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Original article

TOXICOLOGICAL STUDY ON THE EDIBLE LARVA OF *CIRINA FORDA* (WESTWOOD)

O.O. AKINNAWO[1], M.O. ABATAN[2] AND A.O. KETIKU[1*]

Departments of Human Nutrition[1] and Veterinary Physiology/Phamacology[2] University of Ibadan, Ibadan, Nigeria.

Toxicity of the aqueous extracts of raw and processed larva of Cirina forda (Westwood) administered orally were studied in white albino mice and albino rats. Preliminary investigation showed that the raw extract was toxic to mice, showing sign of irritability and muscular tremor. An LD_{50} value of 7,000mg/kg body weight was obtained for the raw extract using mice. The effects of sub lethal dose of the extract on hematological and serum biochemical parameters were also studied in rats for 14 days. No significant effect was observed on most of the hematological and biochemical indices estimated (P> 0.05). Activities of some serum enzymes were normal in all the rats. However, the serum total protein and globulin levels were significantly higher in the control and the group that received processed larva than in the group administered with the raw larva (P<0.05). The albumin level was not affected by the extracts. Boiling of the larva in water followed by sun drying treatment (processed larva) was associated with an increase in the serum total protein and globulin levels in rats. However, the neurotoxic nature of the raw extract needs further investigation.

Keywords: *Cirina forda, larva, toxicity, rats*

**Author for correspondence*

INTRODUCTION

Edible insects constitute a very important food source in many developing Countries. Studies have shown that they are good sources of high quality proteins, fats and minerals (Ene 1963, Ashiru 1988, DeFoliart 1989, 1992). Mass rearing of insects as alternative protein sources has seen advocated by many workers (De Foliart 1996, Ramos – Eloraling 1997).

However, some insects contain powerful pharmacologically active substances, which are known vertebrate toxins. Some insects are also known to secrete toxic metabolites or toxins for defense and other purposes. Other insects secrete chemical compounds as alkaloids, for example fire ants (Solenopsis) venom contains 2, 6– di-akylpiperidimes (MacConnell *et al* 1971); toluene and 0-cresol in longhrn beetles (Moore and Brown, 1971); anabolic steroids from species in the family Dytiscidal (Schildknecht, 1970) as well as cyanogenic glycosides from the larva of the moth *Zyaena trifolii* (Jones *et al* 1962).

Other insects sequester secondary chemicals from their host plants and this phenomenon has been reported in six orders of classification of insects. Substances sequestered include alkaloids, aristocholic acids and glucosinolates (Berenbaum, 1993). Also insects are sources of various types of allergens such as injectaut, ingestaut, constractant and inhalant allergens (Wirtz 1984, Gorham 1991). Many of these substances are known to produce various symptoms in man.

Adamolekun (1993) reported a seasonal ataxic syndrome associated with the consumption of the edible larva of *Anaphe venata* (Butler) in south-west Nigeria. This syndrome is characterized by sudden outset of severe muscular tremors and gait ataxia. Another popular and widely consumed insect larva is that of *Cirina forda* (westwood). The insect is a pest of *Butyrosperuum paradoxuum*, the sheabutter tree and the larva is boiled in water and sun dried into the form that is widely marketed and consumed as an essential ingredient in vegetable soup (Fasoranti and Ajiboye 1993). Because of the wide acceptability and consumption of the larva of *Cirina forda* (westwood) in Nigeria, there is need for toxicological evaluation of the larva of the insect.

MATERIALS AND METHODS

Sample Collection: The larva of *Cirina forda* (westwood) were handpicked from the crowns of sheabutter tree, *Butyrospernum paradoxum* in Batati village, Lavum Local Government Area of Niger state, Nigeria. The identity of the larva was confirmed at the Department of Crop Protection and Environmental Biology, University of Ibadan, Ibadan.

Sample Preparation: 250g of the raw insect larva was boiled in 500mls of distilled water on a low heat for 2hrs and the boiled larva were then sundried for 72hours. After removing the body hairs, the larva samples were milled into powder.

Another 250g of raw larva were killed by freezing (finke *et al*, 1989). The frozen larvae were thawed and dried in an air-drying oven at 40^0c for 48hours. The body hairs of the larva sample were removed before milling into flour. 150g of each flour was extracted separately with 100mls of distilled water at room temperature. The extract was obtained by filtering using a fine cheesecloth of unknown mesh.

Pilot Toxicity Study: Adult mice of both sexes weighing between 20-30g were obtained from Department of Physiology, University of Ibadan. The animals were divided into groups of five mice each and were kept in cages and fed with standard mice cubes. The animals were allowed free access to feed and water. After a of acclimatization, the groups were randomly assigned to the following dose levels; 2,500, 3,750, 6,250, 10,000 and 12,500mg/kg bodyweight using raw and processed larva extracts administered fire and received distilled water by oral administration. Clinical signs were observed post oral administration at 0, 5, 10, 15 and 30minuted and at 1,2,3,4,24 and 48hours. Mortality was recorded in all the groups after 48hrs and a graph of percentage mortality and log Dose (mg/kg) was plotted to obtain the $LD_{50.}$

Sub-lethal Dose Administration.: Adult albino rats of both sexes weighing between 150-200g were used for the study. The rats were divided into groups of six rats each and were allowed free access to feed and water. After one week of acclimatization, the rats were dosed with raw and processed larva extracts at sub lethal levels of 1,750, 2,250 and 3,750mg/kg bodyweight. The doses were administered orally for fourteen (14) days and the control group was also given distilled water by oral administration clinical signs and mortality were observed in all the groups.

Blood Collection and Analysis: At the end of the experiment, the rats were anaesthetized with chloroform and blood was collected by cardiac puncture. 2mls of blood from each rat was put into sample bottles containing disodium EDTA and used to determine heamatological parameters. The packed call volumed (pcv) was determined by the hematocrit method of schalm *et al* (1975) and heamoglobin by cyanmethaemoglobin method (Wintrone *et al* 1981). Red Blood count (RBC) and White Blood Count (WBC) were analysed using a coulter counter heamoglobinometer. Another 3ml of blood form each rat was collected into a sample bottle and allowed to clot. The sera separated from the clot by centrifugation were used in determining the biochemical parameters. Total bilirubin was determined by the method of Jendrassik and by Grof (1938) total serum protein concentration by Biuret reaction and the serum albumin measured using bromocresol green (Donmas *et al* 1971). The serum globulin was obtained from deduction of albumin from the total serum protein. Alanine aminotranferase (ALT), Aspartate aminotransferase (AST) and Alkaline phosphate (ALP) were determin by the methods of International Federation of Clinical Chemistry (IFCC, 1986).

Statistical Analysis

Means and Standard deviations of parameters were calculated using stat pac Gold statistical Analysis Package (1992). To test difference between group means, student's t-test was used. Analysis of multiple treatment effects were conducted using one way analysis of variance (ANOVA).

RESULTS AND DISCUSSION

The result of the pilot toxicity study is presented in Table 1. Increased motor activity and muscular tremors were the physical and signs observed in all the mice the test groups on post oral administration of the raw extract. Partial paralysis and circling motion were observed when the tail held mice given the higher doses. In the group receiving the highest dose, a death occurred within an hour of oral administration preceded by convulsion and twitching of the tail. All deaths recorded in the mice occurred within 24hrs of oral administration.

The clinical signs observed suggest that the raw extract was toxic and the central nervous system appears to be the target of action of the toxin. The value of LD_{50} obtained for the raw extract is 700mg/kg. The processed larva extract did not produce any clinical symptoms in the mice and the mortality observed in some groups treated with the processed larva extract cannot be explained.

Table 1: Dose – Response Evaluation of Aqueous Extract of Raw and Processed Larva of *Cirina forda* (Westwood) in mice

Dose mg/kg	No. of Mice per dose	% Mortality Raw Extract	% Mortality Processed Extract
2,500	5	20	0
3,750	5	40	20
6,250	5	40	0
10,000	5	40	0
12,500	5	80	20
Control	5	0	0

Table 2 : Heamatological Parameters of rats after oral Administration of Extracts of Larva of *Cirina forda* (Westwood). Values are means ± SD (n=6)

Dose mg/kg	PCV %		Hb g/100ml		RBC 10^6/mm^3		WBS 10^3/ mm^3	
Control	41.5[a]		13.8[a]		7.0[a]		8.5[a]	
	R	P	R	P	R	P	R	P
1,750	44.6[a]	41.6[a]	14.9[a]	14.0[a]	7.8[a]	7.2[a]	8.5[a]	8.6[a]
2,250	44.2[a]	43.6[a]	14.6[a]	14.7[a]	7.7[a]	7.5[a]	8.9[a]	8.7[a]
3,750	43.0[a]	41.5[a]	14.2[a]	13.8[a]	7.4[a]	7.0[a]	9.7[a]	8.0[a]

R = Raw Extract; P = Processed Extract; Values along the vertical column for each parameter without a common superscript are significantly different (P<0.05)

Table 3: Serum Biochemical Parameters in Rats after orals Administration of Extracts of Larva of *Cirina forda* (westwood). Values are means ± SD (n = 6)

Dose mg/kg	Total bilirubim mg/dl		Alkaline Phosphase iu/l		Aspartate Amino Transferase iu/l		Alanine Amino Transferase iu/l		Total Protein g/dl		Albumin g/dl		Globulin g/dl	
Control	0.13		329		156		92		7.5		2.9		4.6	
	R	P	R	P	R	P	R	P	R	P	R	P	R	P
1,750	0.11	0.11	336	324	156	149	81	89	6.4	7.1	2.7	2.7	3.7	4.4
2,250	0.11	0.13	282	313	140	150	95	87	6.7	7.5	2.9	2.8	3.8	4.7
3,750	0.10	0.13	309	337	135	143	88	85	7.0	7.6	2.9	2.9	4.1	4.7

R = Raw Extract; P = Processed Extract Values along the vertical column for each parameter without a common superscript are significantly different (P<0.05)

The results of the heamatological parameters estimated are presented in Table2. All the parameters measured were not significantly different (P>0.05) between the raw sample and the control groups. Similarly, there was no significant difference) (P > 0.05) when heamatological parameters in the raw sample treated group and the processed sample treated group were compared.

Table 3 presents the serum biochemical parameters measured in the study. The total bilirubin, alkaline aminotransferase (ALT), Asparate aminotransferase (AST) and Alkaline phosphates (ALP) were not significantly different (P> o.o5) between the control group and those given the raw larva extract. Similarly, there was no significant difference (P > 0.05) in the levels of enzymes when the control group was compared with those given the processed sample. Also when the group receiving the raw and the group receiving the processed extract were compared, these parameters did not indicate any significant difference (P> 0.05). The serum total protein and globulin values were significantly (P<0.05) lower in the group receiving the raw extract compared to the control group for each dose. Also, these parameters were significantly lower in the group receiving the raw extract compared to those receiving the processed extract (p<0.05) for each dose. The albumin level was not significantly different (P> 0.05) in all the groups.

Many insect-derived compounds have been identified (Blum, 1981) but little is known about their toxicology visa-vis human except for compounds like vesicants that are of obvious public health significance. In this study, increased motor activity and muscular tremors were observed after oral administration of the raw extract of the larva of *Cirina forda* to the mice. The processed larva extract did not produce such effects. The boiling and sun drying treatment of the larva may have eliminated the possible neurotoxin in the larva. However, both raw and processed extracts had no effect on the hematological and serum biochemical indices estimated. The normal levels of serum enzymes in all the rats suggest normal functioning of the liver. Thus the larva extracts are considered not hepatotoxic.

The boiling of the larva followed by sun drying treatment was associated with an increase in the serum total protein and globulin. Processing like heat treatment has been shown to improve nutritional quality of proteins and cause partial inactivation of protease inhibitors (Marickar and Paltabiraman, 1988).

This study shows that the processed larva of *Cirina forda* (Westwood) is neither neurotoxic nor hepatotoxic to mice and rats. Previous work by the

authors has shown that the larva of *Cirina forda* (Westwood) has the potential to provide substantial amounts of proteins mineral and polyunsaturated fatty acids to the diets are usually deficient in animal protein (Akinnawo and Ketiku 2000). Since this larva is very popular and widely consumed, its consumption could help to alleviate the incidence of malnutrition especially among people in the low social –economic group. However, human studies still need to be carried out to obtain new data on the potential toxicity of the larva of *Cirina forda* (Westwood).

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