

## RESEARCH ARTICLE

COMPARATIVE EFFICACY OF CLOVE OIL AND 2-PHENOXY ETHANOL ON SERUM BIOCHEMICAL CHANGES AND HISTOLOGICAL STUDIES IN *CHANNAPUNCTATUS*\*G. Chella durai<sup>1</sup>, J. Mohan raj<sup>1</sup>, S. Subbu lakshmi<sup>2</sup><sup>1</sup>Department of Advanced Zoology & Biotechnology, Kamaraj College, Thoothukudi, Tamilnadu, India<sup>2</sup>Department of Microbiology, Kamaraj College, Thoothukudi, Tamilnadu, India

\*Corresponding author: G.chelladurai, chellam.zoo@gmail.com. 9629484026

## ABSTRACT

The aim of the present study was to investigate the comparison of effect of clove oil and 2-phenoxy ethanol for *Channapunctatus* and using values of serum biochemical profile and histological tissue studies, to assess the effects of fish exposure to that anaesthetic: A total of 60 *C.punctatus* of 60.23±5.02g were divided into five groups (12 fish per each): 1<sup>st</sup> treatment as control (no anaesthetic), 2<sup>nd</sup> 0.2ml/1 2-phenoxy ethanol, 3<sup>rd</sup> 0.3ml/1 2-phenoxy ethanol, 4<sup>th</sup> 30mg/1 clove oil and 5<sup>th</sup> 40mg/1 clove oil. Biochemical blood profile of *C.punctatus* were taken 15 min and 24 hrs. After Anaesthesia induction, the factors used to evaluate the serum biochemical profile included the glucose (GLU), total protein (TP), albumin (ALB), total globulin (GLOB), alkaline phosphatase (ALP), serum Glutamic oxaloacetic Transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), histological tissue examination of tissue like gills. Ten minutes exposure to 2-phenoxy ethanol and clove oil caused an increase in concentration of glucose and alkaline phosphatase 15 min after Anaesthesia induction. Histological examination showed. Capillary ectasia of gill filaments immediately after clove oil and 2-phenoxy ethanol, showed swelling of primary and secondary lamella. Twenty-four hours after anaesthesia, no ectasia was observed. No histopathological changes were demonstrated in gills: Our result showed that The 2-phenoxy ethanol at 0.20ml/1 and clove oil at 30mg/1 concentration may be used as an efficient and safe anaesthetic for *Channapunctatus*.

**Key words:** Clove oil, 2-Phenoxy ethanol, *Channapunctatus*, Serum biochemical profile, histological examination of tissues.

## INTRODUCTION

Anaesthetics are one of the groups of pharmaceutical preparation. They have been used extensive and intensive fish culture to reduce the effects of stress on fish and to lower mortality after handling and transporting large stock of fish. General anaesthetics have been applied for many years in fisheries. The anaesthetics are used with increasing frequency on aquaculture, mainly to reduce the stress and to prevent mechanical damage to fish during handling. The most widely used anaesthetics include MS222, (Munday and Nilson, 1997; Ross and Ross, 1999). 2-Phenoxy ethanol, quinaldine, quinaldine sulphate and clove oil, modern fish anaesthetics must meet a number of general requirements, e.g. They must be highly soluble in water, take effect quickly be safe for both fish and human's have broad safety margins, allow anaesthesia intensification of anaesthesia with possibility of spontaneous recovery and they must leave no residues. Clove oil is dark brown liquid, a distillate of flowers stalks and leaves of the clove tree. *Eugenia aromatic* (Soto and Burhanuddin, 1995). At the present clove oil is used for short-term immobilization of the fish before artificial spawning. The recommended concentration for anaesthetic purposes is 30mg/1 of water both (Svoboda and Kolarova, 1999; Hamackova et al., 2001).

The first use of 2-phenoxy ethanol to anaesthetize salmonids was reported in 1963 from Canada (Sehdev et al., 1963; Boel 1964). 2-Phenoxy (Ethylene Glycol mono phenoxy ether) is used for short term immobilization of fish before artificial spawning and generally recommended concentration is 0.20ml/1 of water bath for big breeding fishes, the recommended concentration is 0.30ml/1 of water bath. At the recommended concentration anaesthesia is induced within 5 to 10 min. When transferred to clean water, fish will recover within 10 min (Svoboda and Kolarova 1999; Hamackova et al., 2001). The efficacy is conditioned by environmental (Temperature, pH, and biological factors (Size, weight, lipid

content and fish species) (Bukraet et al., 1997; Ross and Ross, 1999). The anaesthesia subsides and physiological reflexes are restored within 10 minutes after the fish are transferred to clean water (Svoboda, J. Kolarova and S. Narratilet et al., 2007). The present study was to evaluate the effects of clove oil and 2-phenoxy ethanol on serum biochemical parameters and histological studies in *Channapunctatus*.

## MATERIALS METHODS

Healthy *Channapunctatus* were transported from Korampallam fish tank. The fish were acclimated in the laboratory for 10 days. The water used in the experiment had the following mean values for water characteristics: temperature 24±0.1, pH 7.4 ±0.1, and the dissolved oxygen (DO) was oxygen was observed above 7mg l<sup>-1</sup>. 1<sup>st</sup> group served as control (no anaesthesia), 2<sup>nd</sup> 0.2ml/1 2-phenoxy ethanol, 3<sup>rd</sup> 0.3ml/1 2-phenoxy ethanol, 4<sup>th</sup> 30mg/1 clove oil and 5<sup>th</sup> 40mg/1 clove oil. The clove oil (90% eugenol) was first dissolved in 95% ethanol at the ratio of 1:2 (Clove oil: ethanol) and then diluted by shaking with water. The solution was added to the experimental tank 30 min before the introduction of fishes. The induction time carried out in two 30 liter aquaria for 10 min the fishes were exposed to anaesthesia.

Changes in the physiological status of anaesthetized fish were assessed in four consecutive stages (Thienpoint and Niemegeers 1965):

1. Acceleration and subsequent declaration of breathing movements, a partial loss of reactivity to external stimuli:
2. Loss of balance breathing movement's very slow, fish still reactive to strong stimuli.
3. Total loss of reactivity, fish are lying at the tank bottom and do not respond to handling.

4. Complete cessation of opercula movements of fish die if left in the bath for too long.

#### Biochemical examination of blood plasma

The biochemical indices in plasma included; glucose (GLU), total protein (TP), albumin (ALB), total globulin (GLOB), alkaline phosphatase (ALP), serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT). Blood plasma was obtained by centrifuge Blood samples in a cooling centrifuge (4 °C, 1000×g). For biochemical analysis of blood plasma, VETTEST 8008 analyzer in vivak laboratories from Tamilnadu, India. The result are presented as mean ±SE. Multiple comparison were performed by ANOVA and followed by the Tukey honestly significant different (HSD) test in all analyses, the level of significance was set to (p<0.05).

#### Histological Examination of tissue

For histological examination of tissue, *channapunctatus* of 60.23±5.02g (mean ±SD), bodyweight and 32±2.01mm body length were used. After blood sampling samples of gills were taken for histological examination. The samples taken were immediately fixed in 10% formaldehyde drained and embedded in paraffin, section were made of the paraffin blocks and stained with haematoxylin –eosin.

**Table1: Effects of clove oil and 2-phenoxy ethanol on serum biochemical parameters, 15min after anaesthesia induction (Mean± SE)**

Group	GLU	TP	Alb	Glob	ALP	SGOT	SGPT
Control	85.72±2.01 <sup>a</sup>	3.17±0.02 <sup>a</sup>	3.21±0.05 <sup>a</sup>	1.62±0.02 <sup>c</sup>	40.17±3.13 <sup>c</sup>	79.01±3.29 <sup>a</sup>	26.31±1.61 <sup>b</sup>
Group 1	91.73±3.17 <sup>a</sup>	3.28±0.01 <sup>b</sup>	3.75±0.02 <sup>b</sup>	1.79±0.05 <sup>a</sup>	119.2±02.71 <sup>a</sup>	78.32±2.32 <sup>a</sup>	26.78±1.05 <sup>a</sup>
Group 2	121.08±4.12 <sup>c</sup>	2.66±0.02 <sup>a</sup>	4.32±0.33 <sup>a</sup>	2.98±0.15 <sup>a</sup>	120.10±3.12 <sup>a,b</sup>	54.34±1.38	14.81±2.05 <sup>a</sup>
Group 3	101.12±3.18 <sup>a</sup>	2.75±0.01 <sup>b</sup>	5.14±0.03 <sup>b</sup>	3.31±0.17	121.33±1.55 <sup>c</sup>	61.53±2.75 <sup>a,b</sup>	19.73±1.78 <sup>a</sup>
Group 4	105.63.19 <sup>a</sup>	2.53±0.01 <sup>a</sup>	4.21±0.04 <sup>b</sup>	2.76±0.17 <sup>a</sup>	23.57±3.31 <sup>b</sup>	60.12±1.32 <sup>a</sup>	18.31±1.29 <sup>qa</sup>

**Table2: Effects of clove oil and 2-phenoxy ethanol on serum biochemical parameters, 24 hours after anaesthesia induction (Mean± SE)**

Group	GLU	TP	Alb	Glob	ALP	SGOT	SGPT
Control	91.42±1.02	2.10±0.20	3.81±0.06	2.01±0.01	40.19±2.13	78.40±2.14	27.31±1.20
Group 1	92.41±2.13	3.14±0.21	3.65±0.01	1.91±0.06	115±2.14	79.11±1.89	27.18±1.04
Group 2	90.31±3.14	2.77±0.20	4.33±0.44	3.14±0.16	121.01±2.14	55.14±1.22	14.11±2.01
Group 3	102.21±2.14	2.86±0.02	6.12±0.04	3.44±0.50	120.03±1.81	60.51±1.77	19.71±.60
Group 4	103.06±3.14	2.65±0.01	4.01±0.01	1.89±2.10	24.31±3.11	59.11±1.30	19.03±1.09

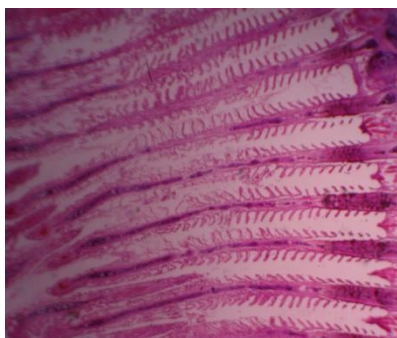


Figure 1A: Control clove oil

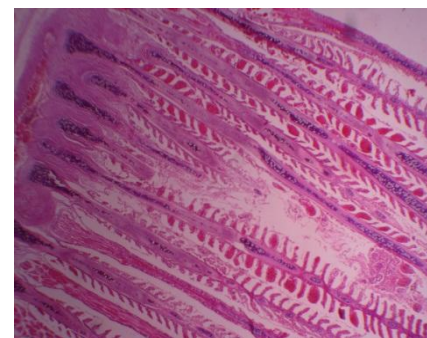


Figure 1B: 15mmts after anesthesia with clove oil

#### DISCUSSION

Anesthetics are necessary for many hatchery procedures in aquaculture. Because species may differ widely in their response to the same anaesthetics screening of dosage of different anaesthetics is often necessary. (Hekla's et al., 1986)

#### RESULT

Ten min exposure to 2-phenoxy ethanol at a concentration of 0.20 and 0.30ml/l cause increase in the concentration of glucose and ALP (alanine phosphatase 15 min after anaesthesia induction (P<0.05). Their values returned back to normal with in 24 hrs. also 10 min exposure to clove oil at concentration of 30 and 40mg an increase in the concentration of glucose. 15 min after anaesthesia induction (p<0.05). Also concentration of 40 mg/l clove oil caused an increase in alkaline phosphatase. (ALP). 15 min after anesthesia induction (p<0.05). Their values returned back to normal within 24 hours, there was no significant changes in other parameter's (TP, ALB, GLOB, SGOT, SGPT).

#### Histological examination of tissues

All specimens of *Channapunctatus*. Showed capillary ectasia (Fig:1A) of gill filaments 15 mints after clove oil. and, twenty four, hours after anaesthesia, no histopathological changes were demonstrated on other tissue of liver. Section of 2 phenoxy ethanol treated with gills showed swelling of primary and secondary lamellae (FIG.2A). after twenty four hours anaesthesia no histopathological changes.

determined in the present study suggest that internal organs and tissues of *C.punctatus* by used presented anaesthetics. In our experiment with *C.punctatus*, a significantly increase ( $p < 0.05$ ) in blood plasma glucose 15 min after the 2-phenoxy ethanol and clove oil anaesthesia was observed. Increase blood plasma glucose level after anaesthesia indicates that the procedure caused some stress in *C.punctatus*. These findings are accord with results of Holloway *et al.*, (2004) and Velisek *et al.*, (2005). The ALP decrease in blood plasma indicates that the anaesthetics does not damage paranchymatus tissues in *C.punctatus*. Anaesthesia with 2-phenoxy ethanol at the concentration of 4000ppm and 2 min exposure time had no effect on tryptophan, 5-hydroxy tryptamine, dopamine or norepinephrine, levels in the brain of rainbow trout (Sloley *et al.*, 1986). Clove oil has been used as an anaesthetic in density for a long time the anaesthetic properties and dosages of clove oil have been tested on different fishes (Griffiths, S.P., 2000 and Tayllor, P.W. and S.D. Roberts *et al.*, 1999). In addition, some physiological reaction of fish exposed to clove oil have also been reported (Keene, J.L. and S.D. Roberts *et al.*, 1998). clove oil may be also

be good for use in aquaculture and during the live transportation of fishes the disadvantage of clove oil is its relatively low therapeutic index, i.e. ratio between the therapeutic and the toxic concentrations. The generally reported optimum ratio is 1:4 or higher (Svobodova and VYKusoya, 1991). A major drawback of 2-phenoxy ethanol is that it requires rather high anaesthetic doses in fishes. The range of effective anaesthetic doses of 2-phenoxy ethanol in most fish species are from about 0.20 to 0.60 ml/l (Guo, F.C., L.H. Teo and Chen., 1995). Ortuno *et al* reported an increase in glucose and cortisol values in *Sparus aurata* anesthetized with 2-phenoxy ethanol. When 2-phenoxy ethanol issued, labor safety regulation should be strictly observed because the anaesthetic is toxic and harmful to human. Furthermore, it should be noted that, as 2-phenoxy ethanol is noted approved for use in food fish, we do not advocate its use in any fish unless MRL values (EEC regulation 2377/190) are set and proper licensing is acquired. In conclusion our results showed that 2-phenoxy ethanol 0.20ml/l and clove oil at 30mg/l concentration may be used as an efficient and safe anaesthetic for *C.punctatus*.

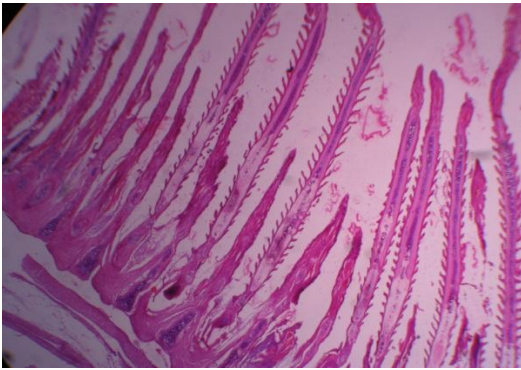


Fig 2A: 15 mins after anaesthesia with 2 phenoxy ethanol

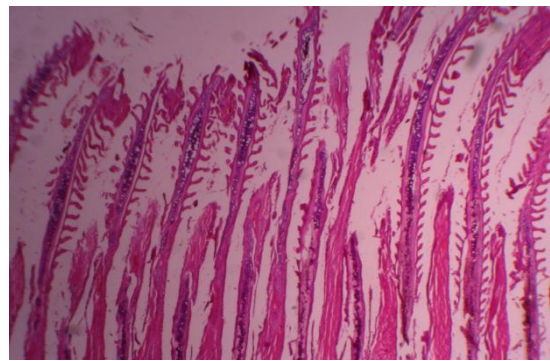


Fig 2B: Control 2 phenoxy ethanol

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