ASSESSMENT OF TOXICITY OF CLOVE OIL TO SPOTTED SNAKEHEAD, CHANNA PUNCTATA (BLOCH), ON THE BASIS OF RESPIRATORY AND BIOCHEMICAL INDICES

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Abstract: The handling of fish out of water pose difficulties and also lead to stress to the fish. Struggling during capture and handling usually has strong effects on both physiology and behaviour. Handling is required for such activities like collection of eggs and milt, tagging, treatment, weighing, and sorting. Hence, it is desirable to immobilise fish before performing such activities. Clove oil is ideal for sedating fish for above purposes. Sedation is a good technique in fish culture to minimize stress or injury caused by crowding, capture, handling and release. The present study investigates the effect of clove oil on the respiratory metabolic parameters such as O2 consumption and opercular activity of snake head fish, *Channa punctata*. Also, influence of clove oil exposure on muscle and liver glycogen content is determined. The concentration of clove oil was selected based on the time of induction of sedation and recovery of the snakehead fish. The concentrations of clove oil in the present study were 50 ppm and 100 ppm.

Keywords: Respiratory Metabolism, Snakehead Fish, Muscle, Liver, Glycogen, Anesthetics, Clove Oil.

Introduction: Fish are easily stressed by handling and transport and stress can result in immuno-suppression, physical injury, or even death. In aquaculture, anesthetics and sedatives are used during transportation to prevent physical injury and reduce metabolism (DO consumption and excretion). They are also used to immobilize fish so they can be handled more easily during harvesting, sampling and spawning procedures.

An ideal anesthetic should induce anesthesia rapidly with minimum hyperactivity or stress. It should be easy to administer and should maintain the animal in the chosen state. When the animal is removed from the anesthetic, recovery should be rapid. The anesthetic should be effective at low doses and the toxic dose should greatly exceed the effective dose so that there is a wide margin of safety[1]. Dissolved oxygen (DO) is the most important factor in any fish-transport system. Proper DO must be maintained throughout transport. Similarly, when fish are handled for treatment or other operations, they tend to be kept out of water, even if for short durations of time. These short spells of oxygen deprivation tend to push the fish deeper into stressful conditions[2]. The same can be said in other conditions where fish may be stocked in smaller tanks, taken out of water for treatment with hormones for induced breeding. Under conditions of breeding, egg/milt harvesting or transport the fish are cut off from oxygen for some time, again putting it under stress.

Clove oil is commonly known to be a local anesthetic but it also acts systemically when absorbed through gills and skin [3]. Its lipophilic nature facilitates absorption across epithelia and into circulation [4].

Table- Stages of anesthesia in fish.

Stage	Condition	Behavior/Response
I	Sedation	Motion & breathing reduced
II	Anesthesia	Partial loss of equilibrium
III	Surgical anesthesia	Total loss of equilibrium, No reaction to touch stimuli
IV	Death	Breathing & heart beat stop, Overdose - eventual death

Clove oil has been widely used as an anesthetic in human dentistry and as a condiment. The major constituent is the oil eugenol. It is an effective anesthesia in fish. In rainbow trout, *Oncorhynchus mykiss*, doses as low as 2 to 5 mg/L produced sedation sufficient to transport the fish, while doses of 40 to 60 mg/L for 3 to 6 minutes gave effective surgical anesthesia. Recovery time increases with higher doses and longer exposure time. Clove oil is also an effective anesthetic for crustaceans at doses of 100 to 200 mg/L[1]. Clove oil is very safe to work with. The major advantage of clove oil is that it is cheap and easily available.

Experimental Fish: Spotted snakehead fish, *Channa punctata* was procured in a healthy condition from local fish supplier. The length of the fish varied from 150mm to 155mm while the weight was between 45 - 58 g. The fish were reared in the laboratory in glass aquarium tanks measuring 50 *x* 25 *x* 25 cm. The water was aerated for 24 hours in a day in all the rearing

tanks. Fifty percent of the water was changed once a week to maintain the water quality parameters at the optimum level. The fish were fed with raw minced beef twice a day. The fish were starved for 48 hrs prior to commencement of the experiment.

Experimental Setup: All the experiments were conducted in 5 liter glass aquaria. Conditions were maintained identical in all containers used for inducing sedation and for recovery afterwards. Predetermined quantities of clove oil was added to the aquaria in each experiment. The dissolved oxygen content was measured in the experimental tank during the initial stage, and at durations of one hour, two hours and three hours into the experiment. The average weight of the snakeheads was 50.82 ± 3.92 g. Appropriate controls were also maintained with the same number of fish in same capacity glass aquarium.

Anesthetization Experiment: Laboratory grade clove oil was purchased from Hi-MEDIA. The range of concentration of clove oil used in the present study was selected after conducting initial anesthetization tests from the minimum to the maximum levels in spotted snakehead fish. The concentration of clove oil used for the initial anesthetization experiment were 50 ppm and 100 ppm.

Clove oil was mixed with water in the experimental tank to achieve the desired concentration. The fish were introduced into the experimental tank with care to avoid stress. The time of induction of anesthesia was noted and when the fish reached the surgical plane of anesthesia, they were then transferred to the recovery tank containing fully aerated water for sacrificing and collection of tissue samples. The rearing tank was covered with a lid without leaving any air bubbles and sealed with petroleum jelly to avoid contact with external air, thereby eliminating the possibility of air-breathing. The opercular movement of the control and experimental fish were noted by visual observation for one minute at an interval of 15 minutes. Water samples were drawn from the experimental tanks using plastic tubing for determination of dissolved oxygen. The dissolved oxygen content of the water samples was estimated by Winkler's method.

The fish were sacrificed at the beginning and at the end of experiment. Muscle and liver tissue samples taken from the sacrificed fish for biochemical analysis. Total glycogen content of the tissues was determined by Anthrone method.

Results:

Clove oil and Respiratory Activity: There is a decreasing trend in opercular beat frequency in *Channa punctata* under the influence of clove oil. In fish exposed to 50ppm clove oil, opercular beat frequency decreased from 11 beats/min at the beginning to 3 beats/min at the end of the exposure period. The same trend is seen in fish exposed to 100ppm clove oil; from 14 to 2 beats/min. Compared to the controls, there was a significant steady decline in the frequency of opercular beats in this fish under treatment (Fig. 1).

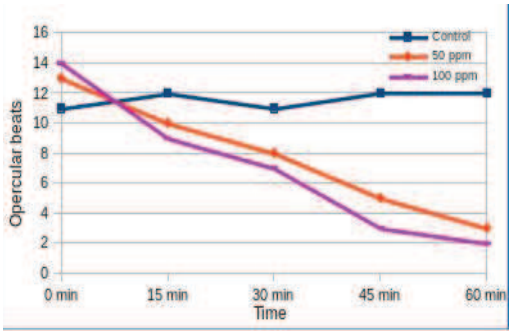

Fig. 1- Effect of clove oil on Channa punctata.

Clove oil and Tissue Glycogen Content: The glycogen content in muscle and liver showed decreasing values from the beginning of exposure period to end. Fig. 2 indicates glycogen content of both muscle and liver of the fish. The normal (control) fish showed a change in glycogen content during exposure period from 1.98±0.13 to 1.6±0.08 mg/g in muscle and from 4.1±0.47 to 3.77±0.35 mg/g in liver.

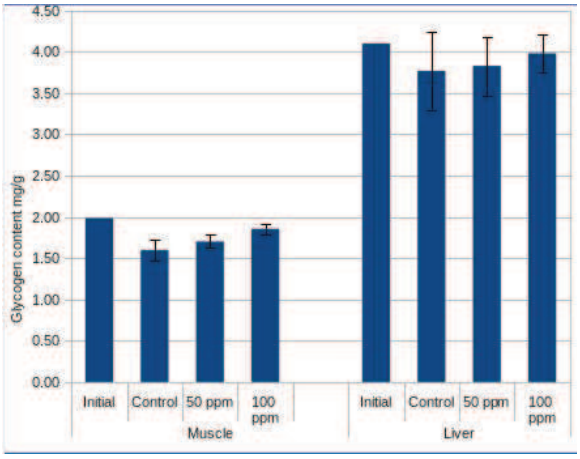

Fig. 2- Effect of clove oil on glycogen content of tissues in Channa punctata.

While in clove oil treated fish, the values were 1.7±0.06 mg/g in 50ppm and 1.85±0.11 mg/g in 100ppm exposure, in comparison to the normal value of 1.98 mg/g. In liver a similar decrease in glycogen utilization was observed. Under control conditions the glycogen content changed from the initial 4.1±0.47 mg/g to 3.77±0.35 mg/g, showing a utilization of 0.33 mg/g in one hour. In treated fish, glycogen content changed from 4.1 to 3.83±0.23 and 3.98±0.23 mg/g in 50ppm and 100ppm clove oil exposed fish respectively. This reflects a decline in

glycogen utilization at 0.27 mg/g and 0.12 mg in 50ppm and 100ppm exposed fish; which could be considered due to a decrease in respiratory rate.

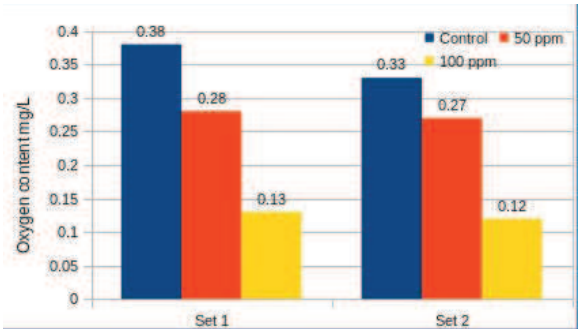

Fig. 3- Effect of clove oil on oxygen consumption in Channa punctata.

Discussion: The present study shows a significant effect of clove oil treatment on respiratory activities and glycogen content in *Channa punctata*. Anesthesia induced decrease in ventilation is strongly established. Diurnal fluctuations in metabolic rate effect of handling can lead to increase in oxygen consumption in fish. Initially the fish may show hyperventilation and increased oxygen consumption as a stress response. Coho salmon and Rainbow trout show recovery of opercular ventilation rates within 1–2 hours after ketamine hydrochloride treatment [5]. Effect of anaesthetics on the oxygen consumption of platyfish, Xiphophorus maculatus, were reported to be dosage and temperature dependent when treated with 2-phenoxyethanol[6]. A state of hypoxia that results from reduced respiratory activities leads to physiological changes such as increased blood glucose[7].

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