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Anaesthetic effects of *Datura stramonium* Leaf on *Heterobranchus bidorsalis* Juveniles

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Abstract
Anaesthetic effects of *Datura stramonium* was investigated using aqueous and ethanol extracts on *Heterobranchus bidorsalis* juveniles as a natural anaesthetic. The treated fish reached anaesthesia within 25 min and recovered in the shortest time 12 min. There was a significant positive correlation between concentration and recovery time of aqueous extract (r=0.990; $p<0.05$) and ethanol extract (r=0.994; $p<0.05$) of *Datura stramonium*. The induction time decreased significantly with increase in concentration of aqueous extract (1.0-5.0 ppm) and ethanol extract (0.5-4.0 ppm) of *Datura stramonium*. Ethanol extract has the shortest induction time 12 min and recovery time 23 min 30 sec. The findings show that *D. stramonium* plant extracts are good anaesthethic agent; they are cheaper and have zero hazard effect on user thus *D. stramonium* could be used as anaesthetic in fisheries management.

Keywords: Anaesthetic, *D. stramonium,* effective dose, *H. bidorsalis,* Recovery time

1. Introduction
The handling of fish out of their natural environment always creates stress which affects their physiology and anatomy. Anaesthetics are often used during these various handlings to reduce metabolic activities. Some researchers carried out some works using plant extracts as natural anaesthetic, because it is cheaper, safer and more effective at lower concentrations when compared with chemical anaesthetics (Akinbulumo, 2005, Agokei and Adebisi 2010) [3, 2] while Popoola *et al.* (2015) [13] also reported the use of plant extracts as substitute for synthetic anaesthetics. *D stramonium* is a common African plant used among the uniform men especially the military men for psycho-active effects Das *et al.* (2012) [6]. Datura contains hyoscine, scopolamine as well as atropine, these are hallucinogenic (substance that courses denseness), alkaloids found in *D. stramonium m* was also reported to be narcotic (Carruthers *et al.,* 2010) [5]. *D. stramonium* plant is a highly effective anaesthetic with no side effects; it is locally available and cheap.
According to Akinbulumo (2005) [3], *Derris elliptica* is a natural plant used as an anaesthetic agent on *O. niloticus* fingerlings showed to be more effective and safer than synthetic anaesthetics. Ramanayaka and Atapattu (2006) [14] reported the anaesthetic properties of some plants; Clove (*Eugnia ariophylum*), Crotalaria (*Crotalaria* spp), *Derris scandens* and *Euphorbia antiquorum* extracts on common carp (*Cyprinus carpio*) while Agokei and Adebisi (2010) [2]; studied the use of aqueous and ethanol extracts of tobacco as an anaesthetic for fish during handling procedures. Popoola *et al.,*(2015) [13]; compared clove oil a synthesis agent with mistletoe (*Visum album)* and Avocado pear (*Pyrus communis*) plants extracts as natural anaesthetics in partial gonadectomy of African catfish. The results of their works showed that some plants extracts are effective fish anaesthetic. The objective of the study was to assess the efficacy of D. *stramonium* extract as fish anaesthesia.

2. Material and Methods
2.1 Preparation of Extracts
The *D. stramonium* leaves were plucked from host plants in Akure environs. The fresh leaves were washed and dried in an oven at 60 ^{0}C for 8 hours per day for 32 hours. The dried materials were milled into powder and sieved using 0.1mm sieve to obtain a fine powder from the leaves and stored in desiccators at room temperature (27 ºC) prior to the extraction.

2.2 Experimental Fish
Four hundred (400) healthy *H. bidorsalis* juveniles (wt. 20.5± 0.86g) were obtained from a fish farm at Akure, Nigeria. The fish were transported life to the Teaching and Research Farm, Department of Fisheries and Aquaculture Technology, The Federal University of Technology, Akure prior to the experiment. The fish were acclimated to laboratory conditions for 48 hours in a glass tank (70cm X 50cm X 40cm) filled with fresh water (20l). Fish were not fed 24 hours prior to the experiment in order to minimize waste product.

2.3 Experimental Procedure
Two experimental procedures were undertaken; aqueous extract experimental set-up and ethanol extract experimental set-up. The experiment was conducted under standard bioassay procedures (American Public Health Association 1971) [4] which involves carefully controlled environmental condition so as to define the responses of the test organisms to *D. stramonium* extract.

2.3.1 Aqueous Extract Experimental Set-up:-A known weight (200g) of powdered leaf of *D. stramonium* was put into two conical flasks containing 1000ml of distilled water and soaked for 24 hours after which it was filtered; the filtrate was used as anaesthetic agent. Ten *H. bidorsalis* juveniles were introduced into 12 aquaria (glass tanks) and each aquarium containing 10 liters of water were covered with netting material to prevent the fish from jumping out. The concentration of *D. stramonium* used for experiment (0.5, 1.0, 2.0, 3.0, 4.0, & 5.0ppm) representing six treatments in duplicates (determined arithmetically) and a control (0.0ppm) were administered to the test fish after obtaining the result of the range finding test.. The experimental set-up was prepared in duplicates. The induction time and recovery time were recorded. Fish that lost balance and ceased movements of their opercula membrane (Anaesthetic phase) were removed immediately and transferred to ten (10) litres fresh water. The recovery time was determined when regular respiratory movement of the opercula starts again.

2.3.2 Ethanol Extract Experimental Set-up:- Ethanol extract was prepared by packing 200g of *D. stramonium* powder into two soxhlet extractor (100g per soxhlet bottle), ethanol was use as solvent for the extraction. The set up was in flow through to reduce heat generated at the extraction, 20g of *D stramonium* crude extract was obtained and was used as the anaesthetic. Six varying concentrations (0.5, 1.0, 2.0, 3.0, 4.0, & 5.0ppm) of *D stramonium* representing six treatments in duplicates (determined arithmetically) and a control (0.0ppm) were administered to the test fish after obtaining the result of the range finding test. The behaviour of the test fish was also monitored, induction time and recovery time was recorded.

2.4 Water Quality Parameter: The temperature of the media were taken using a mercury in glass thermometer, pH values were determined using pH meter, Dissolved Oxygen was determine using Dissolved oxygen meter inserted into the sample glass tanks after standardization in three different buffers per use.

2.5 Statistical Analysis
Data obtain were analysis using SAS program version 8.0, Pearson's correlation coefficient to test level of interaction between doses/concentrations, recovery and induction times.

3. Results
3.1 Aqueous Extract Experiment: Fish exhibited partial loss of equilibrium within the first 15min in the lower concentrations (0.5. 1.0, 2.0, 3.0 ppm) while at concentrations 4.0 and 5.0 ppm, they responded to anaesthesia immediately and lay at the bottom of the aquaria. The fish were removed into recovery tanks immediately they sense no response to movement. Fish exposed to concentration 0.5ppm show no visible anaesthetic reaction throughout the experimental period (60 min). At concentration 1.0ppm of *D. stramonium*, average induction time was 25 min and average recovered time was 30 min after transferring them into recovery tank. At concentration 2.0ppm the test fish reached anaesthesia within 3 min and recovered 32 min after. While at concentrations 3.0ppm and 4.0ppm, 100% of the test fish reached anaesthesia within 20 – 22 min and they all recovered within 34-37 min respectively. In the highest concentration (5.0ppm), they all reached anaesthesia within 18 min and 80% recovered in 40 min (10% did not recover while 10% recovered to die after 40min), Table 1

Table 1*:* Concentration, Induction time, Recovery time and Mortality of *H. bidorsalis* juveniles anesthetized with aqueous extracts of *D. stramonium*

Experimental Treatments	Anaesthetic dose (ppm)	Induction Time (Min)	Recovery Time (Min)	Mortality (%)
1	0.0(control)	-	-	-
2	0.5	Ineffective	-	-
3	1.0	25.00 ± 0.50^{d}	30.00 ± 0.50^{a}	-
4	2.0	23.00 ± 1.00^{cd}	32.00 ± 0.50^{ab}	-
5	3.0	22.00 ± 0.00^{c}	34.00 ± 0.00^{b3}	-
6	4.0	20.00 ± 0.00^{b}	37.00 ± 0.00^{c}	-
7	5.0	18.00 ± 0.00^{a}	40.00 ± 0.50^{d}	20

*mean ± standard error. Superscripts a-d indicate significant different in rows, superscripts ab and bc indicate significant correlation in row among variable

Table 1 shows no significant correlation between dose (concentration) and induction time, increase in concentration led to decrease in duration of expose to anaesthetic (induction time), the correlate value (r) is 0.989 at significant level 0.05 (r=0.989; $p>0.05$). While there is significant correlation between concentration and recovery time, increase in concentration also led to increase in duration of the fish coming back to life, the correlate value (r) is 0.988 (r=0.988; $p<0.05$).

3.2 Ethanol Extract Experiment: Table 2 shows various ethanol concentrations, induction time, recovery time and percentage mortality of *H. bidorsalis* exposed to different concentrations of ethanolic *D. stramonium* extract. At concentration 0.5 ppm induction time was at average 17 min to recover after transferring them into recovery tank at average 23 min 30 sec. At concentration 1.0 ppm, average induction time was 15 min to recover at 25 min. In concentrations 2.0ppm and 3.0 ppm (significant correlation),

average induction time was 14 min and 12 min while their recovery times were 27 min and 28 min respectively with close correlation. At concentrations 4 ppm and 5.0 ppm, the fish were anaesthetized between 7 min to 10 min to recover within 30 min and 68 min respectively, 10% of the test fish died in concentration 4.0 ppm and 40% mortality was recorded in concentration 5.0 ppm.

Table 2: Concentration, Induction time, Recovery time and Mortality of *H. bidorsalis* anaesthetized with ethanol extracts of *D. stramonium*

Experimental Treatments	Anaesthetic dose (ppm)	Induction Time (Min)	Recovery Time (Min)	Mortality (%)
1	0.0(control)	-	-	-
2	0.5	17.00 ±1.0^{c}	23.50± 0.05^{a}	-
3	1.0	15.00 ±0.50^{bc}	25.00 ± 0.00^{ab}	-
4	2.0	14.00 ± 0.00^{b}	27.00 ± 0.05^{b}	-
5	3.0	12.00 ± 0.00^{ab}	28.00±0.75^{bc}	-
6	4.0	10.00 ± 0.20^{a}	30.00 ± 1.05^{c}	10
7*	5.0	7.00 ± 0.20^{a}	68.00 ± 1.05^{d}	40

Mean± standard error, except concentration of 5.0 ml

There is also no significant correlation between concentration and induction time in ethanol extract. Increase in concentration decrease the duration of exposure of the test fish to anaesthetic, correlations value (r) is 0.977 at significant level of 0.05 (r=0.977; p>0.5) while there is significant correlation between concentration and recovery time, increasing in concentration also increases recovery time of the fish from anaesthetic (r = 0.984; p<0.05).

Analysis of variance (ANOVA) between aqueous and ethanol extract indicates there is no signature different between them, both extracts could be used as anaesthesia agent in fisheries management.

3.3 Water Quality Parameters: Temperature decreased from 23.00–21.60 ^{0}C and there is significance different (p<0.05) between the temperature in the control experiment and ethanol extract. The pH values obtained also decreased from 6.70–6.20, there is significant difference (P<0.05) in the pH obtained between the value before and after the experiment. There is significant difference in Dissolved oxygen (P<0.05) which decreased from 8.30-7.60 before and after the experiments (Table 3).

Table 3: Water Quality Parameters values obtained before and after subjection of *H. bidorsalis* to different concentrations of *D. stramonium* extracts

Water Parameter	Before Subjection	After Subjection
Temperature (^{O}C)	23.00±0.09^{a}	21.60±0.20^{c}
pH	6.70±0.04^{a}	6.20±0.49^{c}
Dissolved Oxygen	8.30±0.11^{a}	7.60±0.14^{c}

Summary Mean±SD, superscripts a-c = indicate significant correlation in rows,

4. Discussions

The results of this study which showed that *D. stramonium* extracts used as anaesthetic agent on fish (*H. bidorsalis*) at various concentrations of *D. stramonium*, induction and recovery time of anaesthetized fish followed the typical patterns of fish anaesthetic as reported by Marking and Meyer (1985) [15], Agokei and Adebisi (2010) [2]. This study agreed with some studies which reported that fish exposed to anaesthetics or toxicants usually exhibit decrease in opercula movement, swimming movements, loss of reflection and hyperactivities (Agbon *et al*., 2002, Akinbulumo, 2005, Olufayo and Fagbenro, 2007, Olufayo and David, 2013) [1, 3 10, 11]. Omoniyi *et al.,* (2002) [12] have reported that decreased in opercula movement was caused by decreased efficiency oxygen uptake or oxygen transport and the behaviour displayed by *H. bidorsalis* juveniles used in the study agreed with these findings.

The presence of active component of hyoscine, scopolamine, atropine as well rotenone found in *D. stramonium* has high potency of hallucinogenic substance which facilities sedation period in *H bidorsalis*. Schmidt (2010) [17] has reported that rotenone-containing plants, *Fabaceae and Solanales* families were crushed and introduced into water body by some indigenous people to catch fish, he explained further that rotenone interfered with cellular respiration of the fish, the affected fish rose to the surface in an attempt to gulp for air, where they were easily caught. Hayes, (1991) [8] has reported that rotenone works by interfering with the electron transport chain in mitochondria, inhibits the transfers of anon air electrons which interferes with Nicotinamide Adenine Dinucleotide (NAD) to Nicotinamide Adenine Dinucleotide Hydride (NADH) during the creation of usable cellular energy Adenosine triphosphate (ATP). Also Hayes (1991) [8] has reported that rotenone works by interfering with the electron transport chain in mitochondria by inhibiting the transfer of electron from iron sulphur centre in complex I to Ubiguinone, this interferes with NADH during the creation of usable cellular energy (ATP) also Ross and Ross (1999)[15] and Histon (2000) [9] reported that rotenone is very insoluble in water and gill have a relatively high lipid content that favour rotenone which blocks electron transport in mitochondria by inhibiting oxidation linked to NADH^{2} which resulting in nerve condition blockage similar to observation in this study, when *D. stramonium* plant extracts was introduce into the water, Nicotinamide adenine dinucleotide (NAD) which is one of the most important coenzymes in the cell, Healthy bodies make all the NADH they need using nicotinamide NAD as a starting point. The NAD coenzyme acts as a hydrogen acceptor in oxidation-reduction reactions. The electron transport chain in cellular respiration of the fish is reduce for energy production and is an excellent illustration of NAD's involvement in redox reactions. The fish swim erratically, gasping for air and moved to bottom of the glass tank. The ventilation rate slow down and they sank to the bottom where they remained motionless (indicate anaesthetized stage).

5. Conclusion

Synthetic anaesthetics are widely used in fishery management but they are costly and not easily available locally and as a result of this, an option to synthetic anaesthetic is plant

anaesthetic which is readily available, cheaper and accessible to the local fish farmers when you compare to anaesthetic such as MS-222. Thus, the use of natural anaesthetic is preferred to synthetic because it is more economically sustainable, environmentally friendly, non-toxicity at low concentration and less persistence in the aquatic environment. The use of *D. stramonium* leaf powder as anaesthetic agent in fish management is viewed positive, therefore *D. stramonium* should be used as an anaesthesia in fish management at concentrations 1.0ppm to 3.0ppm.

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