EFFECTS OF EXTRACTS OF DRIED SEEDS OF TOLOACHE, *DATURA INNOXIA* AS ANAESTHESIA ON THE AFRICAN CATFISH *CLARIAS GARIEPINUS* FINGERLINGS

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ABSTRACT

The effects of crude extract. pure extract. aqueous. fraction of pure and lipid fraction of pure extract of dried seeds of toloache, Datura innoxia as anaesthesia on the African catfish, Clarias gariepinus fingerlings were studied. The fish were exposed to various doses of the extract in aquaria tanks and the time taken for each fish to reach anaesthesia was recorded. The fish were anaesthetized up to 3.00g/l fingerlings reached anaesthesia is significantly (p<0.05) shorter time (1.004 minutes at 0.50 gl) in pure unseparated extract than in crude extract (58.50 minutes at 3. 00g/l concentrated). The time to reach anaesthesia decreased with an increase in concentration of the seed extract. Out the two fractions, the lipid fraction had significantly (P<0.05) bettor anaesthetic on the fish. The control produced no observable anaesthetic effect on the fish within three hours. This suggests that the anaesthetizing active ingredient resided in the lipid fraction. All fish recovered from anaesthesia, swam and fed actively and no mortality was observed throughout the exposure period and thereafter. It is therefore recommended for use on C. gariepinus fingerlings.

INTRODUCTION

Anaesthetics are widely used in fisheries science and management to immobilize fish for handling so as to reduce stress and physical damage. For example, capture by nets or hook and line, subsequent measuring fin, clipping, collection of scale samples and insertion of identifying tags constitutes a level of handling that will stress a fish. The level of stress a fish undergoes may affect the animal's immune response and can make it vulnerable to disease. The use of anaesthetics in fish has spanned more than the last five few decades and may compounds have been employed for this purpose, but MS – 222 (tricaine methane sulfonate) benzocaine and quinaldine sulfate (2- methylquinoline) are the most widely used (Randall and Hoar 1971) Each of those compounds has certain disadvantageous toxic effect on fish for instance fish treated with MS-222 must be held for 21 days before release to allow the anaesthetic to leave the fishes body. Because of these drawbacks there is need for an alternative anaesthetic that is effective, low cost, available to third world countries, good margin of safety for fish and is not toxic to humans at concentrations used. Therefore the main goal of this present study was to verify the efficiency of crude and pure extracts of *D innoxia* on fingerlings of the African catfish *Clarias gariepinus* and ascertain whether the active ingredient resided in water-soluble or lipid soluble fractions of *D. innoxia* seed extract.

MATERIALS AND METHODS

Graded series of air-dried seed of *D. innoxia* were soaked in fresh declorinated tap water (0.5, 1.0, 1.5, 2.0, 2.5, 3.0g/l) for 24 hours at room temperature (28°C), after which the mixture was filtered. Before sedation, fish were fasted 24 hours and aeration was provided to anaesthetic bath. Ten healthy actively swimming *C. gariepinus* fingerlings (mean weight 10.08 ±0.02g) were individually immersed into the anaesthetic solutions and behavioural responses carefully monitored and classified according to Ross and Ross (1999) for a period of 180minutes at 28°C.

Each concentration had three replicates. If anaesthesia was attained, the weight and length of each fish was taken thereafter the fish was then placed in a 60L aquarium containing 40L of aerated dechlorinated tap (fresh) water for recovery and time taken to recover notes. Immediately following recovery blood was drawn from the caudal peduncule with heparinized syringe from nine fish at each concentration (three from each replicate) of *D. innoxia* seed extract. Red blood cell count, white blood cell count, packed cell volume, haemoglobin, and erythrocyte sedimentation rate were measured.

Fifty grammes of the air-dried sample of *D. innoxia* seed sample were macerated twice in 240ml chloroform-methanol (2:1) mixture for 24 hours and filtered through No. 1 Whatman filter paper. The filtrate was used as the
unseperated extract. To separate this extract into different fractions, the filtrate was mixed with 0.2 volume of distilled water and separated into lipid and non-lipid layers in a separating funnel. The layers were dried over a heating block and weighed and the concentrations calculated. The lipid layer was dissolved with ethanol and the non-lipid layer dissolved in water. To test the anaesthetic effects of these on the fingerlings, *C. gariepinus* were exposed to (1) the unseparated methanol-chloroform extract, (2) the lipid and (3) non-lipid fractions of the alcohol extracts. Ten fingerlings were individually exposed to dosages of 0.5, 1.0, 1.5, 2.0, 2.5, 3.0g/l of serially diluted solutions and the fish monitored as in the crude aqueous filtrate tests above.

RESULTS

Clarias gariepinus fingerlings exposed to various extracts of *D. innoxia* seed passed sequentially through the various stages of anesthesia. The behavioral responses were excess mucus secretion and apathy, air gulping, distension of the mouth and opercula, erratic swimming and loss of balance. Table I shows that the crude extract of *D. innoxia* seed was able to induce fish to loose reactivity to stimuli at concentration 3.00g/l within 58.50 (0.95) minutes. No such observations were made at concentrations less than 3.00 (i.e. 2.50, 2.0,1.50,1.0, 0.50 and 0.00g/l). The fingerlings were able to recover within two minutes of immersion into fresh aerated water.

Similar results were obtained from fish exposed to unseperated chloroform-methanol extract and fraction of chloroform-methanol extract though the time taken to reach anaesthesia in the alcoholic extract was significantly ($P>0.05$) shorter. To induce fish to a total loss of equilibrium, a dosage of 0.5 g/l was required within 1.27 (0.47) minutes. However, at this dosage, a relatively longer time (1.69 minutes) was needed to induce the minimal opercula movement. To induce fish to minimal opercula needed for surgical anaesthetic state within 59 seconds, more dosage of 3.00 g/l is required. Recovery time increased with increase in concentration of the extract. The non-lipid fraction was able to induce loss of reactivity in *C. gariepinus* within 0.42 (0.26) and 0.35 (0.38) minutes at 2.50 and 3.00g/L respectively.

Haematological values with standard errors obtained for *C. gariepinus* fingerlings exposed to *D. innoxia* sedation are presented in tables II. The mean haemoglobin concentration, Red blood cell count and white blood cell count showed no significant difference ($P>0.05$) between treatment and within treatment for all the extracts (crude, unseparated chloroform-methanol, lipid fraction and non-lipid fractions of the chloroform-methanol). Similarly, mean erythrocyte sedimentation rates and packed cell volume had no significant difference between the control groups and the various concentrations of the crude, unseparated chloroform-methanol, lipid and non-lipid fractions of the chloroform-methanol of the seed extracts.

DISCUSSION

The crude aqueous seed extract of *D. innoxia* was found to be a potent anaesthetic for *C. gariepinus* fingerlings at 3.00g/l within 58.50minutes. The efficacy of *D. innoxia* seed extract was influenced by the dissolution of its active ingredients in chloroform-methanol (2:1) as evidenced

by the significantly ($P<0.05$) shorter time and lower concentration of the unseperated chloroform-methanol extract for *C. gariepinus*. Similar high efficacy was achieved using the lipid fraction of the chloroform-methanol extract. It was observed that anaesthetic time was influenced by dose concentration, and extraction medium (aqueous, unseparated chloroform-methanol, lipid and the non lipid fractions of chloroform-methanol extracts). However, these effects and variations are not unlike the results of other anaesthetic used on fish as observed by Ross ad Ross (1999), Edwards *et al.* (2000), Prince and Powell (2000), Hovda and Linley (2000), Gomes *et al.* (2001), Kazun and Siwicki (2001), Browser (2001), Ortuno *et al.* (2002), Walsh and Pease (2002), woody *et al.* (2002) and Wagner *et al.* (2003).

In general, the lower the dose the longer the time for the anaesthesia, but the faster the recovery. The recovery from anaesthesia seems to be extremely rapid as evidence in the haematological values, which were not significantly different from those of their controls. Thus indicating that the fish recovered fully from anaesthetic effect of *D. innoxia*, which suggests minimal stress during the exposure period. This also indicates that external stimuli influence was minimized. Stress response has been reported with different anaesthetic drugs, such as MS-222 and quinaldine (Ross and Ross 1999) and normally is associated with exposure to high anaesthetic dosage or a prolonged exposure. Such result was not observed with *D. innoxia* extracts.

In earlier work, Eze (1991) reported that *C. gariepinus* were anaesthetic in 4g/l aqueous extract of air-dried ground leaves of suaveolens, recovered and behaved normally after 24 hours. Mgbenka and Ejiofor (1998) reported that *C. gariepinus* and *Heterobrachus longifilis* fingerlings were anaesthetized in up to 3.5g/l crude extract of air dried *Erythrophelum suaveolens* and recovered in fresh water. According to these authors, the two clariids, when exposed to pure unseparated extract were anaesthetized in significantly ($P<0.05$) shorter time than in the crude extract.

D. innoxia seed anaesthesia was not toxic to *C. gariepinus* tissue at concentration 3g/l and below within 180 minutes as indicated by absence of haematological changes and mortality. In all the tests, *C. gariepinus* fingerlings recovered and exhibited no abnormal behaviours. From this study, it was inferred that *D.innoxia* seed extracts anaethesized *C. gariepinus* and no mortality was observed during and after the exposure period. It is therefore recommended that *D innoxia* seed extracts be used as an anaesthetic for *C. gariepinus*.

TABLE 2: HAEMATOLOGICAL VALUES (WITH STANDARD ERRORS) OBTAINED FOR C. GARIEPINUS EXPOSED TO D. INNOXIA SEED SEDATION

Concentration (g/l)	Haematological Parameters				
	Hb(g/l)	RBC ($X 10^6$ mm^{-3})	WBC ($X 10^3$ mm^{-3})	ESR (mm/h)	PCV (%)
0.00	8.88	2.25	38.42	2.34	45.30
	(0.33)	(0.33)	(0.57)	(0.19)	(0.19)
0.50	8.86	2.25	38.41	2.34	45.30
	(0.61)	(0.38)	(0.54)	(0.16)	(0.15)
1.00	8.90	2.25	38.42	2.34	45.29
	(0.56)	(0.26)	(0.38)	(0.28)	(0.15)
1.50	8.91	2.26	38.41	2.35	45.29
	(0.82)	(0.42)	(0.91)	(0.15)	(0.16)
2.00	8.87	2.27	38.43	2.35	45.28
	(0.86)	(0.29)	(0.29)	(0.29)	(0.12)
2.50	8.88	2.27	38.42	2.35	45.28
	(0.75)	(0.30)	(0.40)	(0.19)	(0.13)
3.00	8.85	2.27	38.43	2.35	45.28
	(0.89)	(0.50)	(0.25)	(0.20)	(0.17)
0.00	8.86	2.25	38.40	2.35	45.30
	(0.86)	(0.26)	(0.30)	(0.22)	(0.13)
0.50	8.87	2.26	38.42	2.35	45.30
	(0.89)	(0.30)	(0.70)	(0.18)	(0.11)
1.00	8.88	2.27	38.42	2.35	45.30
	(0.56)	(0.28)	(0.61)	(0.21)	(0.10)
1.50	8.89	2.27	38.41	2.36	45.29
	(0.27)	(0.27)	(0.58)	(0.23)	(0.12)
2.00	8.88	2.25	38.42	2.35	45.29
	(0.30)	(0.34)	(0.40)	(0.25)	(0.13)
2.50	8.89	2.26	38.41	2.34	45.29
	(0.25)	(0.33)	(0.45)	(0.24)	(0.14)
3.00	8.90	2.26	38.42	2.36	45.30
	(0.35)	(0.25)	(0.28)	(0.20)	(0.23)
0.00	8.87	2.26	38.41	2.34	45.30
	(0.39)	(0.20)	(0.50)	(0.23)	(0.10)
0.50	8.88	2.25	38.41	2.35	45.30
	(0.33)	(0.18)	(0.25)	(0.19)	(0.16)
1.00	8.85	2.26	38.41	2.35	45.30
	(0.63)	(0.13)	(0.38)	(0.17)	(0.11)
1.50	8.86	2.26	38.41	2.35	45.30
	(0.45)	(0.26)	(0.41)	(0.30)	(0.20)
2.00	8.86	2.25	38.42	2.34	45.29
	(0.24)	(0.28)	(0.32)	(0.21)	(0.17)
2.50	8.87	2.25	38.42	2.34	45.29
	(0.29)	(0.11)	(0.36)	(0.24)	(0.21)
3.00	8.88	2.26	38.42	2.34	45.30
	(0.20)	(0.33)	(0.70)	(0.24)	(0.17)
0.00	8.88	2.26	38.41	2.35	45.30
	(0.55)	(0.28)	(0.40)	(0.22)	(0.16)
0.50	8.87	2.26	38.41	2.35	45.30
	(0.29)	(0.23)	(0.39)	(0.22)	(0.21)
1.00	8.88	2.26	38.42	2.35	45.30
	(0.35)	(0.19)	(0.28)	(0.25)	(0.19)
1.50	8.87	2.26	38.41	2.35	45.30
	(0.52)	(0.22)	(0.35)	(0.28)	(0.15)
2.00	8.85	2.26	38.41	2.36	45.29
	(0.63)	(0.25)	(0.30)	(0.22)	(0.13)
2.50	8.87	2.26	38.42	2.35	45.29
	(0.42)	(0.19)	(0.33)	(0.21)	(0.22)
3.00	8.89	2.26	38.42	2.35	45.29
	(0.44)	(0.20)	(0.29)	(0.20)	(0.12)

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