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EFFECT OF CLOVE OIL AND QUANALDINE AS AN ANESTHETIC AGENTS ON SOME HEMATOLOGICAL AND BIOCHEMICAL PARAMETERS IN SILVER CARP (*HYPOPHTHALMICHTHYS MOLITRIX*) DURING SPAWNING SEASON

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Abstract

In this study, the anesthetics of interest were clove oil and quanaldine to comparing their anesthetic efficacy and select the most suitable anesthetic for the species silver carp (*Hypophthalmichthys molitrix*) broodstock. Thirty broodstock silver carp; fifteen female with an average weight of 2.500-2.700 kg and mean total length of 60-65 cm and fifteen male with an average weight of 2.300-2.400 kg and mean total length of 56-58 cm were divided into five groups. Different concentrations of clove oil G1,G2,G3 (5, 7.5 and 10 ml/100l) respectively, quanaldine G4 (2.5 ml/100 l) and control group G5 were used to determine the anesthesia time, recovery time and their impact on reproductive performance during controlled spawning. The effect of two anesthetics on haematological indices and biochemical parameters were studied. Also, the histopathological changes that occurred in some internal organs of different groups were recorded. Results of the current study indicated that using of clove oil at a rate (5 ml / 100 l) was the optimal concentration causing general anesthesia at (6.0 min) and an average recovery time (3min, 3sec) with successful spawning rate (100%) and latency period (9h). No significant differences between values of haematological and biochemical parameters of clove oil and control one, while quanaldine resulted significant increase in glucose and cortisol levels as compared with the control group. The histopathological changes that occurred in some internal organs of different groups were studied.

INTRODUCTION

Fishes during aquaculture practices and stocking activities are confront with several stressors which are often associated with disease outbreaks such as immune suppression, physical injuries or even death in cultured fish.

Silver carp *Hypophthalmichthys molitrix* were first imported into Egypt in the early 1980s for food security purpose; it is considered one of the most important fish used in polyculturing the earthen fish ponds in semi-intensive because of fast growth rate and lower cost of feeding in comparison with other fish species. Also, silver carp control of plankton in aquaculture ponds, reservoirs, and sewage treatment lagoons (Woo and Bruno, 1999).

Anaesthesia is a biological state with the partial or complete loss of sensation or loss of voluntary neuromotor control induced by chemical or nonchemical means (Summer felt and Smith, 1990). Anaesthetics in fish farms are used to minimize motility during handling and transport. This may reduce susceptibility to pathogens and infection (Matin *et al*., 2009 and Olufayo and Ola 2010). Also, they are used in fish during artificial spawning, weighing, tagging, grading, blood sampling, surgery and surgical procedures (Anderson *et al*., 1997). A number of considerations are important during choosing an anaesthetic such as efficacy, cost, availability and ease of use, as well as toxicity to fish, humans and the environment (Soto and Burhanuddin, 1995 and Akbulut *et al*., 2011). A perfect anesthetic should cause anesthesia quickly with minimum hyperactivity or stress. It should be easy to supply and should preserve the animal in the selected state. When the animal is eliminated of the anesthetic, recovery should be quick. The anesthetic should be efficient at low doses and the toxic dose should greatly exceed the effective dose so that there is a vast border of security (Coyle *et al*., 2004).

Quinaldine is commonly and widely used anesthetic in the aquaculture industry, but there are doubts on its safety because of reported associations with thyroid abnormalities in humans and mice (Munday and Wilson, 1997).

Clove oil is a natural product and relatively inexpensive. Its active ingredients are eugenol (4-allyl-2-methoxyphenol) and iso-eugenol (4-propenyl-2-methoxyphenol) (Ross and Ross, 2008). Eugenol is rapidly absorbed and metabolized after oral administration, and it is almost completely excreted in the urine within 24 h with no apparent ill effects in fish; non-

carcinogenic and non-mutagenic so, it is safe for laboratory use (Fischer *et al*., 1990).

Clove oil is considered more attractive as an anesthetic in aquaculture than the other agents because of its availability, handler, safety and low cost (Keene *et al*., 1998; Detar and Mattingly 2004; Neiffer and Stamper, 2009). Also, it has shown to have low intoxication, mortality risks (Soto and Burhanuddin, 1995; Griffiths, 2000 and Inoue *et al*., 2011).

Fish health and disease condition are known to be reflected in blood physiology, and in many cases may be characterized by a general hematological failure prior to a disease occurrence, such as changes in the Hb, Hct, RBC, etc. (Phani Kumar and Sree Ramulu, 2013). Cortisol and glucose circulating levels are also suitable parameters to assess the fishes' stress (Barton, 2002).

The aim of the current study is to compare effects of two anesthetics (clove oil and quanaldine) on haematological, biochemical parameters and reproductive performance of silver carp (*Hypophthalmichthys molitrix*) and determine the optimal concentrations of clove oil causing general anaesthetia in order to select an anesthetic with the lowest risk and less expensive to be used in aquaculture.

MATERIALS AND METHODS

Fish.

A total number of thirty apparently healthy broodstock silver carp (*Hypophthalmichthys molitrix*); fifteen female with an average weight of 2.500-2.700 kg and mean total length of 60-65 cm and fifteen male with an average weight of 2.300-2.400 kg and mean total length of 56-58 cm were obtained from El-Abbassa Fish Hatchery belonging to the General Authority for Fish Resources Development, El-Abbassa, Sharkia Governorate, in May 2016. The fish were transported to circular fiberglass tanks with capacity of 11.5 m^3 and supplied with irrigation water and well oxygenated. The fish were fed twice daily with pelleted food (30 % crude protein) in a rate of 3% of their body weight.

All of the physico-chemical conditions of water such as (temperature, pH, total ammonia, and dissolved oxygen) were controlled and kept at optimal levels during the experiment as shown in (Table 1). The physicochemical analysis of water samples were carried out in accordance to standard analytical methods (APHA, 2005). Fishes were stored in fiberglass tanks for 12 hrs. after harvest to prevent stress effects before the beginning of the experiment.

Table 1. Physico-chemical conditions of water during the experiment.

Parameter	Means
Temperature (°C)	24±0.2°C
PH	7.50±0.01
Total ammonia	0.5±0.1
Oxygen (mg/l)	4.5±0.5

Anesthetic solution Preparation.

Clove oil was marketed by the Kulich Company (Jan Kulich, Hradec Králové/Říčany, Czech Republic). Because of the incomplete solubility of the clove oil in water it was first dissolved in ethyl alcohol, one ml was dissolved in 4 ml ethyl alcohol (95%) (Stock solution), then the desired concentration prepared by adding the desired dose to 10 liter of irrigation water. The quanaldine was marketed from Al-Gomhoria Company, and prepared by adding one ml to 40 liter irrigation water (Gibson, 1967).

Experimental Design.

The fishes were divided into five groups each one contains three females and three males. The design of the experiment was shown in (Table 2). Fish were anesthetized via bathing in small tanks and then transferred to larger tanks to recover from anesthesia.

At the end of experiment female (*H. molitrix*) were transported to tanks of 3m water volume filled with accreted and filtered fresh water for spawning. Each tank contains spawners. Females were injected with carp pituitary extract (CPE) at dose of 3mg /kg two doses as ovulation stimulators. Prior to injection, fish were anesthetized according to (Bowser 2001). Eggs were collected by

stripping in buckets pooled milt was added for fertilization and incubated in fiberglass tanks supplied with continuous water flow at temperature of 27ºc. Egg weights and ovulation rate were estimated according to (Szabo et al. 2002). Latancy period which is period from injection till the onset of ovulation (hrs) was also recorded.

-**Ovulation rate** = No. of ovulaed female/ No. of injected female x 100

-**Gonadosomatic index (GSI)** = weight of striped egg mass/ Body weight of female x100

Table 2. Design of the experiment, means time of anaesthesia and recovery of different treatments.

Group No.	Type of anaesthesia	Dose	Time of anaesthesia (min)	Time of recovery (min)
G1	Clove oil	5 ml /100 l	6.0	3.3
G2	Clove oil	7.5 ml/100 l	4.5	4.5
G3	Clove oil	10 ml/100 l	1.1	9.1
G. 4	Quanaldine	2.50 ml / 100 l	4.5	4.5
G. 5	Control	-	-	-

Samples collection.

Blood samples were collected from the fish caudal vein by a sterile syringe which was previously rinsed with EDTA solution (as anticoagulant) and shaken gently to prevent hemolysis of blood which is used for hematological analysis. The indices used to evaluate the haematological profile include the erythrocytes count (RBCs), haemoglobin concentration (Hb), Packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin (MCHC) were determined according to the method of (Houston 1990; Dacie and Lewis 1991 and Stoskopf, 1993).

Serum was obtained by centrifugation of blood at 3000 rpm for 15 min and non-haemolyzed serum was stored in deep freezer for further biochemical analyses. Total protein content (TP) was measured according to the method of

(Grant *et al*., 1987). The serum albumin level (ALB) was estimated according to the method of (Doumas *et al*., 1981). Urea level, creatinin and uric acid were measured according to the method of (Patton and Crouch, 1977). Glucose concentration was measured according to (Trinder, 1969). Cortisol level was quantified according to (Patrono and Peskar, 1987). Activities of Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) were determined according to the method of (Thomas, 1998).

Histopathological examination.

Samples for histopathological examination were freshly taken from liver of studied fish. Histopathological techniques were carried out according to (Roberts 2001).

Statistical analysis.

All values from chemical analyses are presented as mean ± SD Data obtained from the experiment were subjected to one way analysis of variance (ANOVA) test followed by Duncan's Multiple Range Test (DMRT) to compare between the hematological and blood biochemistry values. In all cases, $P \leq 0.05$ was the accepted significance level. The software SPSS, version 10 (SPSS, Richmond, Virginia, USA) was used as described by (Dytham 1999).

RESULTS

As shown in Table (2), clove oil at concentration (5 ml/100 l) causing general anaesthetia at (6.0 min) and fast recovery (3.3 min). While, the higher dose of clove oil (10 ml/100 l) gives shorter time anaesthesia (1.1 min) and longer time of recovery (9.1 min), clove oil at concentration (7.5 ml/100 l) and quanaldine at concentration (2.50 ml / 100 l) gives time of anaesthesia (4.5 min) and time of recovery (4.5 min).

The highest percentage of ovulation (100%) was observed in G1 and G4, followed by (80%) in G2 and G5, while, G3 was (60%). The latency period ranged between 9-10hrs for the five ovulated groups.G2 and G3 showed the longest latency period (10h), while G1, G4 and G5 showed the lowest latency

period (9h). The Gonadosomatic index (GSI) showed significant difference ($P < 0.05$) among different groups with the lowest values (9,8±0.3) in G3 and the highest value (13,7±0,6) was observed in G1 (Table 3).

Table 3. The effect of clove oil and quanaldine anaesthesia on spawning parameters in of *Hypophthalmichthys molitrix*.

Group No.	Ovulation rate (%)	Latency time (h)	GSI
G1	100	9	13.7±0.6[a]
G2	80	10	10.4±0.2[b]
G3	60	10	9.8±0.3[b]
G4	100	9	11.2±0.3[ab]
G5	80	9	12.6±0.2[a]

Different letters indicate there is a significant difference at $p \leq 0.05$.

Table 4. Effect of clove oil and quanaldine anaesthetia on blood hematological indices of *Hypophthalmichthys molitrix*.

Experiment / Indices	Hb g/dl	RBC $10^6/mm^3$	PCV %	MCV µ3	MCH pg	MCHC g/dl
G. I	8.2± 0.07[a]	2.4± 0.01[a]	27.6± 0.5[a]	124.7±1.5[ab]	35.8± 0.3[b]	37.9± 1.2[b]
G. 2	6.5±0.2[c]	2.0±0.04[c]	23.0 ±0.6[c]	123.5±2.2[b]	32.5±1.9[c]	37.9± 0.3[b]
G. 3	6.9±0.1[b]	1.9±0.02[c]	24.8± 0.4[b]	126.6±2.6[ab]	36.3±2.6[a]	39.5±0.6[a]
G 4	7.8 ±0.2[b]	2.1±0.04[c]	25.8±0.2[b]	129.9±1.6[a]	36.5±0.5[a]	39.9±0.7[a]
G 5	7.7±0.1[b]	2.2±0.00[b]	25.2 ±0.5[b]	124.1±1.9[ab]	36.6±0.4[a]	38.9±0.1a[b]

Different letters indicate there is a significant difference at $p \leq 0.05$.
Hemoglobin concentration (Hb), Packed cell volume (PCV), Mean cell volume (MCV), Mean cell hemoglobin(MCH), Mean cell hemoglobin concentration (MCHC) and Red blood cells(RBC).

Table (4) reveals hematological parameters of *H.molitrix*. Hb and PCV increased significantly ($p \leq 0.05$) in G1(8.2±0.07[a] and 27.6±0.5[a]), respectively, while G2 decreased significantly ($p \leq 0.05$) (6.5±0.2[c] and 23.0 ± 0.6[c]), respectively. Also, Hb and PCV showed non-significant difference in G3, G4 and G5. RBC_S increased significantly ($p \leq 0.05$) in G1 (2.4± 0.01[a]) and decreased significantly ($p \leq 0.05$) in G4, G2 and G3 (2.1± 0.04[c], 2.0± 0.04 [c] and1.9±0.02[c]), respectively. The MCV results showed that the high significant difference appear in G4 (129.9± 1.6[a]) followed by G3 (126.6±2.6[ab]), while G2 showed significant decrease (123.5±2.2 [b]). About MCH G5 showed the highest value while the lowest one was recorded in G2. No significant difference were

recorded between G1and G2 (37.9± 1.2^{b} and 37.9±0.3^{b}), respectively in MCHC, as well as G3, G4 (39.5± 0.6^{a} and 39.9± 0.7^{a}), respectively.

Table 5. Effect of clove oil and quanaldine anaesthesia on biochemical parameters of *Hypophthalmichthys molitrix*.

Experiment / Indices	Glucose mg/dl	TP g/dl	ALB g/dl	AST u/l	ALT u/l	Creatinine mg/dl	Urea mg/dl	Uric acid mg/dl	Cortisol µg/dl
G 1	101.5± 1.8^{d}	3.5± 0.06^{b}	1.8± 0.02^{a}	9.4± 1.1^{b}	8.6± 0.3^{b}	0.43± 0.01^{b}	7.5± 0.2^{a}	1.4± 0.03^{b}	15.9 ± 0.6^{c}
G 2	102.8± 2.5^{d}	3.9± 0.03^{a}	1.7± 0.03^{a}	12.7± 1.2^{a}	12.7± 2.3^{a}	0.48± 0.01^{ab}	7.6± 0.6^{a}	1.6± 0.1^{b}	18.5± 0.8^{b}
G 3	133.3± 5.4^{b}	3.4± 0.05^{b}	1.66± 0.04^{b}	12. 6± 1.4^{a}	10.5± 1.0^{ab}	0.48± 0.03^{ab}	6.1± 0.17^{b}	1.5 ± 0.1^{b}	18.6± 0.4^{b}
G 4	172.6± 3.0^{a}	3.5± 0.08^{b}	1.50± 0.02^{b}	13.5± 0.57^{a}	8.4 ± 0.8^{b}	0.49± 0.02^{ab}	7.0± 0.1^{ab}	1.6± 0.1^{b}	25.8± 0.7^{a}
G 5	119.5± 1.1^{c}	3.3± 0.05^{b}	1.55± 0.02^{b}	13.9± 0.33^{a}	11.9± 0.57^{a}	0.53± 0.03^{a}	7.0± 0.17^{ab}	2.3± 0.01^{a}	16. 5± 0.6^{b}

Different letters indicate there is a significant difference at p≤ 0.05.
Total protein (TP), Albumin (ALB), Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT).

Regarding results of biochemical parameters (Table 5). Glucose concentration showed highly significant difference (p≤0.05) between G4 (172.6± 3.0^{a}) compared with G1 (101.5± 1.8^{d}), G2 (102.8± 2.5^{d}), G3 (133.3± 5.4^{b}) and G5 (119.5 ± 1.1^{c}). TP results showed higher significant difference (p≤0.05) in G2 (3.9±0.03^{a}) while other groups showed non-significant difference (p≥0.05). The Higher values of Alb were observed in G1 (1.8±0.02 ^{a}) while G2, G3, G4 and G5 were (1.7±0.03^{a}, 1.66± 0.04^{b}, 1.50± 0.02^{b} and 1.55± 0.02^{b}), respectively. AST showed the highest value in G5 (13.9 ± 0.33 a) while G1 showed the lowest value (9.4± 1.1^{b}). The results of ALT showed that G2 (12.7± 2.3^{a}) had the higher values while G4 showed the lower value (8.4± 0.8^{b}). Creatinine results revealed that G5 (0.53± 0.03^{a}) was highly significant than others which have no significant difference (p≥0.05). Urea showed higher values in G2, G1, G4 and G5 (7.6± 0.6^{a}, 7.5± 0.2^{a}, 7.0± 0.1^{ab} and 7.0± 0.17^{ab}), respectively and finally G3 showed the lowest value (6.1± 0.17 ^{b}). Uric acid concentration observed higher values with a high difference in G5 while,

no significant difference was observed between G1, G2, G3 and G4. Cortisol concentrations showed higher significant difference ($p \leq 0.05$) in G4 (25.8 ± 0.7^{a}) comparative with G2 and G3 (18.5 ± 0.8^{b} and 18.6 ± 0.4^{b}); G1and G5 (15.9 ± 0.6^{c} and 16.5 ± 0.6^{b}).

The histopathological alterations in the liver observed in the present study revealed that liver tissues of silver carp not altered by clove oil anaesthesia with different concentrations and no histopathological changes were demonstrated while in quanaldine group showed vacuolar degeneration in the hepatocytes, focal areas of necrosis, thrombosis formation, congestion in central veins, dilation and congestion in blood sinusoids (Plate1).

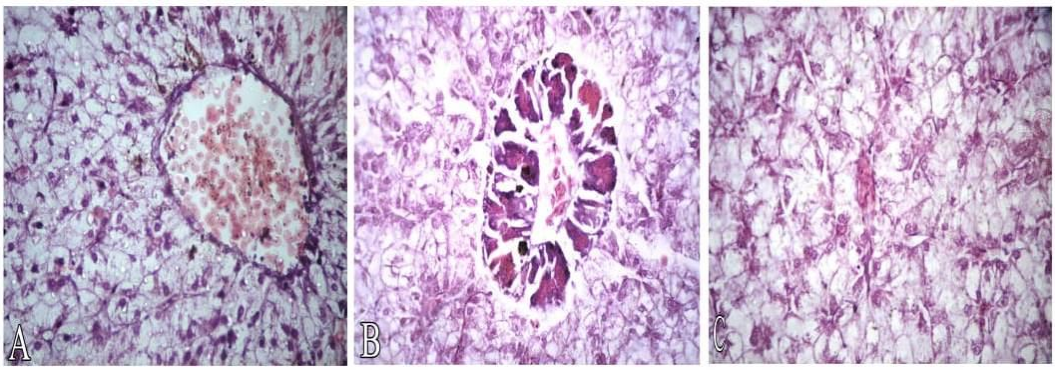

Plate1. A- Photomicrograph of normal liver of *Hypophthalmichthys molitrix* control groups and anesthetic by clove oil groups showed nuclei, kupffer cells, sinusoids and central vein. (H&E stain x400). B- Photomicrograph of liver of *Hypophthalmichthys molitrix* anesthetic by quanaldine showed congested central vein, necrosis and degeneration in the hepatocytes. (H&E stain x400). C- Photomicrograph of liver of *Hypophthalmichthys molitrix* anesthetic by quanaldine showed dilation and congestion in blood sinusoids. (H&E stain x400).

DISCUSSION

Anaesthetic act on the central nervous system and induces calming effect, loss of equilibrium, mobility, consciousness and finally loss of reflex action in an organism exposed to higher concentrations.

The concentration of the anesthetic in the central nervous system determines the stage of anaesthesia (Shaluei *et al*., 2012). Induction time is the number of minutes required to reach a given stage of anesthesia. Procedures that require long exposure may result in a prolonged recovery time sever oxygen debt, anoxia, or subsequent death of the subject (Schoettger and Julin 1967). Present results indicate that increasing the anesthetic dose significantly decreased induction time and increase the recovery time. This was agree with that reported about *Cyprinus carpio* (Velisek *et al*., 2005) who recorded that the higher the concentration of anaesthetic, the shorter the induction time and the longer the recovery time. The longer recovery time observed in fish anaesthetized with clove oil in higher concentrations could be as a result of stress incurred from the anaesthetic influence, metabolic rate، oxygen consumption, branchial respiration and the blood pressure of the test fish. So, in the present study clove oil at a concentration of 5 ml /100 l is an efficient anesthetic for silver carp. These results less than values reported by (Waterstrat, 1999) who stated that 100 mg/l clove oil is considered a safe concentration for anaesthesia of channel catfish, (Velisek *et al*., 2005) in common carp who use of clove oil at a concentration of 30 mg/l. This difference could be due to the different biological and environmental factors that might affect the efficacy of an anesthetic agent or may be due to differences in the proportion of eugenol content in clove oil. (Iversen *et al*., 2003 and Abdolazizi *et al*., 2011).

The employment of hematological techniques, including evaluation of erythrocytes count, hemoglobin concentration, hematocrit and leucocytes count, has provided valuable knowledge for fishery biologists in the assessment of fish health (Blaxhall, 1972) and in monitoring stress responses (Soivio and Oikari, 1976). These results reflect the health status of fish cultured with all treatments. Hb, RBCs and PCV values in this study vary within different concentrations of clove oil and quanaldine.

So, it is assumed that the decrease or increase may be attributed to a defense reaction against which occurs by stimulation of erythropoiesis. In the

present study, Hb, RBCs and PCV values in G1 were significantly higher than other groups. (Sudagar *et al*., 2009) reported that clove powder caused a significant increase in hematocrit, hemoglobin and total erythrocyte count in *Rutilus rutilus*. Results of (MCV, MCH and MCHC) in the present study showed no significant differences in clove oil groups in compare with quanaldine anaesthesia and control groups. Similar results were recorded by (Mazandarani *et al*., 2015) who stated that RBC and MCV of the anesthetized fish with clove oil were significantly higher than those of non-anesthetized fish, whereas there was no significant difference in MCHC. (Velisek *et al*., 2005) recorded that clove oil anesthesia had no effect on the hematological profile of *Cyprinus carpio* and *Oncorhynchus mykiss*. Blood indices are particularly important for the diagnosis of anemia in most animals (Coles, 1986). The decrease of hematological parameters in quanaldine and control groups in this study may be due to high stress and struggling that led to hypoxia and exhaustion resulting in adverse effects on the fish health.

The analysis of blood indices has proven to be a valuable approach for analyzing the health status of fish as these indices provide reliable information on metabolic disorders, deficiencies and chronic stress status before they are present in a clinical setting (Bahmani *et al*., 2001). Blood glucose levels are considered as the main source of energy for fish cells and rapid increase of blood glucose follows acute stress because after stress, plasma glucose increases to provide a demand in energy to cope with stressful conditions and the maintenance of whole body homeostasis. (Barton, 2002). In the present study glucose concentration showed highly significant difference in quanaldine anaesthesia (G4) in compare with control and other group's clove oil anaesthesia. These findings are in keeping agreement with results of those reported by (Thomas and Robertson 1995) who stated that quanaldine exhibited dose related increase in glucose level. Also, (Mazandarani *et al*., 2015) recorded that the non-anesthetized fish had significantly higher glucose levels compared to the anesthetized group with clove oil. While, (Iverzen *et al*., 2003) found no changes in glucose concentrations of Atlantic salmon following clove oil

anaesthesia, while (Velisek *et al*., 2005 and Zapryanova *et al*., 2017) stated that significant increase in blood plasma glucose clove oil anaesthesia and indicated the presence of stress during the procedure.

Blood albumin showed significantly increase in G1 and G2 while the study of (Mavadati and Habibian, 2011) on the anesthetic effects of clove oil upon rainbow trout revealed no significant changes in the blood albumin after 15 min and 24 h of anesthesia.

The liver enzymes AST and ALT are localized within the tissue cells of the liver and other organs. Measurement of their activities in blood is one

of the most valuable diagnostic tools because they reflect the physiological conditions of fish (Shahsavani *et al*., 2010). In this paper AST showed decrease in all groups while ALT increased significantly in all groups. However, (Velisek *et al*., 2011) showed a significant increase in AST enzyme levels in the blood and no changes in ALT was found in rainbow trout after clove oil exposure.

Kidney function has been reported using creatinine, urea and uric acid levels (Abdel-Tawwab and Wafeek, 2008). Uric acid showed significantly decreased in all groups comparing the control group while

Creatinine showed significant decrease in G4 and G1 and Urea significantly increase in G1 and G2 while decrease significantly in G3.

Cortisol is known as the most important indicator of stress in animals. In this study, the amount of cortisol in the blood of fish showed a significant difference with higher values in quanaldine anaesthesia group (G4) in compare with control and other group's clove oil anaesthesia. (Wagner *et al*., 2003) recorded that decreased cortisol levels in the blood of rainbow trout (*Oncorhynchus mykiss*) treated with clove oil indicating diminished stressful conditions. (Small, 2003) study the effects of clove extract on the channel catfish and found it very effective in suppressing the secretion of cortisol hormone in the fish, while (Holloway *et al*., 2004) and (Velisek *et al*., 2005)

recorded a significant increase in blood plasma glucose with clove oil anaesthesia in rainbow trout. Also, (Mazandarani *et al*., 2015) recorded that the non-anesthetized fish had significantly higher cortisol levels compared to the anesthetized group with clove oil. The discrepancies between the results of different studies may be due to differences in species, gender, age, size, and conditions of the culture media, the type dose of the anesthetics and the anesthesia technique applied. This probably means that anesthesia with clove oil is effective in minimizing fish stress.

In this study, anesthesia had no negative effects on reproduction performance. Moreover, clove oil showed a positive function on stress mitigation and positive effect on the health of silver carp (*Hypophthalmichthys molitrix*). Similar results were obtained by (Mazandarani *et al*., 2015) who reported that clove oil had no effects on reproduction performance of the Persian sturgeon breeders.

Histopathological changes have been widely used as biomarkers for evaluating degree of pollution or toxic effect with the habitat of an organism. Limited studies are available on histopathology as biomarker in

anaesthetic treatments (Padiyoor *et al*., 2017).

The histopathological alterations in the liver observed in the present study revealed that liver tissues of silver carp not altered by clove oil anaesthesia with different concentrations and no histopathological changes were demonstrated, this results were agree with (Velisek *et al*., 2005) who confirmed that there was no histopathological changes showed in tissues of common carp (*Cyprinus carpio* L.) anaesthetized clove oil and (Padiyoor et *al*., 2017) who suggest that the use of clove oil anaesthetic at acceptable dose does not causes irreversible damage in the test fishes. On the other hand quanaldine group showed vacuolar degeneration in the hepatocytes, focal areas of necrosis, thrombosis formation, congestion in central veins, dilation and congestion in blood sinusoids. These changes may be attributed to direct anesthetic effects on hepatocytes, since the liver is the site of detoxification of all types of toxins and chemicals (Soufy *et*

al., 2007). Congestion indicates an increase in the blood flow in the hepatic. Thus, congestion can be considered an indicator of stress in fish due to the presence of xenobiotic chemicals (Rezende *et al*., 2014 and Marchand *et al*., 2009). Necrosis is strongly associated with oxidative stress (Li *et al*., 2000). Oxygen deficiency being the most common cause of the cellular degeneration in the liver this may be due to effect of anthestic on the gills (Eder and Gedigk, 1986). So, according to the present study clove oil is an efficient and relatively safe anaesthetic this results in parallel with (Taylor and Robers 1999 and Simões *et al*., 2011). Whereas (Cuesta *et al*., 2004) recorded that quinaldine has been used to anesthetize fish for many years, but its mode of action is unknown.

Based on the current study, clove oil was physiologically good anaesthesia for silver carp (*Hypophthalmichthys molitrix*). Furthermore, it is inexpensive and easily obtained all this features make it attractive to be a widely-used anesthetic for fish. This finding is in keeping with (Iverzen et al., 2003; Park *et al*., 2009 and Zapryanova *et al*., 2017).

CONCLUSION

In conclusion, the present results indicated that clove oil at a concentration of 5 ml /100 l was the most effective anesthetic for silver carp (*Hypophthalmichthys molitrix*) broodstock fish which had no effects on reproduction performance in addition to its low price and less persistence in aquatic environment which encourages using it as medicinal plants with anasthetic active compound than synthetic anaesthetic such as quanaldine.

REFERENCES

Abdel-Tawwab, M. and M. Wafeek, 2008. Response of Nile tilapia, *oreochromis niloticus* (l.) to environmental cadmium toxicity during organic selenium supplementation. 8th International Symposium on Tilapia in Aquaculture, 415-430.

Abdolazizi, S.; E. Ghaderi; N. Noshin Naghdi and B.B. Kamangar, 2011. Effects of Clove Oil as An Anesthetic on Some Hematological Parameters of *carassius auratus*. J. Aquac. Res. Development, 2 (1): 1-3.

Akbulut, B.; E. Cakmak; N. Aksungur and Y. Cavdar, 2011. Effect of exposure duration on time to recovery from anaesthesia of clove oil in juvenile for Russian sturgeon. Turkish J. Fish Aquat. Sci., 11: 463–467.

Anderson, W.G.; R.S. Mckinley and A.M. Colavecchi, 1997. The use of clove oil as an anesthetic for rainbow trout and its effects on swimming performance. North American Journal of Fisheries Management, 17 (2): 301-307.

APHA, 2005. Standard methods for the examination of water and waste water, 21th edition, Washington, American Public Health Association.

Bahmani, M; R. Kazemi and P. Donskaya, 2001. A comparative study of some hematological features in young reared sturgeons (*Aci-penser persicus* and *Huso huso*). Fish Physiol. Biochem., 24: 135–140.

Barton, B.A., 2002. Stress in fishes: A diversity of responses with particular reference to changes in circulating corticosteroids. Integr. Comp. Biol. 42: 517-525.

Blaxhall, P.C., 1972. The haematological assessment of the health of freshwater fish. J. Fish Biol., 4: 593-605.

Bowser, P.R., 2001. Anesthetic Options for Fish, In: Recent Advances in Veterinary Anesthesia and Analgesia: Companion Animals, Gleed, R.D. and Ludders, J.W. (Eds.), Publisher: International Veterinary Information Service (www.ivis.org), Ithaca, New York, USA.

Coles, E.H., 1986. Veterinary Clinical Pathology 4th Edition. W.B. Saunders Co. Philadelphia, 17-19.

Coyle, S.D.; R.M. Durborow and J.H. Tidwel, 2004. Anesthetics in Aquaculture. Southern Regional Aquaculture Center Publication No. 3900.

Cuesta, A.; J. Meseguer and M.A. Esteban, 2004. Total serum immunoglobulin M levels are affected by immunomodulators in seabream (Sparus aurata L.). Vet Immunol Immunopathol, 101: 203-210.

Dacie, V. and S.M. Lewis, 1991. Practical Hematology 7th ed. Churchill Livingstone; London, pp 556.

Detar, J. E. and H. T. Mattingly, 2004. Response of southern red belly dace to clove oil and MS-222: effects of anesthetic concentration and water temperature. Proc. Annu. Conf. Southeast. Assoc. Fish and Wildl Agencies, 58: 219-227.

Doumas, B.T.; D.D. Bayso; R.J. Carter; T. Peters and R. Schaffer, 1981. Determination of total serum protein. Clin. Chem., 27: 1642-1643.

Dytham, C., 1999. Choosing and using statistics: A Biologist's guide Blackwell Science Ltd., London, United Kingdom, p. 147.

Eder, M. and P. Gedigk, 1986. Lehrbuch uer Allgeminen Pathologie und der Pathologischem. Springer, Berlin.

Fischer, I.U.; G.E. Unruh and H.J. Dengler, 1990. The metabolism of eugenol in man. Xenobiotica, 20 (2): 209–222.

Gibson, R.N., 1967. The use of the anaesthetic quinaldine in fish ecology. Journal of Animal Ecology, 36: 295–301.

Grant, G.H.; L.M. Silverman and R.H. Christenson, 1987. Amino acids and proteins. In: Tietz NW (Ed.). Fundamentals of clinical chemistry, 3rd Edn. WB Saunders Co., London., pp. 291-345.

Griffiths, S.P., 2000. The use of clove oil as an anaesthetic and method or sampling intertidal rockpool fishes. Journal of Fish Biology, 57 (6) 1453-1464.

Holloway, A.; J. Keene; D. Noakes and R. Moccia, 2004. Effects of clove oil and MS 222 on blood hormone profiles in rainbow trout *Oncorhynchus mykiss*,Walbaum. Aquaculture Research, 35: 1025-1030.

Houston, A.H., 1990. Blood and Circulation. In Methods in Fish Biology. Schreck, C.B. and Moyle, P.B. (Eds). American Fisheries Society, Bethesda, Maryland, pp. 273-334.

Inoue, L.A.K.A.; C.L. Boijink; P.T. Ribeiro; A.D. Silva and E.G. Affonso, 2011. Avaliação de respostas metabólicas do tambaqui exposto ao eugenol em banhos anestésicos. Acta Amazonica, 41 (2): 327-332.

Iverzen, M.; B. Finstad; R.S. McKinley and R.A. Eliassen, 2003. The efficasy of metomidate, clove oil, Agui-Sand Benzoak as anaesthetics in Atlantic salmon stress – reducing capacity. Aquaculture, 221: 549–566.

Keene, J.L.; D.L.G. Noakes; R.D. Moccia and C.G. Soto, 1998. The efficacy of clove oil as an anaesthetic for rainbow trout, Oncorhynchus mykiss Walbaum. Aquacult. Res., 29: 89-101.

Li, Q.; M.H. Yeo and B.K. Tan, 2000. Lipid peroxidation in small and largephospholipid unilamellar vesicles induced by watersoluble free radical sources. Biochem. Biophys. Res. Commun, 273: 72-76.

Marchand, M.J.; J.C. Van Dyk; G.M. Pieterse; I.E.J. Barnhoorn and M.S. Bornman, 2009. Histopathological alterations in the liver of the sharptooth catfish Clarias gariepinus from polluted aquatic systems in South Africa. Environ Toxicol., 24: 133-147.

Matin, S.M.A.; M.A. Hossain and M.A. Hashim, 2009. Clove oil anaesthesia in singhi (Heteropneuses fossilis) and lata (Channa punctatus) fish. Bangladesh Vet., 26 (2): 68–73.

Mavadati, A. and R. Habibian, 2011. Comparision of effect of clove oil and 2-phenoxy ethanol on serom biochemical parameters in *Onchorhynchus mykiss*. Word Journal of fish and marine sciences, 3 (4): 318-322.

Mazandarani, M.; S.M. Hoseini and R. Shahriari, 2015. Anesthesia of wild female Persian sturgeon, Acipenser persicus (Borodin, 1897) breeders during controlled propagation: effects on hematological parameters,

stress response and reproductive performance. Journal of applied Ichthyology, 31 (6): 997-1001.

Munday, P.L. and S.K. Wilson, 1997. Comparative efficacy of clove oil and other chemicals in anaesthetization of *Pomacentrus amboinensis*, a coral reef fish. J. Fish Biol., 51: 931–938.

Neiffer, D.L. and M.A. Stamper, 2009. Fish sedation analgesia, anesthesia, and euthanasia: considerations methods, and types of drugs. ILAR Journal, 50, 343-360.

Olufayo, M.O. and O.O. Ola, 2010. The use of Clove oil as anaesthesia on African Catfish: Women perspective. Proceedings of TWOWS Fourth General Assembly and International Conference: Women Scientists in a Changing World held in Beijing, China, 27th -30th.

Padiyoor, B.A.; F.G. Benno Pereira and V. Jayaprakas, 2017. Assessment of clove oil and Benzocaine Anaesthesia on Haematological and Histopathological profile of Haludaria fasciata. J. Bio. Env. Sci., 10 (4): 27-37.

Park, M.O.; S.Y. Im; D.W. Seol and I.S. Park, 2009. Efficacy and physiological responses of rock bream, *Oplegnathus fasciatus* to anesthetization with clove oil. Aquaculture, 287: 472-430.

Patrono, C. and B.A. Peskar, 1987. Radioimmunoassay in basic and clinical pharmacology. Heidelberg, Springer-Verlag, 313.

Patton, C.J. and S.R. Crouch, 1977. Calorimetric determination of blood urea. Analytical Chemistry, 49: 464-469.

Phani Kumar, M. and P. Sree Ramulu, 2013. Hematological changes in *Pangasius hypophthalmus* infected with *Aeromonas hydrophyla*. Int. J. Food Agric. Vet. Sci., 3: 70–75.

Rezende, K.F.; R.M. Santos; J.C. Borges; L.M. Salvo and J.R. Silva, 2014. Histopathological and genotoxic effects of pollution on Nile Tilapia

(Oreochromis niloticus, Linnaeus, 1758) in the BillingsReservoir (Brazil). Toxicol Mech Methods, 24: 404-411.

Roberts, R.J., 2001. Fish pathology 3rd ed. WB. Saunders, Philadiphia, PA. U.S.A., 472 pp.

Ross, L.G. and B.R. Ross, 2008. Anaesthetic and sedative techniques for aquatic animals. Blackwell Science, Oxford: Blackwell Science. 240p.

Schoettger, R.A. and M. Julin, 1967. Efficacy of MS-222 as an anesthetic on four salmonids. Invest. Fish Control U.S. Dept. Int., 13: 1 –15.

Shahsavani, D.; M. Mohri and H. Kanani, 2010. Determination of normal values of some blood serum enzymes in Acipenser stellatus Pallas. Fish Physiology and Biochemistry, 36 (1): 39-43.

Shaluei, F.; A. Hedayati; A. Jahanbakhshi and M. Baghfalaki, 2012. Physiological responses of great sturgeon to different concentrations of 2-phenoxyethanol as an anesthetic. Fish Physiology and Biochemistry, 38: 1627–1634.

Simões, L.N.; D.C. Lombardi; A.T.M. Gomide and L.C Gomes, 2011. Efficacy of clove oil as anesthetic in handling and transportation of Nile tilapia, *Oreochromis niloticus* (Actinopterygii: Cichlidae) juveniles. Zoologia 28: 285–290.

Small B.C., 2003. Anesthetic efficacy of metomidate and comparison of plasma cortisol responses to tricaine methanesulfonate, quinaldine and clove oil anesthetized channel catfish *Ictalurus punctatus*. Aquaculture, 218: 177-185.

Soivio, A. and A. Oikari, 1976. Haematological effects of stress on a teleost, Esox lucius L. J. Fish Biol., 8 (5) 397-411.

Soto, C.G and S. Burhanuddin, 1995. Clove oil as a fish anaesthetic for measuring length and weight of rabbitfish (*Siganus lineatus*). Aquaculture, 136:149–152.

Soufy, H.; M. Soliman; E. El-Manakhly and A. Gaafa, 2007. Some biochemical and pathological investigations on monosex Tilapia following chronic exposure to carbofuran pesticides. Global Veterinaria, 1: 45-52.

Stoskopf, M.K., 1993. Fish medicine. Sounders Company, U.S.A pp 882.

Sudagar, M.; A. Mohammadizarejabad; R. Mazandarani and S. Pooralimotlagh, 2009. The efficacy of clove powder as an anesthetic and its effects on hematological parameters on Roach (*Rutilus rutilus*). Journal of Aquaculture feed science and nutrition, 1: 1-5.

Summer felt, R.C. and L.S. Smith, 1990. Methods for fish biology. In: Schreck CB, Moyle PB (eds) Anaesthesia, surgery and related techniques. American Fisheries Society, Bethesda، 213–272.

Szabo, T.; C.S. Modgyasszay and L. Horvath, 2002. Ovulation induction in nase (*Chondrostom anasus*). Using pituitary extract or GnRH analogue combined with domperidone. Aquaculture, 203: 389-395.

Taylor, P.W. and S.D. Roberts, 1999. Clove oil: An alternative anaesthetic for aquaculture. North American Journal of Aquaculture, 61: 150–155.

Thomas, L., 1998. Clinical laboratory diagnostics. TH-Books Verlagsgesellschaft, Frankfurt, pp 231–241.

Thomas, P. and L. Robertson, 1995. Plasma cortisol and glucose stress responses of red drum (*Sciaenops occllatus*) to handling and shallow water stressors and anesthesia withMs-222, quinaldine sulfate and metomidata. Aquaculture, 96: 60- 86.

Trinder, P., 1969. Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. Annal. Clin. Biochem., 6: 24-27.

Velisek, J.; A.L. Stara; S. Silovska and J. Turek, 2011. Comparison of the effects of four anaesthetics on blood biochemical profiles and oxidative stress biomarkers in rainbow trout. Aquaculture, 310: 369-375.

Velisek, J.; Z. Svobodova and V. Piackova, 2005. Effect of clove oil anaesthesia on rainbow trout (*Oncorhynchus mykiss*). Acta Veterinaria Brno, 74: 139–146.

Wagner, G.N.; T.D. Singer and R.S. Mckinley, 2003. The ability of clove oil and MS-222 to minimize handling stress in rainbow trout (*Oncorhynchus mykiss*, Walbaum). Aquaculture Research, 34: 1139-1146.

Waterstrat, P.R., 1999. Induction and recovery from anesthesia in channel catfish (*Ictalurus punctatus*) fingerlings exposed to clove oil. Journal of the World Aquaculture Society, 30: 250–255.

Woo, P.T.K. and D.W. Bruno, 1999. Fish Diseases and Disorders. Vol. 3: Viral, Bacterial, and Fungal Infections. CABI Publishing. New York, NY. Pp. 559.

Zapryanova, D.; A. Atanasoff and G. Sandeva, 2017. Effects of clove oil on some blood biochemical parameters in carp (*Cyprinus carpio*). 5th international conference sustainable postharvest and food technologies– Inoptep and 29th national conference processing and energy in agriculture , Vršac, Serbia.

تأثير زيت القرنفل والكوانالدين كعوامل مخدرة على بعض المعلامات الهيماتولوجية والبيوكيمائيه للدم في المبروك الفضي خلال موسم التفريخ

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الملخـص العربــي

تتناول الدراسة الحالية تأثير زيت القرنفل والكوانالدين كمخدرين لأمهات المبروك الفضى اثناء موسم التفريخ. تم استخدام ثلاثين سمكه من أمهات المبروك الفضي، خمسه عشر أنثى بمتوسط وزن يتراوح بين 2.500–2.700 كيلوجرام ومتوسط طول يتراوح بين 60–65 سم وخمسه عشر ذكر بمتوسط وزن يتراوح بين 2.300–2.400 كيلوجرام ومتوسط طول يتراوح بين 56–58 سم. قسمت هذه الأسماك إلي خمس مجموعات. تم إستخدام تركيزات مختلفة من زيت القرنفل (5 مل/ لتر ، 7.5 مل/ لتر، 10 مل/ لتر) للمجموعة الاولى و لمجموعة الثانية والمجموعة الثالثة على التوالى وتركيز 2.5 مل/ لتر من الكوانالدين للمجموعة الرابعة وكانت المجموعه الخامسه هى المجموعه الضابطه. تم تحديد وقت التخدير، وقت الإفاقة والأداء التناسلى للأسماك. تم دراسة التأثير على بعض المعلامات الهيماتولوجية والبيوكيمائيه للدم. كما تم دراسة التغيرات الهستوباثولوجية التى حدثت فى بعض الأعضاء الداخلية للمجموعات المختلفة. أشارت نتائج الدراسة إلى أن استخدام زيت القرنفل بمعدل (5 مل/ 100 لتر) كان التركيز الأمثل الذي أدى إلى التخدير الكامل عند (6 دقائق)، وقت الإفاقة (3 دقائق ، 3 ثوانٍ) مع معدل التفريخ الناجح (100٪) وكانت الفتره بين الحقن وإنطلاق البيض (9 ساعات). كما لم توجد هناك اختلافات معنويه بين مقاييس الدم المختلفه والمجموعه الضابطه. بينما استخدام الكوانالدين أدي إلى إختلافات معنويه كبيره بين مقاييس الدم المختلفه وخاصة الجلوكوز والكورتيزول مقارنة مع المجموعة الضابطه.