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ORIGINAL RESEARCH

Anesthetic efficacy of clove oil and its impact on hematological and biochemical changes in *Channa striatus* (Bloch, 1793)

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ABSTRACT:

Channa striatus is one among the dominant group of air breathing freshwater fishes in Southeast Asian countries. In the present study, fish anesthetic clove oil was used to study the hematological and biochemical changes at different concentration (400 ppm, 450 ppm and 500 ppm) and time interval (0 h, 1h and 24 h) in *C. striatus*. The induction and recovery time was noted for each treatment groups. Erythrocyte count (T/L), Hemoglobin and Hematocrit values showed elevated levels when compared to control. RBC, Hb and Ht values significantly increased 1 h after anesthesia and returned to normal after 24 h. Anesthetic treated fishes exhibited marked decrease in WBCs when compared to control group. The rest of the indices (MCV, MCH, MCHC, Lymphocytes, Monocytes, Neutrophils) were at comparable levels in all groups. The anesthetic treated fishes were found to show a significant increase in the concentration of glucose. The rest of the indices (TP, ALB, GLOB, ALT, AST) were at comparable levels in all groups. Results of the study suggested that the use of clove oil at the concentrations of 400, 450 and 500 ppm does not cause irreversible damage on the blood parameters as well as biochemical profile in *C. striatus*.

Keywords:
C. striatus, anesthesia, induction and recovery time

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INTRODUCTION:

Murrels, commonly called snakeheads are important air breathing freshwater fishes and are highly regarded as food fish in the South and Southeast Asian countries. They belong to the family Channidae (Ophiocephalidae) (Wee *et al*., 1982). Murrels can be kept alive for several hours outside water under slight moist condition which facilitates transportation to distant markets in good condition. Even then often fishermen meet heavy loss due to death of murrels during transport. Hence, sedation or use of anesthetics can be beneficial to calm excitable fish during bulk transportation of fish stocks, especially over long distances and high density.

In aquaculture practices and research activities, fish handling are a common source of stress. Hence, a variety of anesthetics are used mainly in order to reduce stress level and to prevent fish injury/ death during their handling. The most commonly used fish anesthetics are tricaine methanesulfonate (MS-222), benzocaine (ethyl paraaminobenzoate) (Kiessling *et al*., 2009), 2-phenoxyethanol, metomidate (Weber, 2009), and carbon dioxide (Pirhonen and Schreck, 2003).

Clove oil is a dark - brown liquid, a distillate of flowers, stalks and leaves of the clove tree *Eugenia aromatica* (Soto and Burhanuddin, 1995) having a mild anesthetic effect on human (Nagababu and Lakshmaiah, 1992; Taylor and Roberts, 1999) and fish (Ross and Ross, 2008). Keene *et al.* (1998) showed that the clove oil is much less expensive than other chemicals including MS222 and recommended the same for fish transport.

Anesthesia may affect blood parameters and hemolyse tissues (McKnight, 1966). Since, hemodynamic is closely related to response of animal to external environment (Fernandes and Mason, 2003). The goal of this study was to assess efficacy of clove oil as an anesthetic through the measurement of multiple blood parameters of striped murrel – *Channa striatus*. The effect of different concentrations of clove oil on induction and recovery periods and mortality are also studied.

MATERIALS AND METHODS:

The striped murrel, *C. striatus* acclimatized in cement tanks (15mX3mX2m) for a period of two weeks at CARE Aquafarm were used for this study. During this period, the fishes were fed twice a day with chicken intestine. Forty fishes (29.7± 1.69 cm and 232 ±14 g) were selected and were divided into four groups each with 10 fishes based on the concentration of clove oil (group I: control [0ppm], group II: 400ppm, group III: 450 ppm and group IV: 500 ppm). Test fishes were starved for 24 hours prior to the experiment and mortality rate (if any) was recorded regularly throughout the course of the study. Stock solution of clove oil was prepared by dissolving 1 ml of clove oil in 9 ml of tap water. The test fishes were exposed to the respective concentrations and they were anesthetized.

The induction and recovery period of fishes as a function of concentration were recorded. Blood was sampled from caudal vein of the anesthetized *C. striatus* with heparin coated syringe. To stabilize the blood samples, aqueous solution of heparin sodium salt (5000 U/ml) was added to blood (Svobodova *et al.,* 1991). The blood sample was divided into two aliquots; one part was transferred to a 2 ml heparinized tube and stored in refrigerator prior to hematological analyses. The other part of aliquots was transferred to 1.5 ml microcentrifuge tubes and centrifuged for 15 min. at 4°C. The plasma was removed and transferred to another 1.5 ml microtube and stored frozen at −70°C for biochemical analyses (Fast *et al*., 2008).

Hemogram was established by estimation of total erythrocyte count (RBC), total white blood cell count (WBC), hematocrit (Ht), hemoglobin concentration (Hb), erythrocyte indices (MCV, MCH, MCHC) and white blood cell differential count (Campbell, 2004). Biochemical indices of blood plasma included glucose

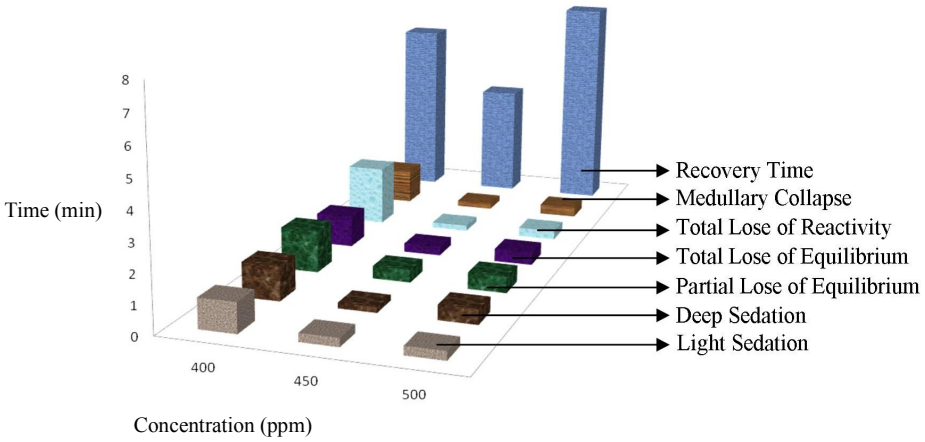

Figure 1: Effect of Clove oil on Induction and Recovery time of *C. striatus*

(GLU), Total Protein (TP), Albumin (ALB), Globulin (GLOB), Aspartate amino Transferase (AST), and Alanine amino Transferase (ALT). Statistical differences between groups if any at each time point (0 h, 1 h and 24 h) were tested using SPSS software and data were presented as mean± SD.

RESULT AND DISCUSSION:

Water quality parameters were measured and recorded as pH 7.0, chloride 200 ppm, total hardness 525 ppm, fluoride 0.5 mg/l, iron 0.5 mg/l, residual chlorine 0 mg/l and nitrate 0.45 mg/l.

All the fish exposed to different concentrations of clove oil recovered well and returned to normal behaviour with respect to feeding, surfacing activity, swimming and respond to external stimuli after the anesthetic treatment. Furthermore, no mortality was noticed within 48 h following recovery from anesthesia.

The technique "anesthesia by immersion" was applied to provide the active ingredient into the fish gills through water flow to travel through bloodstream to the central nervous system. Thus, the fish goes through several stages of anesthesia viz ; light sedation, deep sedation, partial loss of equilibrium, total loss of equilibrium, loss of reflex reactivity and ultimately medullary collapse as described by Bowser (2001).

Induction of anesthesia was assumed to be complete when the fish lost its response to external stimuli. As per the results obtained, the optimum concentration to anesthetize *C. striatus* was found to be 450 ppm. The fishes underwent anesthesia through six stages as described by Bowser (2001). The first stage of anesthesia was light sedation which involved: Slight loss of reactivity to external stimuli; opercular rate slightly decreased with normal equilibrium. This was followed by the next stage i.e., deep sedation involving total loss of reactivity to all but strong external stimuli; slight decrease in opercular rate with normal equilibrium. The signs of next stage included: Partial loss of muscle tone and swimming was erratic; increased opercular rate; reactivity only to strong tactile and vibration stimuli. Total loss of muscle tone and equilibrium; slow but regular opercular rate; loss of spinal reflexes especially loss of equilibrium observed in the fourth stage. The changes that were noticed in the fifth stage were total loss of reactivity; opercular movements were slow and irregular, heart rate was very slow and loss of all reflexes was noticed. The final stage during anesthesia was medullary collapse (stage of asphyxia) and the anesthetized fishes showed opercular movements to cease; cardiac arrest followed quickly. The time duration for each stage was shown in the figure 1.

Increase in clove oil concentration resulted in decrease in induction time whereas the recovery time

increased significantly. However, the recovery and induction time was also concentration dependent. The mean induction time for *C. striatus* 400, 450 and 500 ppm clove oil was found to be 7.10 ± 1.10 min., 2.36 ± 0.42 min. and 2.02±0.52 min. respectively. The mean recovery time for each concentration was 5.21±1.1 min., 3.20±0.40 min. and 6.38±1.15 min. respectively. The induction time was longer at the lower dose whereas it is quick and short at higher dose. In the same manner, the recovery time was quick in lower dose whereas it elapsed for a longer duration at higher dose. For morphological evaluations, biopsy and stripping, long handling periods are required and hence, anesthetizing with clove oil would be an added advantage which produces longer recovery time (Seol *et al*., 2007; Park *et al*., 2009).

It was observed that if the exposure was prolonged until the fish become anesthetized; the recovery was concentration-independent and lasted for about four minutes. In the present study, the tested concentrations met the efficacy criteria specified by Marking and Meyer (1985) and hence, dose of 450 ppm of clove oil can be suggested for transport or grading of *C. striatus.* This finding is also in accordance with the results reported by Woody *et al.* (2002) where higher dose produced a rapid and uniform response in all size classes, suggesting that a dose of 400 ppm might be well over the effective concentration for sockeye salmon (*Oncorhynchus nerka*).

Similar observations were made by Inoue *et al*. (2003) on juveniles of matrinxa, *Brycon cephalus*, where, the recovery in the experiment with a prolonged exposure was longer at all concentrations, but with 30 and 40 mg /L, it was still below 10 min. Our results showed that increasing the anesthetic dose significantly decreased induction time but prolonged recovery time. This was in agreement with reports of *Salmo salar smolts* (Iversen *et al*., 2003), *Cyprinus carpio* (Velisek *et al.,* 2005) and *Silurus glanis* (Velisek *et al.,* 2006).

As reported by Matin *et al*. (2009), when the fish were put into induction tray containing clove oil, they became excited and hypermotile followed by bubbling; the gill and fin movements progressively decreased, the fish lost equilibrium and started swimming laterally. Finally, the fish became immobile with full loss of equilibrium and consciousness. After transfer to recovery tray, reappearance of gill movement was noticed first. This was followed by fin and then tail movement. The fish started moving laterally. Gradually full equilibrium was regained and normal behaviour was restored at 5.21±1.1 min., 3.20±0.40 min. and 6.38±1.15 min. as a function of 400 ppm, 450 ppm and 500 ppm clove oil respectively. Similar, behavioural changes during induction and recovery as a function of anesthesia have been reported by McFarland (1960).

Hematological and biochemical profiles of blood are necessary to provide vital information about internal environment of organism. Erythrocyte count (T/L) showed increased values in anesthetic treated groups (1.18-1.21) when compared to control (1.14) (Table 1). Similarly, Hemoglobin and Hematocrit also showed elevated levels in anesthetic induced fishes (Hb: 59.93 – 64.826 g/dl and Ht: 0.179 – 0.194 l/l) as compared to control (Hb: 59.58 – 63.24 g/dl and Ht: 0.178 – 0.189 l/l respectively). RBC, Hb and Ht values significantly increased 1 h after anesthesia and returned to normal 24 h post anesthesia.

Anesthetic treated fishes exhibited marked decrease in WBCs (0.876 – 1.109 g/L) when compared to control group (0.981 – 1.138 g/L) (Table 1). The rest of the indices (MCV, MCH, MCHC, Lymphocytes, Monocytes, Neutrophils) were at comparable levels in all groups. Results of the study suggested that the use of clove oil at the concentrations of 400, 450 and 500 ppm does not cause irreversible damage on the blood parameters in *C. striatus*.

Tort *et al*. (2002) reported clove oil altering hematocrit concentrations in rainbow trout. Velisek *et al*. (2005) observed the same in rainbow trout and carp, both

Table 1: Effect of Clove Oil on Hematological Indices of *C. striatus*

S. No.	Hematological Indices	Control			400 ppm			450 ppm			500 ppm		
		0 h	1 h	24 h	0 h	1 h	24 h	0 h	1 h	24 h	0 h	1 h	24 h
1	Erythrocyte (RBC) (T/L)	1.18±0.07	1.14±0.08	1.14±0.05	1.18±0.08	1.20±0.09	1.18±0.06	1.19±0.09	1.20±0.12	1.18±0.05	1.19±0.06	1.21±0.07	1.18±0.08
2	Hb (g/dl)	59.33±1.2	59.35±1.72	59.50±1.28	59.60±1.32	63.24±0.84	60.23±0.30	59.93±0.89	64.28±0.81	58.39±1.21	60.22±0.53	64.83±0.60	60.37±0.42
3	Ht (l/l)	0.17±0.003	0.14±0.005	0.16±0.004	0.18±0.004	0.19±0.003	0.18±0.30	0.18±0.003	0.19±0.002	0.18±0.004	0.18±0.002	0.19±0.002	0.18±0.001
4	MCV (fl)	151.91±8.98	156.20±12.36	156.53±8.90	150.59±10.23	157.76±21.01	152.67±6.98	150.80±17.26	160.43±21.57	148.31±6.37	151.85±8.68	159.93±37.22	153.26±12.58
5	MCH (pg)	50.58±2.98	52.08±4.09	52.21±2.99	50.42±3.47	52.79±7.08	51.09±2.34	50.49±5.72	53.43±7.20	49.48±2.10	50.52±2.92	53.44±12.36	51.12±4.12
6	MCHC (g/L)	332.96±0.53	333.44±0.52	333.55±0.69	334.82±0.71	334.60±0.63	334.63±0.52	334.80±0.34	333.05±0.44	333.63±0.3	332.27±0.45	334.15±0.57	333.55±0.53
7	Leukocytes (WBC) (g/L)	1.07±0.01	1.14±0.02	0.98±0.01	0.99±0.02	0.92±0.02	0.93±0.02	0.96±0.01	0.90±0.02	1.11±0.02	0.91±0.17	0.88±0.26	0.98±0.02
8	Lymphocytes %	83.4±3.05	83.6±2.41	84.1±2.71	81.6±1.14	81.2±1.14	83.1±1.58	82±1.58	80.4±1.14	81.8±0.84	81±1.58	80.6±1.14	82.4±1.14
9	Neutrophils %	14.4±1.58	14.0±2.08	13.5±1.14	16.6±0.71	16.0±1.48	14.9±1.52	13.4±1.14	15.6±0.89	13±1.58	13.8±1.30	14.8±0.84	11.8±1.10
10	Monocytes %	2.2±0.84	2.4±1.14	2.4±1.52	1.8±0.84	2.8±0.84	2.0±1.0	1.6±0.89	3.0±1.22	2.2±0.84	1.2±0.45	2.6±0.89	2.0±0.71

Table 2: Effect of Clove Oil on Blood Plasma Indices of *C. striatus*

S.No	Biochemical Indices	Control			400 ppm			450 ppm			500 ppm		
		0 h	1 h	24 h	0 h	1 h	24 h	0 h	1 h	24 h	0 h	1 h	24 h
1	Glucose (mmol/L)	6.13±0.22	6.49±0.18	6.41±0.14	7.28±0.10	8.42±0.21	6.41±0.07	7.20±0.11	7.51±0.25	6.25±0.18	7.32±0.10	7.62±0.07	6.31±0.21
2	Total Protein (g/L)	69.08±0.18	68.29±0.42	69.31±0.49	70.38±0.71	71.30±0.42	69.0±0.54	70.25±0.51	71.22±0.31	69.15±0.58	69.4±0.53	70.22±0.57	69.80±0.99
3	Albumin (g/L)	4.09±0.19	4.51±0.28	4.24±0.40	6.26±0.32	5.32±0.50	5.11±0.25	7.96±0.30	7.08±0.42	5.38±0.23	13.11±0.61	15.37±0.24	11.33±0.37
4	Globulin (g/L)	64.99±0.27	63.78±0.49	65.07±0.86	64.12±0.72	65.85±0.36	63.89±0.35	62.29±0.36	64.54±0.93	63.77±0.67	56.29±1.09	54.84±0.72	58.46±0.87
5	AST (IU/L)	8.33±0.28	6.6±0.45	7.34±0.44	7.60±0.35	5.04±0.20	8.31±0.33	6.27±0.27	8.18±0.30	14.14±0.32	12.33±0.33	16.26±0.45	18.18±0.26
6	ALP (IU/L)	12.44±0.38	14.52±0.36	13.42±0.39	8.59±0.36	12.63±0.29	19.85±0.34	13.79±0.33	10.49±0.30	13.39±0.31	16.52±0.33	21.54±0.15	23.07±0.26

of them also exhibited significantly increased plasma glucose concentrations after longer exposure periods (10 min.).

The increase in RBC level after anesthetic treatment showed that fish's body is kept in touch with insufficient gas when anesthetized. When oxygen becomes a limiting factor, RBCs increased for carrying more oxygen to the cells. Hemoglobin (Hb) is an effective index of taking more oxygen in the blood and hence Hb increased for obtaining more oxygen; the average volume value (MCV) with a red blood cell increases after anesthetization and showed that dissolved oxygen amount decrease in fish's blood. So MCV is strengthened by holding more oxygen, and keeping the basic physiological function to fish's body; the average concentration of hemoglobin of red blood cells (MCHC) increase after anesthetization and show that dissolved oxygen amount is decreasing in fish's blood, so MCHC increase for combining more oxygen (Wu *et al*., 2000).

The respiratory actions lower resulting in reduced O_2 for circulation for breathing and survival creating a hypoxic environment which results physiological changes altering the blood factors like glucose and Hematocrit (Ht). Hyperglycemia (increased blood glucose), elevated HB and Ht were similar to other fishes anesthetized by Pirhonen and Schreck (2003); Park (2009); Gomes *et al*. (2001). Reports by Velisek *et al*. (2005) on *Cyprinus carpio* and *Oncorhynchus mykiss* suggests that clove oil anesthesia at 30 mg/l concentration and 10 min exposure did not produce any marked changes in the blood parameters after 24 h. However Sudagar *et al*. (2009), reported that a 7-min exposure to clove powder resulted in significant reversible increase in Ht, Hb and RBC immediately after anesthesia in Roach *Rutilus rutilus.*

Increase in the numbers of leukocyte (WBC) increase after anesthetization showed the unexpected changes of the living water environments or invasion of outside material (Wu *et al*., 2000). The fish immune system was evaluated by the changes in White Blood Cell (WBC). It showed a decline trend associated with arresting in anesthetic. Increase in plasma cortisol concentration which is a glucocorticoid hormone, is also acting as an immunosuppressive (Fast *et al*., 2008), so it could suppress humoral factors and lead in declining circulating WBC along with elevating cortisol.

The anesthetic treated fishes were found to show a significant increase in the concentration of glucose i.e., 7.196 – 7.318 mmol/l at 0 h and 7.514 – 8.422 mmol/l at 1 h following anesthetic treatment. The glucose values of anesthetic treated fishes returned back to control values after 24 h post treatment (6.134 – 6.486 mmol/l). The rest of the indices (TP, ALB, GLOB, ALT, AST) were at comparable levels in all groups (Table 2).

These results revealed the significance of exposure time and dosage on some physiological indicators of anaesthetized fish. Post-exposure mortality and lack of biochemical alteration in the present study could be due to the short induction time after which blood was collected. These results indicated the need to study possible physiological changes occurring in different fish species exposed to different doses of clove essence.

Plasma cortisol as well as glucose is a physiological indicator of stress in fishes and their interactive effects on metabolism during recovery from stress have recently become a subject of more intense study (Tytler and Hawkins, 1981; Woody, 2002; Weber *et al*., 2009). Clove oil was found to block the activity of cortisol, although not completely, in *Brycon cephalus* (Inoue *et al*., 2005). Although the mechanism is not well known, Iverson *et al*. (2003) suggested that it blocks transmission of impulses to the Hypothalamus-Pituitary Interregnal axis (HPI).

In our experiments with murrel, an increase in blood plasma glucose immediately after clove oil anesthesia was observed. Increased glucose level returned to normal 24 h after anesthesia. Increased blood

plasma glucose level after anesthesia indicates that the procedure caused some stress in the experimental fish. These findings are in accordance with results of Holloway *et al*. (2004) and Velisek *et al.* (2005) who also detected increase of glucose concentration in rainbow trout (*Oncorhynchus mykiss*) following clove oil anesthesia. On the other hand, Iverzen *et al.* (2003) found no change in the concentration of glucose in Atlantic salmon (*Salmo salar*) following clove oil anesthesia.

CONCLUSION

According to these results, clove oil at a concentration of 450 ppm could be an efficient and relatively safe anesthetic agent, but further studies are required to detect any possible toxicity effect on fish. In conclusion, clove essence was found to be safe and can be effectively and easily applied in used dosages to anaesthetize various size groups of murrels with minimal disruption in the physiological indicators studied and with zero mortality. Hence, considerations should be given to the use of clove essence as a replacement for synthetic forms of anesthetics.

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