africa.2nd Edn and S Livingstone Ltd,1962, P:118.

3. Dalziel, J.M: The useful Plants of West Tropical Africa, volume 6, Crown Agents for the Colony, London, 1937,P:612.

4. Comley, J.C.W., **Trop.Med.Parasit**, 1990 41(1), 1.

5. Fagbule, M.O. and Olatunji, G.A.,**Cellul.Chem.Tech.**, 1984,18,293.

6. Choudhury,M.K.and Haruna, A.K.,**Indian J.Pharm.Sci.**, 1994 56,230.

7. Kela, S.L., Ogususi,R.A., Ogbogu, V.C. and Nwude, N.,**Rev.Elev.Med.Vet.Fays.Trop.**, 1989, 42, 195.

8. Kahina,R., Personal communication.

9. Theakston, R.D.C and Reid, H.A.,**Bull.W.H.O.**, 1983,61,949.

10. Miller, L.C. and Trainter, M.L., **Proc.Soc.Expt. Biol.Med.**, 1944, 57, 261.

11. Warrel, D.A. Manson's Tropical Diseases. Balliere Tindall, 1987, P:855.

12. Haruna, A.K., Ph.D. Thesis, Ahmadu Bello University, Zaria, 1994, P:216.

Simultaneous Spectrophotometric Determination of Dipyrone and Caffeine in Pharmaceutical Formulations.

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A simple and rapid spectrophotometric method has been developed for simultaneous determination of dipyrone and caffeine without prior seperation.

DIPYRONE in combination with caffeine is used as an analgesic antipyretic drug.Dipyrone or caffeine in binary combination withother analgesics has been determined by titrimentry[1-3] and spectrometry[4-9]. Inthis study, the absorbance ratiotechnique was applied to determination of dipyrone-caffeine mixtures.

In this method, it is necessary to choose the two wavelengths to be used in the analysis. There are wavelength at which one of the two substances exhibits maximum absorption and the isoabsorptive point. At the isoabsorptive point, dipyrone and caffeine have the same asbsorbancy index values. In this study, there are two isoabsorptive wavelengths (235.1 and 255.2 nm in 0.1 N HCl). Caffeineexhibits maximum absorption at271.4 nm in 0.1 N HCl (**Fig1**).

At the choosen wavelengths,standart solutions of dipyrone and caffeine obey Beer's Law in the concentration range of 2-24 μg. ml^{1} and 1-18 μg.ml^{1} respectively.