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RESEARCH PAPER

Effect of Different Anaesthetics on Hematology and Blood Biochemistry of *Labeo rohita*

Syed Sikandar Habib[1], Saira Naz[2], Aima Iram Batool[1], Muhammad Fayyaz Ur Rehman[3], Mujeeb Ullah[4], Osman Sabri Kesbiç[5], Giulia Maricchiolo[6,*], Francesco Fazio[7]

[1]Department of Zoology, University of Sargodha, 40100, Sargodha, Punjab, Pakistan.
[2]Institute of Molecular biology and Biotechnology, University of Lahore Sargodha Campus, 40100, Sargodha, Punjab, Pakistan.
[3] Department of Chemistry, University of Sargodha, 40100, Sargodha, Punjab, Pakistan.
[4] Department of Zoology, Islamia College University, Peshawar 25120, Khyber Pakhtunkhwa, Pakistan.
[5] Department of Animal Nutrition and Nutritional Diseases, Veterinary Faculty, Kastamonu University, 37150, Kastamonu, Türkiye.
[6] CNR, Institute for Marine Biological Resources and Biotechnologies (IRBIM), 98122, Messina, Italy.
[7] Department of Veterinary Sciences, University of Messina, 98168 Messina, Italy.

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Corresponding Author
Tel.: +390906015411
E-mail: giulia.maricchiolo@cnr.it

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Abstract

The current study aims to determine the effect of four different anesthetics such as MS-222 (100 mg/L), tobacco extract (50 mg/L), propiscin (1.0 ml/L) and clove oil (40 mg/L) on the hematology and blood biochemical parameters of Labeo rohita. Blood was sampled 10 minutes after the anesthesia was administered and again after 24 hours. Except for the clove oil 24h measurements, there was a significant difference in the number of blood cells in all anesthetic applications ($P<0.05$). Hemoglobin and hematocrit values were not altered after 24 hours of treatment with clove oil and propiscin. MCV, MCH and MCHC values were not significantly affected in any application except clove oil 24h. Biochemical parameters, including total protein and glucose level, significantly changed ($P<0.05$) in propiscin and clove oil group. Enzymatic activities such as ALT changed significantly with MS-222, and AST values were non-significant ($P>0.05$) with tobacco, Change has been detected in ALP with MS-222 and tobacco calcium and magnesium showed non-significant differences with anesthetics. Overall all the parameters were affected by anesthetics used in the current research, however, it was established that 24 hours following the administration of clove oil, the discrepancies in blood values had vanished to a great extent.

Introduction

Nowadays, in modern aquaculture, the common practice is using non-stressful anaesthetics. Some plant-based products are mostly used as an anaesthetic during all aquaculture practice, handling, surgery and tagging, minimizing stress-inducing issues like a decrease in feeding rate and immune function. (Aydın & Barbas, 2020). Various research on different types of anaesthetics, such as clove oil, MS-222, methomidate, benzocaine, 2-phenoxyethanol and quinaldine sulphate, have been held globally to evaluate the performance of anaesthetics employed in addressing these difficulties (Velí & Ek, 2004; Velíšek & Svobodova, 2004; Velíšek et al., 2005). Several important properties must be considered when selecting a particular agent as an anaesthetic for a specific purpose, such as availability, physiological disturbances, comfort use, cost, effectiveness, toxicity effects on fish and ease of use (Readman et al., 2013). Environmental factors (salinity, temperature, hardness, pH) and biological factors (fish species, weight and size) also directly related to and influenced the effectiveness of anaesthetics (Weber et al., 2009). A suitable anaesthetic can lead to anaesthesia

within 3-4 minutes and let them recover within 5 minutes after introducing fish into clean freshwater (Hasimuna et al., 2021). According to different studies, the varied concentration of anaesthetics and responses towards the different anaesthetics greatly varied from species to species (Pawar et al., 2011; Zahl et al., 2012). Generally, the fish's physiological responses to stress are primary and secondary (Iwama et al., 2004). All of these are related to changes in particular haematological and biochemical indices, which are the indicators of induced stress. In other words, changes in blood parameters could be a useful tool to reflect the stress caused by anaesthesia (Skomal & Mandelman, 2012). Hence, the primary response is the elevated blood cortisol concentration, while the secondary responses are increased glycemia and haematological alteration (Caruso et al., 2010).

From ancient times, anaesthesia has been utilized in aquaculture for multiple purposes, research purposes and many more. Moreover, these anaesthetic drugs are also used for *Labeo rohita* to reduce stress during handling and other routine procedure like vaccination, weighing, tagging, blood sampling, transporting, experimental or medical surgeries and different other veterinary procedures (Størkersen et al., 2021). The famous aquaculture specie *Labeo rohita* selected in the current study is mostly cultured species in intensive, semi-intensive and polyculture systems in South Asia. It is a highly consumed and nutritionally important specie. The current study aimed to examine the four different anaesthetics effect on haematological and biochemical profiles of *Labeo rohita*.

Materials and Methods

The anaesthetics (MS-222, tobacco extract, propiscin and clove oil) used in the current study were purchased from the chemicals company (Sigma-Aldrich). The experimental animal (*Labeo rohita*) of 27 fish was purchased from the local fish farm in the district Mianwali, Punjab, Pakistan. All the fish were carefully observed before selection and were found healthy with no sign of infection. The average body weight was 340.31±78.45 g, and the length was 33.23±3.57 cm. Experimental fishes were transported to the Zoology research laboratory at the University of Lahore for the experiment. The fish were kept in fibreglass tanks with 300 L of water capacity for 14 days of acclimatization (L-12 and D-12). During this period, commercial feed (Supreme company (Pakistan), crude protein: 30% and crude lipid: 10%) at an appropriate rate was provided twice a day to maintain proper growth. Throughout the experimental period (30 days), the water quality parameters were stable and measured daily. The mean water temperature was 28±0.7°C, pH 7.4, and DO was 5.6±0.03 mg/L. After every three days, fresh tube well water was added to the experiment tanks, which contained a fixed aerator.

After the acclimatization period, the fish were divided into nine groups. One group was kept as the control group (without anaesthesia), and the other eight groups were designed as experimental groups. Each group contained three individuals for each anaesthetic drug. Blood was sampled (0.5cc) from the control group using a sterile syringe needle size (22 Ga) from the caudal vein, while anaesthetics including MS-222 (100 mg/L), tobacco extract (50 mg/L), propiscin (1.0 ml/L) and clove oil (40 mg/L) was given to the four groups respectively, and then the blood was sampled immediately after 10 minutes. Further, blood was again collected 24 hours after being induced with 10 minutes' anaesthesia from the last four groups. During the experimental period, no mortality was recorded in all groups. The same quantity of blood was drawn from each group using the same instruments. After sampling, poured the blood into the sterile Eppendorf tubes containing an anticoagulant (heparin). The dose of all anaesthetics drugs was selected according to the available literature (Coyle et al., 2004). Behavioural changes during anaesthesia and recovery are given in Table 1.

The haematological parameters such as RBC and WBC count were done through Neubauer hemocytometer as described by Goldenfarb et al. (1971). Haemoglobin (HB) was determined through a photoelectric colorimeter (AE- 11M) with an absorbance of 540 nm (Mallesh et al., 2015). The microhematocrit centrifuge (HERMLE Z-230-H) determined the hematocrit value. Heparinized blood (50µL) through capillary action was transferred into the hematocrit capillary tubes during this process. After that, the tubes were centrifuged for 5 min at 12000 rpm. The values were measured by using a scale. Erythrocyte indices were determined by using the following formulas (1,2,3):

Table 1. Behavioural changes during anesthesia and recovery stages of fish

Stage	Anesthesia	Recovery
I	Reduced responsiveness to outside stimuli and sedation	Maintaining instability, ocular movement, and a lack of reaction to environmental stimuli
II	Partial loss of balance, sluggish swimming, and ataxia symptoms	Partial return to equilibrium, no swimming activity
III	A total loss of balance, a halt to movement, and a lack of responsiveness to environmental stimuli	Regaining full balance, swimming normally, and responding to environmental stimuli
IV	Medullary collapse	

$$\text{Mean Corpuscular Volume} = \frac{Packed\ cell\ volume(pct)}{Erythrocytes(million\ per\ mm\ cube)} \times 100 \quad (1)$$

$$\text{Mean Corpuscular Hemoglobin} = \frac{Hemoglobin(gram\ per\ deciliter)}{Erythrocytes(million\ per\ mm\ cube)} \times 100 \quad (2)$$

$$\text{Mean Corpuscular Hemoglobin Concentration (pct)} = \frac{Hemoglobin(gram\ per\ deciliter)}{Packed\ cell\ volume(pct)} \times 100 \quad (3)$$

To determine biochemical parameters, plasma was separated from the sampled blood through a centrifuge (4000 rpm) for 3 minutes. For further analysis, the plasma was stored at -20°C. In the current study, an analyzer (YSD-100 vet) was used for the determination of biochemical indices, including total protein (TP), albumin (ALB), glucose (GLU), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), calcium (Ca^{2+}) and magnesium (Mg) were determined. Biochemical parameters were examined following the technique of Kolářová and Velíšek (2012).

The data were subjected to statistical analysis using SPSS software (version 22.0). In tables, data are presented as mean ± standard deviation. The Kolmogorov-Smirnov test was applied initially to normalize the data. After that, One-way ANOVA was applied with a 5% significant level. Significant difference among the groups was compared by applying the Duncan multiple range test.

Results

Haematological Parameters

The findings of haematological parameters of *Labeo rohita* after exposure to different anaesthetics such as MS-222, a tobacco extract, propiscin and clove oil can be seen in Table 2.

A significant difference (P<0.05) was determined in RBC count with MS-222, a tobacco extract, and propiscin after 10 min and 24 hr of blood sampling, while clove oil only showed significant results after 10 minutes but no significant difference (P>0.05) was recorded in RBC of fish after exposure to clove oil (24 h) compared to the control group. Besides this, a significant difference (P<0.05) was observed in WBC count (10 min and 24 h) with MS-222, a tobacco extract, propiscin and clove oil. Haemoglobin and hematocrit levels were significantly higher with MS-222, tobacco extract and propiscin (10 min and 24 h) and clove oil (10 min). No significant difference was recorded in haemoglobin and hematocrit value with propiscin and clove oil (24 h). Moreover, the MCV value was significantly higher (P<0.05) with tobacco extract (10 min and 24 hr) and no significant difference at 24 h with MS-222, clove oil and propiscin compared to the control group. Values of MCH were significant (P<0.05) at 10 min with clove oil, MS-222, tobacco extract and propiscin. In contrast, the values were non-significant (P>0.05) at 24 h with MS-222, clove oil, tobacco extract and propiscin. Apart from this, the values of MCHC were non-significant with MS-222 and propiscin (10 min and 24 h) and with tobacco extract and clove oil (24 h)., while a significant difference was observed only in tobacco extract (10 min).

Biochemical Parameters

The result of the biochemical parameters of fish (*Labeo rohita*) with different anaesthetics is given in Table 3. The result revealed that the level of total protein showed no significant difference (P>0.05) with MS-222, tobacco extract (10 min and 24 h) and with propiscin and clove oil (24 h), while a significant difference (P<0.05) was recorded with propiscin and clove oil (10 min) compared to control. The albumin value presented no significant difference compared to the control group after anaesthesia with MS-222, a tobacco extract, propiscin and clove oil (10 min and 24 h). The level of glucose was non-significant (P>0.05) after induced anaesthesia with MS-222 and tobacco extract; however, with propiscin and clove oil, the glucose level was significantly higher (P<0.05) than in the control group. After anaesthesia with tobacco extract, propiscin, clove oil (10 min and 24 h) and MS-222 (24 h), the activity of alanine aminotransferase showed no significant (P>0.05) difference compared to the control group, but a significant difference was observed with MS-222 (10 min). The activity of aspartate aminotransferase after induced anaesthesia with MS-222, propiscin, clove oil (10 min and 24 h) and tobacco extract (24 h) revealed no significant difference (P>0.05) from than control group. However, their activity was significantly higher (P<0.05) with tobacco extract (10 min). The level of alkaline phosphatase was significantly higher (P<0.05) after anaesthesia with MS-222 and

Table 2. Effect of different anaesthetics on haematological parameters of *Labeo rohita*

Parameters	Control group	MS-222		Tobacco extract		Propiscin		Clove oil	
	0	10 min	24 h	10 min	24 h	10 min	24 h	10 min	24 h
RBC ($\times10^6$ mm^{-3})	80.6±4.2^a	72.5±3.2^b	75.3±2.5^c	73.5±6.2^b	76.7±7.2^c	75.3±9.4^b	77.5±4.6^c	73.2±1.4^b	78.3±4.9^a
WBC ($\times10^3$ mm^{-3})	135.6±5.34^a	98.5±3.66^b	108.7±5.46^c	94.7±2.64^b	105.4±9.88^c	105.2±4.55^b	122.6±2.43^c	108.8±4.65^b	96.2±3.45^c
Hemoglobin (g dL^{-1})	8.3±0.33^a	6.1±0.12^b	7.2±0.23^c	6.2±0.78^b	7.5±0.56^c	6.8±0.11^b	7.9±0.34^a	6.5±0.33^b	7.9±0.34^a
Hematocrit (%)	43.45±0.36^a	32.86±0.46^b	37.56±0.71^c	34.87±0.24^b	39.75±0.25^c	34.46±2.64^b	41.23±0.66^a	36.22±0.68^b	41.53±0.34^a
MCV (fl)	463.7±1.22^a	446.8±2.45^b	461.5±9.43^a	444.9±7.24^b	456.8±9.44^c	451.3±4.64^b	461.6±9.35^a	441.4±9.23^b	461.6±2.75^a
MCH (pg)	93.4±1.43^a	87.3±1.23^b	91.3±2.34^a	88.3±3.24^b	92.45±5.12^a	86.5±3.24^b	91.2±1.53^a	81.3±3.24^b	91.5±2.53^a
MCHC (g dL^{-1})	19.4±0.04^a	17.34±0.02^a	18,9±0.64^a	16.3±0.43^b	18.3±0.01^a	17.43±0.03^a	19.1±0.05^a	18.9±0.01^a	19.2±0.02^a

Note: *Means with different superscripts are significantly different* (P<0.05)

tobacco extract (10 min), but no significant difference with propiscin, clove oil (10 min and 24 h) and MS-222 and tobacco extract (24 h) compared to the control group. The values of calcium and magnesium compared to the control group showed no significant difference (P>0.05) after anaesthesia with MS-222, a tobacco extract, propiscin and clove oil (10 min and 24 h).

Discussion

Different fish species have a wide range of responses to anaesthetics, so dose assessment is frequently required. Different anaesthetics examined in the current study were active as a sedative for routine evaluating and assessing methods and handling for breeding. The analysis of haematological and biochemical profiles is an important tool for understanding the health status of the aquatic organism (Fazio, 2019; Habib et al., 2021). The haematological and biochemical parameters are mostly studied to know the effect of the anaesthetics. Very limited data on anaesthetics, including MS-222, a tobacco extract, propiscin and clove oil, is available. The current research found a significant difference in RBC and WBC count after exposure to anaesthetics due to chemical stress. A similar result was reported by Abdolazizi et al. (2011) who evaluated the effect of clove oil on haematology of *Carassius auratus*. Another study was carried out by Mohammadi and Khara (2015) on haematological parameters of *Oncorhynchus mykiss* by using different anesthetics (tobacco, tricaine methanesulfonate, clove oil and ketamine). They observed a significant difference in the values of RBCs, WBCs, haemoglobin and hematocrit, which is according to the findings of the current study. Kristan et al. (2012) evaluated the effect of four different anaesthetics (2-phenoxyethanol, MS 222, clove oil and propiscin) on the blood profile of pikeperch. Results revealed that MCH values were significant with propiscin (10 min and 24 h) as in the current study and non-significant with clove oil, 2-phenoxyethanol and MS 222 (10 min) compared to the control group. In contrast to current studies, Lepic et al. (2014) discovered no alterations in the haematological parameters of *Vimba vimba* under anaesthetics MS-222, clove oil, 2-phenoxyethanol, and propiscin. Changes in PCV and MCHC were observed by (Velisek et al., 2007) with 2-phenoxyethanol on sheatfish.

The current research outcomes revealed that the anaesthetics influenced the biochemical parameters. A significant difference was observed in total protein with propiscin and clove oil (10 min) than the control group. It is thought that these observed differences originate from the effect of anaesthesia leading to hemolysis of RBCs. The strongest evidence for this argument is that clove oil had a hemolytic impact in an in-vitro assessment of the effect of clove oil on blood (Nagaraju et al., 2021). Similar to our study the value of total protein was significantly higher with 2-phenoxyethanol (10 min) (Lepic et al., 2014). Velíšek et al. (2005) evaluated the effect of plant-based anaesthetics on fish (*Cyprinus carpio*) biochemical indices. In the results of the studies, total protein levels were non-significant; it's the same as in our findings on clove oil and propiscin (24 h), MS-222 and tobacco (10 min and 24 h). No significant difference was recorded in albumin with MS-222, 2-phenoxyethanol, clove oil and propiscin (Lepic et al., 2014) and with MS-222 (Rożyński et al., 2019) compared to the control group which is according to the findings of the current study. This low albumin level might be due to reduced liver function due to stress. The well-known truth, glucose is the primary energy supply required to regain homeostasis after a stressor injury. Therefore, blood glucose levels are the most helpful instrument for detecting stress-related physiological changes in fish. Increased glucose level with propiscin and clove oil (10 min and 24 h) than the control group due to emotional and physical stress. Kristan et al. (2012) also reported a higher level of glucose in pikeperch with clove oil and MS-222. Other studies, including Rożyński et al. (2019), detected a high level of glucose in pikeperch with MS-222 and (Ortuño et al., 2002) in *Sparus aurata* observed a higher level of glucose. Changes in the activity of the enzymes that signal liver health in the blood plasma might be a good predictor of stress since the liver is a prominent marker for the endocrine activity that helps fish deal with problems physiologically. Significant changes in AST and ALT indicate tissue damage which may be due to the stress of anaesthetics.

In the current study, a significant change was observed in ALT and AST with all four types of

Table 3. Effect of different anaesthetics on biochemical parameters of *Labeo rohita*

Parameters	Control group	MS-222		Tobacco extract		Propiscin		Clove oil	
	0	10 min	24 h	10 min	24 h	10 min	24 h	10 min	24 h
TP (g L^{-1})	42.42±2.63^{a}	43.42±2.76^{a}	44.01±3.42^{a}	42.30±3.42^{a}	43.45±2.42^{a}	46.01±1.3^{b}	42.35±1.24^{a}	46.23±0.5^{b}	44.15±0.73^{a}
ALB (g L^{-1})	8.18±1.85^{a}	7.74±0.46^{a}	7.54±0.76^{a}	8.21±1.56^{a}	7.76±0.94^{a}	9.31±1.43^{a}	8.01±1.43^{a}	8.73±0.84^{a}	8.13±0.57^{a}
GLU (mmol L^{-1})	4.63±0.74^{a}	6.51±1.32^{a}	4.82±1.43^{a}	6.83±1.42^{a}	4.84±2.31^{a}	7.63±1.53^{b}	8.53±0.98^{c}	8.83±1.65^{b}	11.32±0.74^{c}
ALT (µkat L^{-1})	0.86±0.42^{a}	0.52±0.32^{b}	0.78±0.53^{a}	0.67±0.73^{a}	0.81±0.33^{a}	0.73±0.23^{a}	0.75±0.34^{a}	0.77±0.64^{a}	0.76±0.43^{a}
AST (µkat L^{-1})	1.72±0.48^{a}	1.23±0.62^{b}	1.41±0.74^{c}	1.02±0.14^{b}	1.59±0.73^{a}	2.01±1.63^{b}	2.83±0.73^{c}	0.94±0.73^{b}	0.74±0.63^{c}
ALP (µkat L^{-1})	0.58±0.23^{a}	0.54±0.33^{b}	0.58±0.53^{a}	0.65±0.74^{b}	0.58±0.21^{a}	0.58±0.32^{a}	0.57±0.23^{a}	0.58±0.21^{a}	0.59±0.43^{a}
Ca^{2+} (mmol L^{-1})	2.28±0.42^{a}	2.25±0.53^{a}	2.40±0.64^{a}	2.35±0.36^{a}	2.58±0.74^{a}	2.38±0.85^{a}	2.63±0.74^{a}	2.47±0.25^{a}	2.71±0.43^{a}
Mg (mmol/l)	1.14±0.23^{a}	1.16±0.04^{a}	1.34±0.03^{a}	1.18±0.07^{a}	1.42±0.41^{a}	1.28±0.03^{a}	1.25±0.05^{a}	1.37±0.08^{a}	1.15±0.43^{a}

Note: *Means with different superscripts are significantly different* (P<0.05)

anaesthetic material compared to the control. Velíšek et al. (2005) observed a significant decrease in AST activity in *Oncorhynchus mykiss* with 2-phenoxyethanol and clove oil. ALP activity in our study was increased with MS-222 and tobacco extract (10 min) due to stress-induced anaesthesia. ALP activity was also enhanced in a study by Mirghaed et al. (2018) on *Oncorhynchus mykiss* with myrcene. No significant difference was detected in calcium and magnesium levels with all types of anaesthetics (10 min and 24 h) in our study, which is according to the findings of Rożyński et al. (2019).

Conclusion

The current study's findings imply that the directly employed anaesthetics predominantly impacted the internal physiology of fish, such as haematology and biochemical blood profiles. However, compared to the control and other groups, clove oil (24 h) had the slightest influence on the haematology of fish. Although the European Union approved MS-222 as an anaesthetic agent for fish, the blood parameters in the case of *Labeo rohita* were found to be altered. Biochemical parameters were also influenced up to some extent by all the anaesthetics. Based on the current study, it may be possible if the concentration of clove oil becomes reduced, it could not alter the internal physiology of fish. Further research is suggested to discover new possible anaesthetics to minimize the stress in fish.

Ethical Statement

All practices during the study were carried out by European Union Directive no: 2010/63 and followed all the valid national rules for the use and care of animals.

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This study involved no external funding.

Author Contribution

SSH: conceptualization, writing, original draft. SN: methodology, analysis, original draft. AIB: proofreading, methodology, supervision. MFUR: resources, proofreading, writing (review and editing). MU: methodology, resources, writing. OSK: proofreading and editing. GM: proofreading and editing. FF: proofreading and editing.

Conflict of Interest

The authors declare no conflict of interest.

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