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Research Article

ASSESSMENT OF LEMON BALM (*Melissa officinalis*) ON COMMON CARP, *Cyprinus carpio*: ANESTHESIA

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Abstract

Transportation, handling, sorting by size, weighing, increased farming density and degradation of water quality are stressors in aquaculture practices which exhibit physiological stress responses in fish. Present study assesses the optimal concentration and the degree of effectiveness of lemon balm as anesthetics of the common carp young, *Cyprinus carpio*. Study contributes the knowledge on some aspects of stress research include the detection of hematological and blood enzymes. Fish total length was 7.82 ± 0.44 cm and total weight 6.04 ± 1.13 gm. Three concentrations were used (550, 600 and 650 mg/l). Induction and recovery times were measured to the nearest second. In the recovery tub, the fish were monitored continuously to study partial and overall anesthesia and recovery time in minute. The blood samples from each fish of the different groups for determination of Red blood cells and White blood cells level, and for determination of ALP, AST, ALT, LDH and CK in Ul/l. Partial and overall anesthesia occurred in all concentrations, the results indicated to the significant differences ($p \leq 0.01$) at concentration of 550, 600 and 650 mg which taking less time for either partial or overall recovery. There are no significant differences ($p \geq 0.01$) for the effect of anesthesia on blood enzymes in all studied parameters which were numerically higher in controls treatments of all parameters, but there are significant differences ($p \leq 0.01$) for the effect of anesthesia on hematological in all studied parameters compared to control group. Based on results of the present study, Lemon balm can be recommended as suitable anesthetics for fishes (650 mg/l). Moreover, as an advantage, it refers to natural substance which has no any side effects on fishes and does not represent any hygienic risks.

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1. Introduction

Anesthetic agents play a fundamental role in both fisheries kinds of research and aquacultures due to it utilized in facilitating various handling procedures, such as weighing, sorting. Piscine researchers put their interesting and research priority to find a novel herbal derived anesthetic for different fish species due to several beneficial properties such as highlighting the fish antioxidant defense and alleviating stress responses in different species. This is the main reason for several plants extracted by researchers rather than using a proper anesthetics agent

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(Velisek *et al*., 2010; Bahrekazemi and Yousefi, 2017).

A lot of considerations should be counted before choosing and using anesthetic agents like efficacy, cost, availability, and ease of use, in addition to its toxicity on fish, humans and the environment (Ucar and Atamanalp, 2010). There are little studies about fish anesthesia in Iraq. Al-Jashami *et al*. (2002) used clove powder (*Eugenia caryophyllata*) as a new anesthetic for the common carp. Fry, mature fish and fingerling had undergone to different concentrations of the anesthetic ranged 120 - 230 mg/l. Al-Obaedy *et al*. (2013) has achieved three experiments to study some clove powder properties (*E. caryophyllata*) as an anesthetic for two fish species. Hassan (2016) has studied the anesthetic efficacy of two kinds of natural anesthetic (mustard and clove) both powder in forms, and also oil was estimated on young common carp. Al-Niaeem *et al*. (2017a, b) studied the anaesthetic the effect of nutmeg powder, *Myrisitca fragrans* on young common carp. Niaeem *et al*. (2019) study the anaesthetic effect of chamomile (*Matricaria chamomilla*) and anise (*Pimpinella anisum*) and a combination of their powders on young common carp. The aim of the present study was to evaluate the anesthetic effects and safe use of natural agent such as lemon balm in health treatments research.

2. Materials and Methods

Common carp were got from fish farms at the University of Basrah, Iraq. Fishes were put in an aquarium (50 × 30 × 30 cm) for three days for acclimation at 26 ºC ensuring that they had sufficiently recovered from possible capture - related or transport stress. The total length of fishes was 7.82 ± 0.44 cm and total weight 6.04 ± 1.13 gm. Three concentrations (550, 600 and 650 mg/l) of lemon balm powder was used alone as anesthetic agent with concentration control. It was prepared according to Al-Niaeem (2006). Each fish was weighed and transferred to recovery aquarium that has been filled with well aerated freshwater at the same time of preparation of the anesthetic baths. In the recovery aquarium, the fish has monitored continuously to determine the time of full equilibrium overall anesthesia, partial recovery and overall recovery time in minute. The time was recorded for: Stage I anesthesia: Partial loss of equilibrium, some body movements and reduced reaction to external stimuli. Stage II anesthesia: No body movements, total loss of equilibrium and no reaction to external stimuli.

Recovery time: Recovery of equilibrium, body movements and response to external stimuli.

The blood samples from each fish were collected by severing of the caudal peduncle to determine blood serum enzymes (ALP, GOT, GPT, CK and LDH in Ul/l). The levels of enzymes were assayed according to the instructions provided with the corresponding enzymatic kits. The number of Red Blood Cells (RBC) and White Blood Cells (WBC) were calculated by using Hemocytometer. The statistical calculations of the results were completed using SPSS version 20, one way (ANOVA) to determine the difference between the means.

3. Results and Discussion

Lemon balm (*M. officinalis*) and its preparations have a mildly soothing, antiviral effect, improve the digestive tract, and relax intestinal spasms (Vejdani *et al*., 2006). Lemon balm is a natural source of Rosmarinic acid (RA). RA is one of the main phenolic acids in the chemical composition of *M. officinalis*, and it determines the pharmacological effect and the medical use of the plant (Zarei *et al*., 2015). The perusal of the result indicates that there is a concentration dependent response in induction and recovery time of lemon balm used in the present study i.e. the highest concentration of anesthetic evoked quickest induction and the longest recovery time.

The Table – 1 showed the induction time of *C. carpio* which decreased with increasing concentrations of lemon balm, *M. officinalis*. At 650 mg/l, the time to reach a complete anesthesia was 15 min which is significantly different ($P \leq 0.01$). There was a clear direct relationship between recovery time and concentrations of the anesthetic with significant lowered ($P \leq 0.01$). The

longest recovery time was 25 min at a concentration of 550 mg/l, while the shorter recovery time was 14 min at a concentration of 650 mg/l lemon balm.

Table - 1: Effect of lemon balm on anesthesia and recovery stages (min) in common carps

Concentration (mg/ l)	Partial anaesthesia time (min.)	Overall anaesthesia time (min.)	Partial recovery time (min.)	Overall recovery time (min)
	(Mean ± SD.)			
550	30 ± 1.03 [a]	55 ± 2.30 [a]	18 ± 1.30 [a]	25 ± 1.21 [a]
600	18 ± 1.32 [b]	25 ± 2.24 [b]	13 ± 2.32 [b]	20 ± 2.43 [b]
650	11 ± 2.12 [c]	15 ± 1.25 [c]	10 ± 2.52 [c]	14 ± 2.51 [c]
P*	0.002	0.005	0.002	0.002

*Different letters in the same column are significantly different (P≤0.01).

The effects of lemon balm on the hematological RBC and WBC count of common carp are shown in Figure - 1. RBCs level was decreased significantly (0.84 × 10^{12} cells/l) when fish inducted to concentration of 600 ml/l of lemon balm compared to other concentrations and control groups. In recovery all three concentrations show increase in RBCs level compared to control group (0.90 × 10^{12} cells/l). These findings agree with study of Abdolazizi *et al*. (2011). This increase may result from the release of immature red cells by the spleen, as the fish were subjected to capture stress, this release could be an immediate response to this acute stress (Tort *et al*., 2011). Anesthetic treated fishes with concentrations of 550, 600 and 650 ml/l showed the significant increase (p≤0.01) in WBC level (190.4, 160.2 and 205.3 × 10^{9} cells/l), respectively, when compared to control group (124.3 × 10^{9} cells/l) as observed in Figure - 2.

In recovery, WBC was increased to (216.3, 195.6 and 230 × 10^{9} cells/ l) for the three doses respectively, compared to control group. These results are in agreement with those of Ucar and Atamanalp (2010) in which the effects of clove oil and 2-phenoxyethanol on cultured rainbow trout (*Oncorhynchus mykiss*) and brown trout (*Salmo truttafario*) are examined in their experiment. The increase in WBC count seen in the present study may have resulted from the excitation of defense mechanism of the fish to counter the effect of the anesthesia as suggested by (Shahi and Singh, 2011). No changes in leucocytes count followed anaesthesia in common carp, rainbow trout, European catfish and Siberian sturgeon (Velíšek *et al*., 2005; Velíšek *et al*., 2006).

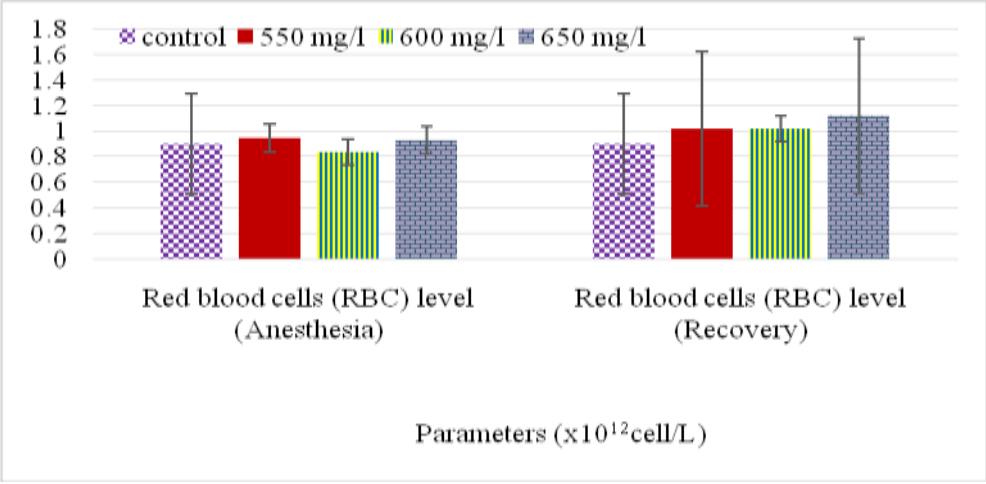

Figure - 1: Effect of lemon balm on Red blood cells level of common carp in anesthesia and recovery

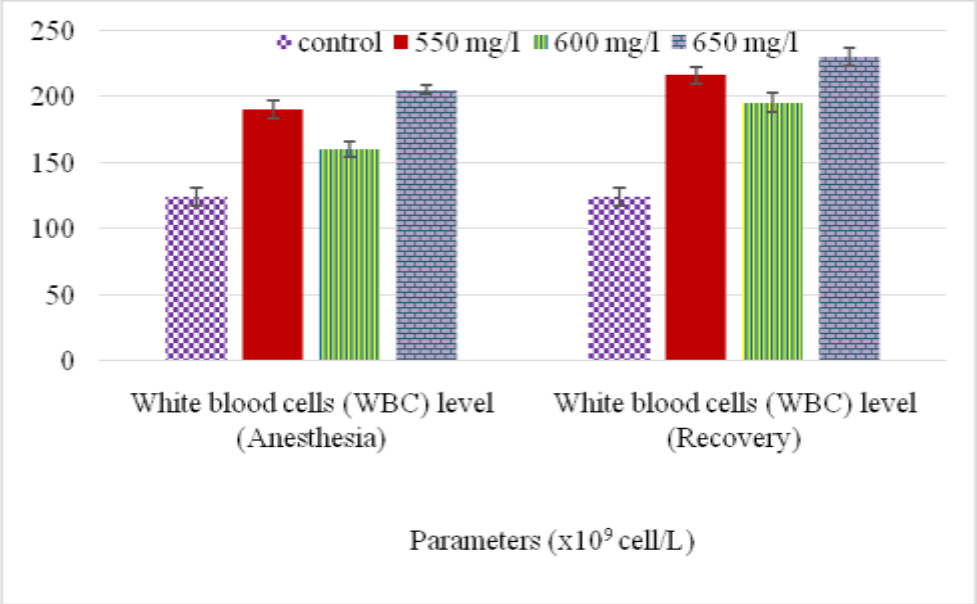

Figure - 2: Effect of lemon balm on White blood cells level of common carp in anesthesia and recovery

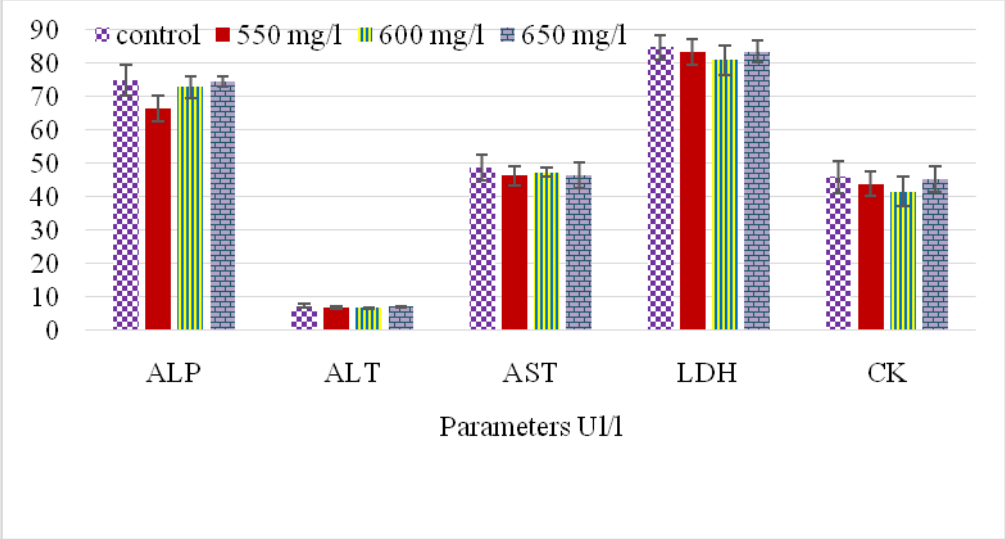

Figure - 3: Effect of lemon balm on blood serum enzymes level of common carp in anesthesia and recovery

The effect of lemon balm concentration in blood serum enzymes (AST, ALT, ALP, LDH and CK) showed no significant differences ($p>0.01$) appeared in all studies parameters as shown in Figure - 3 but the control numerically was higher in all parameters.

The above information clearly underlines the need to determine the optimum dose for anaesthetization for each anaesthetic on species - basis. Further, the optimum dose should be compared taking into account not merely the induction and recovery time but also the biochemical parameters during the process of anaesthetization. In summary, the present study

clearly established that 650 mg/ l is the optimum anaesthetic dose of lemon balm in on common carp which is relatively safe since it does not cause any architectural disruption or stress-induced changes in the target organs such as gills and buccal epithelium. This information will be of impartment to aquaculturists.

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