African Journal of Biomedical Research, Vol. 8 (2005): 89 - 93
ISSN 1119 – 5096 © Ibadan Biomedical Communications Group
Available online at http://www.bioline.org.br/md

Full length Research Article

Sub-Acute Toxicity and Biochemical Effects of extracts of *Anaphe venata* larvae in mice

E.O.Iwalewa[1i], O.A Onayade[2], O.O Oyedapo[3], O.M Daniyan[1]

Departments of Pharmacology[1], Pharmacognosy[2] and Biochemistry[3], Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria.

Received: February, 2005
Accepted: May, 2005

ABSTRACT
Ataxia syndrome which is characterized by sudden onset of severe muscular tremor and gait ataxia has been shown to be associated with the consumption of the larvae of *Anaphe venata* in South Western part of Nigeria. In this report, the sub-acute toxicity and biochemical effects of polar and non-polar extracts of *Anaphe venata* larvae were investigated in mice. The sub-acute toxicity study showed an increase in the behavioural components as tremor, jerking and stretching, after the administration of the extracts for 7 days, an indication of Ataxia syndrome. Also, no significant difference in these components occurred between polar and non-polar extracts, an indication of similarity in the chemical composition and level of toxicity. There was a corresponding increase in enzymatic activities coupled with increase in weight of essential organs investigated, but not significantly different from the controls. However, the involvement of receptors and neurotransmitters, in the action of the extracts to cause ataxia syndrome require further investigation.

Keywords:
Anaphe venata, larvae, toxicity, Free radicals, I*n vitro*, *Cissus quadrangularis*, CCl_4, Antioxidant activity

INTRODUCTION

Studies have shown that in many developing countries of the world, insects are a good source of quality proteins, fats and minerals (Ene 1963 and Defoliart 1989, 1992) as such it mass rearing has even been advocated.

Anaphe venata larvae (African Silkworm) found commonly in Western part of Nigeria during the period of July-September each year, have been shown to be of high protein content (Umoh et al. 1980, Ashiru 1988) Other known constituents of the larvae include amino acids, fats and sterols (Onayade and Adamolekun, 1996).

However, a seasonal ataxia syndrome associated with the consumption of these larvae has been reported (Adamolekun 1993a). This syndrome is characterized by sudden onset of severe muscular tremor and gait ataxia, and it occurrence coincides with the period of it wide availability in the market.

Based on its wide acceptability and it high seasonal availability, coupled with high consumption rate, in Western Nigeria, it is therefore necessary to evaluate the sub-acute toxicity and biochemical effects of the polar and non-polar extracts of the larvae of *Anaphe venata* on certain serum biochemistry of mice.

MATERIALS AND METHODS

Sample Collection: The larvae of *Anaphe venata* were obtained as commercial sample from a local market in Ile Ife, Nigeria and authenticated by comparison with known sample with similar vernacular names at the National History Museum of the Obafemi Awolowo University, Ile Ife.

Sample Preparation: *Anaphe venata* larvae (1kg) were parboiled in water to which ashes have been added. They were dried and roasted in hot white sand to remove their numerous tough and long tufts of hair. The larvae were then cooked in salt water followed by sun dried for preservation.

Extraction Procedure: The extraction procedure followed the earlier described method

of Onayade and Adamolekun (1996). It involved the crushing of dried larvae to powder followed by maceration in absolute ethanol for 48 hours in the dark. The resulting alcoholic extract was then partitioned in ethyl acetate to give the aqueous (polar) and ethyl acetate (non-polar) fractions. The fractions were concentrated in vacuo at room temperature and freeze dried.

Animals: Adult Swiss Albino mice weighing between 18-24g were obtained from the animal house of the Department of Pharmacology, Obafemi Awolowo University, Ile Ife, Nigeria. They were fed with standard diets (Pfizer mouse cubes) with regular supply of water ad libitum. They were kept within a lighted environment on 12 hours light-dark cycle maintained at room temperature.

Sub-Acute Toxicity Testing: Adult Swiss Albino mice (10) were used in this study. The animals were randomly divided into two groups of 5 mice each for polar and non-polar extracts administration. A sub-lethal dose of 500mg/kg body weight for polar and 750mg/kg body weight for non-polar were used. Intraperitoneal administrations of both extracts were performed once daily consistently for seven days at 24 hours interval. The animals were observed for behavioural display, which include freezing, forward walking, rearing, grooming, cycling, sniffing, hyperactivity, tremor, licking, respiratory distress, stretching, jerking and raising of tail, for 15 minutes before the administration, 5 minutes for drug stability and then for the next 40 minutes. The animals were kept under close observation and treatment for the 15 days. The observed behavioural components were scored on a rating scale of 0-4 depending on the intensities or frequencies of the observe response as shown in Table 1:

Table 1: Ratings of observed behavioural components in mice

Score	Behavioural frequencies
0	Absent
1	Mild intensity, present 1-4 times.
2	Moderate intensity, present 5-8 times.
3	High intensity, present 9-12 times.
4	Severe, present for a prolong period i.e. > 12 times

Biochemical study: Adult Swiss Albino mice (27) of both sexes were used. They were randomly divided into nine groups of 3 mice each. Group 1 serves as control while the other eight groups were divided into two of four groups each for polar and non-polar extract administration. The extracts were administered intraperitoneally at varying doses of 200, 800, 3200 and 12,800mg/kg body weights for both polar and non-polar extracts. All the groups were observed continuously for the first 24 hours and daily for the next 15 days. At the peak of convulsive episode which shortly precedes death in any of the groups, the set of mice in the group were quickly sacrificed blood samples collected into an anticoagulant tube containing 3.8%w/v sodium citrate followed by centrifugation at 5000rpm for 10 minutes. The plasma samples were stored at -20^{o}C until analysed. Also, essential organs- lung, spleen, brain, heart, kidney and liver- were aseptically removed, weighed and kept in the deep freezer. Meanwhile, all the animals that survived were sacrificed on the 15th day and blood as well as the essential organs were collected and treated as earlier described. Finally, the plasma samples were analysis for Total protein (Gornorlet et al. 1949), Aspertate / Alanine aminotranferase (Retman and Frontal 1957), and Alkaline Phosphatase (Saini and Von-Etten, 1979, Oyedapo, 1996).

Statistical Analysis: The variations in the observed behavioural components among the mice in each group (polar and non-polar) were tested using Kruskal Wallis Analysis of Variance (ANOVA). The Wilcoxin sign ranked test for paired observations was used to test the hypothesis of no difference between the effects produced before and after extracts administration. Mann-Witney U test was used to test the difference in the effects produced by polar and non-polar extracts on mice. Analysis of multiple biochemical treatments effects and pathophysiological changes on essential organs were conducted using one way analysis of variance (ANOVA). All analysis was performed using PRIMER statistical software.

RESULTS AND DISCUSSIONS

The behavioural effects elicited by the polar and non-polar extracts of *Anaphe venata* larvae in response to seven days sub-lethal dosage administration (Fig.1) showed no significant difference among the treatment groups. This implied that the mice gave similar responses to the effects of extracts at the tested doses. However, when compared with the observed behavioural responses before the administration of the extracts (Table 2) a significant difference were observed. Such behavioural activities like tremor, jerking and stretching were conspicuously present, which were consistent with our earlier preliminary findings (Onayade et. al. 2004). Maricker and Paltabiraman (1988) have shown that heat treatment of foods increases the nutritional values of protein and partial inactivation of protease inhibitors.

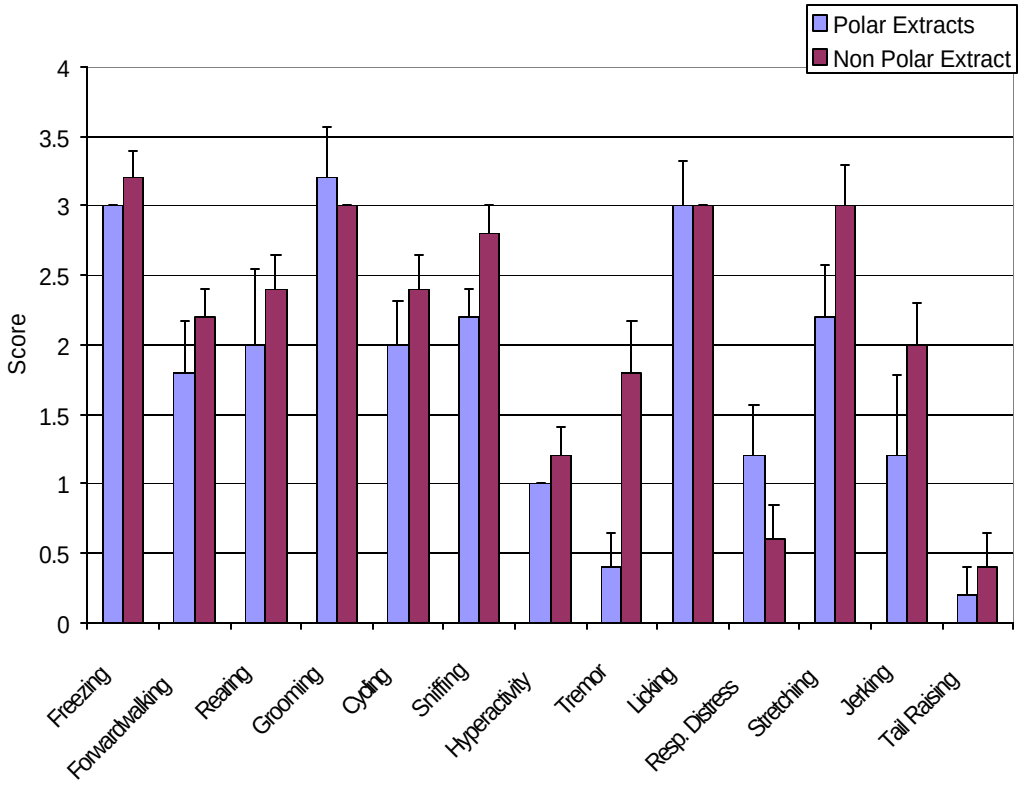

Fig. 1
Summary of effects of polar and non-polar extracts of Anaphe venata larvae on mice behaviour.
Polar: H=2.047 with df =4 and p=0.727, Non-Polar: H=1.477 with df =4 and p=0.831; Observed p>a=0.05 in both extracts, therefore there is no significant difference in the observed activity among the mice used.

Table 2:
Behavioural components exhibited by mice after i.p administration of polar and non-polar Extracts of *Anaphe venata* larvae.

Components	**Polar Average Ratings**		**Non-Polar Average Ratings**		**Polar Vs Non-Polar**	
	A1	**B1**	**A2**	**B2**	**B1**	**B2**
Freezing	0.2	3	0.2	3.2	3	3.2
Forwardwalking	3	1.8	3	2.2	1.8	2.2
Rearing	3.8	2	3.4	2.4	2	2.4
Grooming	1.8	3.2	2.4	3	3.2	3
Cycling	0.2	2	0.8	2.4	2	2.4
Sniffing	3.6	2.2	3	2.8	2.2	2.8
Hyperactivity	2.2	1	2.4	1.2	1	1.2
Tremor	0	0.4	0.2	1.8	0.4	1.8
Licking	0.6	3	1.4	3	3	3
Resp. Distress	0	1.2	0	0.6	1.2	0.6
Stretching	0	2.2	0	3	2.2	3
Jerking	0	1.2	0	2	1.2	2
Tail Raising	0	0	0	0.4	0	0.4

A1/A2 and B1/B2 signify rating scores before and after extract administration. Each value represents Mean ± SEM

Polar: W=-35.0, n=12 (1 ties deleted) and p>0.06; Non-Polar: W=-53.0, n=13 and p>0.06 and Polar Vs Non-Polar: T= 195.5, n_1=n_2=13.

Probability associated with the observed values of W is greater than a=0.05, thus, there is a significant difference in the observed activities after extracts administration. Also, observed T(195.50 > T table(137.0), indicating no significant difference in the actions of Polar and Non-Polar extracts in mice

TABLE 3:
Summary of biochemical parameters in mice after i.p administration of polar and non-polar Extracts of *Anaphe venata* larvae.

Biochemical Parameters	Polar Extracts (mg/kg body weight)					Non-Polar Extracts (mg/kg body weight)				
	control	200	800	3200	12800	control	200	800	3200	12800
AST (unit/ml) (N=6)	154.00 ±9.42	200 ±58.67	75.3 ±10.66	118.0 ±25.70	162.0 ±10.80	154.00 ±9.42	140.0 ±25.94	145.3 ±16.43	72.7 ±2.45	190.7 ±60.93
ALT (unit/ml) (n=6)	86.00 ±14.17	87.3 ±2.87	105.3 ±9.81	178.0 ±33.89	184.0 ±20.07	86.00 ±14.16	137.3 ±41.35	127.3 ±14.88	56.0 ±9.42	208.0 ±12.33
ALKPase (umol/ml/min) (n=6)	9.01 ±1.19	22.71 ±4.25	99.79 ±51.69	108.7 ±29.56	222.2 ±9.66	9.01 ±1.19	178.4 ±60.24	231.7 ±32.71	95.61 ±42.9	211.1 ±55.73
AciPase (umol/ml/min) (n=6)	2.956 ±0.40	27.04 ±5.88	25.02 ±1.89	128.4 ±35.20	145.6 ±17.06	2.956 ±0.398	112.0 ±60.94	217.1 ±33.26	58.98 ±26.00	213.4 ±46.66
Total Protein (g/dl) (n=9)	0.276 ±0.01	0.454 ±0.03	0.738 ±0.03	2.955 ±0.76	3.547 ±0.20	0.276 ±0.01	3.655 ±0.88	3.130 ±0.21	3.546 ±1.26	8.433 ±1.16

Each value represents Mean ± SEM.
Polar:* *F = 1.53(p = 0.231) < F table (4, 20) =2.87 (p<0.05);* ***Non-Polar: F =* *2.53(p = 0.072) < F table (4, 20) =2.87 (p<0.05). The observed probability levels in both extracts are greater than a (0.05) level; therefore there is no significant difference among the treatment groups and the control.*

TABLE 4:
Effects of polar and non-polar extracts of *Anaphe venata* larvae on different weights of organs (Each value represents Mean ± SEM, n=3)

Weights (g)	Polar Extracts (mg/kg body weight)					Non-Polar Extracts (mg/kg body weight)				
	control	200	800	3200	12800	control	200	800	3200	12800
Mice	20.00 ±0.00	21.67 ±1.36	21.67 ±1.36	21.67 ±1.36	25.00 ±0.00	20.00 ±0.00	22.50 ±4.3	25.00 ±2.36	25.00 ±0.00	25.00 ±2.36
Lungs	0.140 ±0.00	0.175 ±0.02	0.184 ±0.01	0.256 ±0.01	0.226 ±0.04	0.140 ±0.00	0.177 ±0.02	0.217 ±0.04	0.178 ±0.02	0.324 ±0.04
Brain	0.416 ±0.00	0.373 ±0.03	0.387 ±0.02	0.468 ±0.02	0.374 ±0.03	0.416 ±0.00	0.370 ±0.02	0.368 ±0.04	0.322 ±0.06	0.352 ±0.04
Liver	0.975 ±0.00	1.115 ±0.02	1.128 ±0.12	1.268 ±0.02	1.405 ±0.05	0.975 ±0.00	1.163 ±0.01	1.186 ±0.06	1.222 ±0.10	1.725 ±0.12
Spleen	0.077 ±0.00	0.150 ±0.03	0.101 ±0.02	0.214 ±0.02	0.109 ±0.02	0.077 ±0.00	0.189 ±0.04	0.131 ±0.01	0.213 ±0.07	0.139 ±0.02
Kidney	0.260 ±0.00	0.362 ±0.05	0.287 ±0.01	0.365 ±0.02	0.388 ±0.02	0.260 ±0.00	0.405 ±0.02	0.298 ±0.02	0.382 ±0.03	0.474 ±0.02
Heart	0.124 ±0.00	0.103 ±0.01	0.107 ±0.01	0.097 ±0.01	0.105 ±0.01	0.124 ±0.00	0.144 ±0.01	0.110 ±0.02	0.140 ±0.02	0.205 ±0.02

Polar:* *F = 0.08(p = 0.987) < F table (4, 25) =2.76 (p<0.05);* ***Non-Polar: F =* *0.18(p = 0.947) < F table (4, 25) =2.76 (p<0.05). The observed probability levels in both extracts are greater than a (0.05) level; therefore there was no significant difference among the treatment groups and the control.*

As observed, the processed larvae of *Anaphe venata* produced significant behavioural activities in mice which revealed that the potential neurotoxic components of the larvae were not affected by heat during processing. This observation was consistent with the finding of Nishimmue et. al. (2000) that Anaphe species contained heat-resistant thiaminase which could be responsible for the various behavioural changes. This might explain its role in the pathogenesis of ataxia syndrome. Moreover, biochemical and pathophysiological studies revealed that increase in concentration of the extracts gave a corresponding increase in the activities of the enzymes assayed and an increase in the weight of organs investigated, with the exception of L-aspartate aminotranferase (AST) and brain, both of which showed decrease in activity and weight respectively. However, these observed effects were not significantly different among the test groups and the control. The extracts did not cause any significant damage to the organs examined during the acute administration. According to Akinnawo et.al. (2002), acute administration of another edible larvae of *Cirina forda* (Westwood) did not also produce significant changes in biochemical parameters in treated animals. Nevertheless, since the sub-acute toxicity test gave an indication of a possible toxicity at the accumulated doses, it may be summarized that the reported ataxia syndrome was actually due to an accumulated toxic effect, arising from continuous consumption

of the larvae over a period of time. This was consistent with the earlier report that ataxia syndrome occurred at a period of the wide availability of *Anaphe venata* larvae and mostly among the low income earners who have a seasonal exacerbation of their thiamine deficiency from thiaminase in seasonal food. (Adamolekun, 1993)

REFERENCES:

Adamolekun B. (1993a) *Anaphe venata* entomology and seasonal ataxic syndrome in South West Nigeria. Lancet 341:629

Adamolekun B. (1993b) Epidemiological studies of the etiology of a seasonal ataxia in Nigeria. Neurology 43: 1419

Adamolekun B., Adamolekun W. E., Sonibare A. D., Sofowara G. (1994) A double-blind, placebo-controlled study of the efficacy of thiamin hydrochloride in a seasonal ataxia in Nigerians. Neurology 44:549-551

Adamolekun B., Ibikunle F. R. (1994a) Investigation of an epidemic of seasonal ataxia in Ikare, western Nigeria. Acta Neurol. Scand. 90:309-311

Ajayi A.A and Ukponmwan O.E (1994) Evidence of Angiotensin II and opiod modulation of novelty-induced rearing in the rat. African Journal of Medicine and Medical Sciences 23: 287-290.

Akinnawo O.O, Abatan M.O, Ketiku A.O (2002): Toxicological study on the edible larva of Cirina forda (Westwood). African Journal of Biomedical Reseach: 5: 43-46

Ashiru M.O (1988) The food value of *Anaphe venata* larvae Buttler (Lepidoptera: Notodontidae). Ecology of Food and Nutrition. 22: 313-320.

Costello C. A., Kelleher N. L., Abe M., McLafferty T. W., Beyloy T. P. (1996) Mechanistic studies on thiaminase I. J. Biol. Chem. 271:3445-3452.

De Follart G.R (1989) The Human used of Insects as food and feed. Bulletin Entomological Society in America. 35:22-35

De Follart G.R (1992) Insects as Human food. Crop Protection 11: 395-399.

Dixon K.C (1958) Fatty deposition; a disorder of the cell Quarterly Journal of Experimental Physiology 43:139-141.

Ene T.C (1963) Insects and Man in West Africa, Ibadan University Press, Ibadan.

Evans W. C. (1975) Thiaminases and their effects on animals. Vitamins & Hormones 33:467-504

Fujita A. (1954) Studies on thiaminase. Vitamins (Japan) 7:1-11

Golding F.S. (1942) The Silkworm in Nigeria Farm and Forest 3: 35-40.

Maricker Y and Paltabiraman T.N (1988): Changes in Protease inhibitory activity in plant seeds on heating. J. Fd Sci. Technol 25(2): 59-62.

Nishimune, T., Watanabe, Y., Okazaki, H., and Akai, H. (2000) Thiamin Is Decomposed Due to *Anaphe* spp. Entomophagy in Seasonal Ataxia Patients in Nigeria. Journal of Nutrition. *130:1625-1628.*

Onayade O.A; Iwalewa E.O; .Adamolekun B and.Omobuwajo O.R (In press): Behavioural Effects and Toxicity Potentials of Non-polar and Polar extracts of *Anaphe venata* larvae in mice. Hamdard Medicus. XLVII (3) July-Sept 2004. (in press)

Onayade O.A and Adamolekun. B (1996) Preliminary entomochemical analysis of the larvae of *Anaphe venata* Butler (Lepidoptere, Notodontidae). African Journal of medicine and medical sciences.

Osuntokun B.O (1972) Epidemic ataxia in Western Nigeria. British Medical Journal 2: 584-589.

Oyedapo O.O. (1996) Studies on bioactivity of the root extract of *Plumbago zeylenica* 34:365-369.

Reitman S and Frankel S. (1957) A Colorimetric method for determination of serum glutamic oxaloacetic and glutamine pyruvic transaminases. American J. Clin. Pathology 35: 58-65.

Saini N.S and Van-Etten L.R. (1979) An essential carboxylic acid group in Human prostate acid Phosphatase. Biochem. Biophys. Acte 568:370-376

Umoh I.B, Ayalonu E.O, Bassir O. (1980) Evaluation of the nutritive value of some lesser known protein sources in Nigerian peasants diets. Ecology of Food and nutrition. 9:81-86.

[i] *Author for correspondence*