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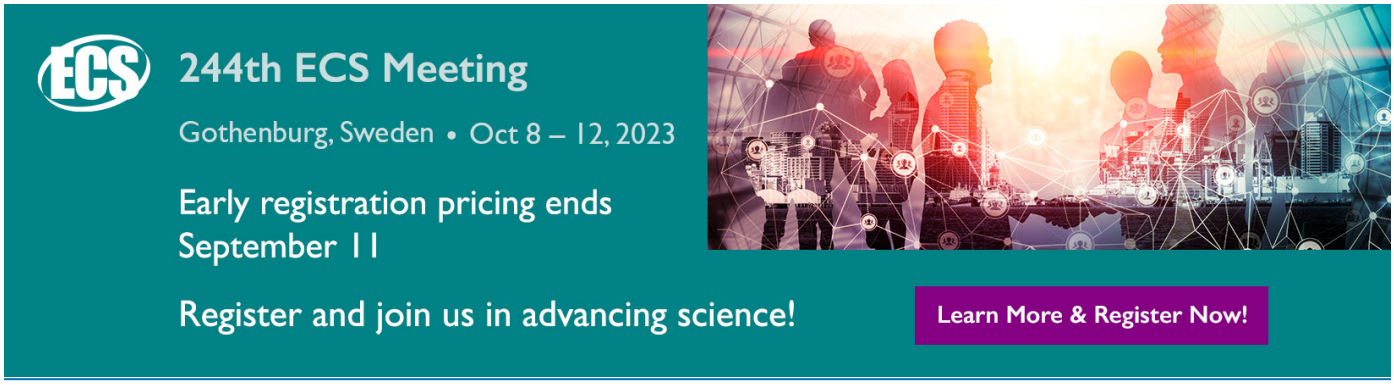

Assessment of Valerian (*Valeriana officinalis*) on Common Carp, *Cyprinus carpio*: Anesthesia

Samer Saleem Alshkarchy[1], Khalidah S. Al-Niaeem[2] and Raaed Sami Attee[3]

[1]Department of Pathology and Poultry Disease, College of Veterinary Medicine, Al-Qasim Green University, Babylon, Iraq.
[2]Department of Fisheries and Marine Resources, College of Agriculture, University of Basrah, Basrah, Iraq.
[3] Department of Animals, College of Agricultural, University of Diyala, Diyala, Iraq.

[1]E-mail: samer.alshakrchy@vet.uoqasim.edu.iq

Abstract. The anesthetic efficacy of Valerian (*Valeriana officinalis*) powders was evaluated on common carp (*Cyprinus carpio* L.)The following concentrations (250, 350, 450) mg/liter were used in three replicates. During the experiment, the period required for partial and total anesthesia and the time required for partial and total recovery was tested, as well as the number of red and white blood cells after and before Anesthesia in addition to blood serum enzymes represented (ALP, GOT, GPT, CK, and LDH in Ul / l). The results showed that the least period of anesthesia occurred in the fourth treatment, the treatment with a concentration of 450 mg / l, as well as the least period required for recovery, while the recovery period was the longest. And anesthesia in the first treatment exposed to a concentration of 250 mg/liter, and it was not noticed that there was a significant difference between each the number of red blood cells, white blood cells in the test fish before the experiment and after treatment with valerian plants, as well as each of (ALP, GOT, GPT, CK, and LDH in Ul / l) There were no significant differences between all treatments before and after exposure to valerian, and it is concluded from the study that valerian is a safe plant for use in anesthetizing fish.

Keywords. Anesthesia, *Valeriana officinalis*, *Cyprinus carpio*, ALP, GOT, GPT, CK.

1. Introduction

Anesthesia is used in fish and other aquatic organisms for several reasons shown by recent research as fish experience pain[1,2], suffer, distress, and respond to stressful and noxious stimuli just like other 'higher' animals, and this applies to adult fish [3,4] and fish larvae [5,6], as well as other invertebrate aquatic animals [7,8]. The sense of pain is a useful and indisputable ability for self-protection and survival for most creatures. Fish have similar rights to protection from suffering and pain as other such as mammals and birds [9]. Proper sedation and anesthesia have the potential to limit the adverse effects caused by the hypothalamic–pituitary–interrenal axis that plays a central role in the physiological stress response. Sedation is indicated to reduce stress and the risk of damage for many procedures or husbandry practices such as handling, crowding, pumping, transport, bath treatments in tanks, brood stock handling, or as premedication to anesthesia, thus facilitating safe handling and improving fish welfare[9]. Induction of anesthesia in fish and recovery from it quickly and with the least amount of stress are among the important characteristics that must be available in a good anesthetic. On the other hand, it must be easy to prepare and use, as well as it must be non-toxic to fish

and not stored in the tissues of their bodies and affect the health of the consumer (human)[10]. The wide margin of safety is one of Other important criteria in selecting an appropriate anesthetic [11]. In the past, various chemicals were used to anesthetize fish, and tricaine methane sulfonate with trade name MS222 was the most commonly used anesthetic drug in fish farming[12].
Nowadays, due to the side effects of this substance and its high price, and difficulty in obtaining it, its use is limited. Also, the side effects caused by chemical drugs led researchers to use plant compounds such as clove powder and its essential oil, due to its ease of preparation, economic efficiency, and effective analgesic as well. Its anesthetic properties are recommended in aquaculture [13]. However, It is worth noting that, in contrast to the aforementioned benefits, the results of some studies have shown that it causes side effects in fish treated with it, such as a decrease in water quality, changes in the physical and chemical characteristics of the water, and causes gill defects, and even death in some cases [14] Valerian (Valeriana officinalis) is an herbal plant of the Valerianaceae family and one of the oldest medicinal plants that have been used since ancient times as a pain reliever and an antidepressant [15]. Biochemical studies show that the valeric acid contained in it inhibits the enzyme responsible for the breakdown of Gamma-Aminobutyric Acid (GABA) and causes an increase in the concentration of GABA in brain tissue, which reduces the activity of the various brain nuclei. It causes sedative effects [15]Also, [16] reported in a study that valerian (*Valeriana officinalis*) root extract is effective in suppressing stress responses in swordfish (*Xiphophorus hellerii*). On the other hand, these authors reported in another study that the use of valerian root extract can play an effective role in reducing stress in rainbow trout (Oncorhynchus mykiss), so the use of this plant extract during the transfer of ornamental fish and rainbow trout was considered appropriate [17], and in another study, compounds of valerian plant were reported to have effective anti-anxiety effects on zebra fish [18] and another study also confirmed the properties of the anticonvulsant effect of valerian extract on Adult zebrafish [19] on the other hand, positive anesthetic effects of valerian extract have been demonstrated on rainbow trout [19]reported that valerian has debilitating effects on the central nervous system and recommended its use for aqueous anesthesia. In another study, an extract of valerian root in wildflowers Pangasius sutchi (Pangasius sutchi) was reported to be effective [20] Therefore, researchers had to find an alternative. It is necessary to use appropriate straws instead of common anesthesia due to their side effects. Therefore, the current study was conducted in order to determine the optimal concentration of valerian plant *(Valeriana officinalis)* as an anesthetic for common carp, being the first breeding fish in Iraq, and to evaluate the possibility of using valerian extracts as a new and alternative anesthetic compared to cloves.

2. Materials and Methods

2.1. Experiment Fish

Fishes were put in an aquarium (50 x 30 x 30 cm) for 14 days for acclimation at 26 ºC ensuring that they had sufficiently recovered from possible capture-related or transport stress. The total length of the fish was 6.42 ± 0.24 cm and the total weight was 5.09 ± 1.25 gm. Three concentrations (250, 350, and 450 mg/l) of Valerian powder were used alone as an anesthetic agent with concentration control. It was prepared according to [21].

2.2. Anesthesia Experiment

Each fish was weighed and transferred to a recovery aquarium that has been filled with well aerated freshwater at the same time as 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 minutes. The time was recorded for:

- Stage I anesthesia: reduced reaction to external stimuli., some body movements, and Partial loss of equilibrium.
- Stage II anesthesia: No body movements, total loss of equilibrium, and no reaction to external stimuli.

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

2.3. Blood Samples

The severing of the caudal peduncle to determine blood serum enzymes (ALP, GOT, GPT, CK, and LDH in Ul / l) collected the blood samples from each fish. The levels of enzymes were assayed according to the instructions provided with the corresponding enzymatic kits. The number of blood cells (RBC) and white blood cells (WBC) were calculated by using Hem cytometer.

2.4. Statistical Analysis

The statistical calculations of the results were completed using SPSS version 23, one-way (ANOVA) to determine the difference between the means.

3. Results

3.1. Anesthesia and Recovery Stages

The results of the statistical analysis showed that there were significant differences between the different concentrations in the time of total and partial anesthesia, where the longest time until the onset of anesthesia was in the first treatment at a concentration of 250 mg/L, as shown in Table (1), where the time until anesthesia was 45 minutes, and until total anesthesia was 62 minutes, while The least time until partial and total anesthesia was reached in the third treatment, which amounted to (10 and 16) minutes, respectively. On the other hand, it was clear from the results of the statistical analysis, as shown in Table 1, that the time for partial and total recovery was the least in the third treatment, with a time of (8.13).) minutes, respectively, and the fish of the first treatment took the longest time for partial recovery, 22 minutes, and the total 29 minutes, as shown in Table 1.

Table 1. Effect of Valerian on anesthesia and recovery stages (min.) in common carp.

Concentration (mg/ l)		Partial anesthesia time (min.)	Overall anaesthesia time (min.)	Partial recovery time (min.)	Overall recovery time (min.)
		(Mean ± SD.)			
T1	250	45 ± 1.15 [a]	62 ± 2.48 [a]	22 ± 1.20 [a]	29 ± 1.51 [a]
T2	350	30 ± 1.54 [b]	48 ± 2.16 [b]	18 ± 1.65 [b]	28 ± 2.37 [b]
T3	450	10 ± 2.25 [c]	16 ± 2.15 [c]	8 ± 1.12 [c]	13 ± 1.14 [c]
P*		0.003	0.002	0.003	0.002

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

3.2. Blood Parameter

3.2.1. Red Blood Cells

The results demonstrated a difference in the count of red blood cells in each of the T1, T2, and T3 when compared to the number of cells that were not treated with valerian (control treatment). A rise was seen in the value of red blood cells in all treatments following recovery when compared to the number of red blood cells in fish during the anesthesia period, and it was the highest increase. The value of red blood cells in the T3 treatment at a concentration of 450 mg/L was 0.93 ($x10^{12}$cell/L) during anesthesia and 1.12 ($x10^{12}$cell/L) after recovery. The T1 and T2 treatments had values of red blood cells at anesthesia of 0.84 $x10^{12}$cell/L and 0.72 $x10^{12}$cell/L, respectively, while the value of red blood cells reached for the same two treatments was reached for the same two treatments after recovery (1.1, 1.1) x 10^{12}cell/L, respectively, with the same value and as shown in Figure 1.

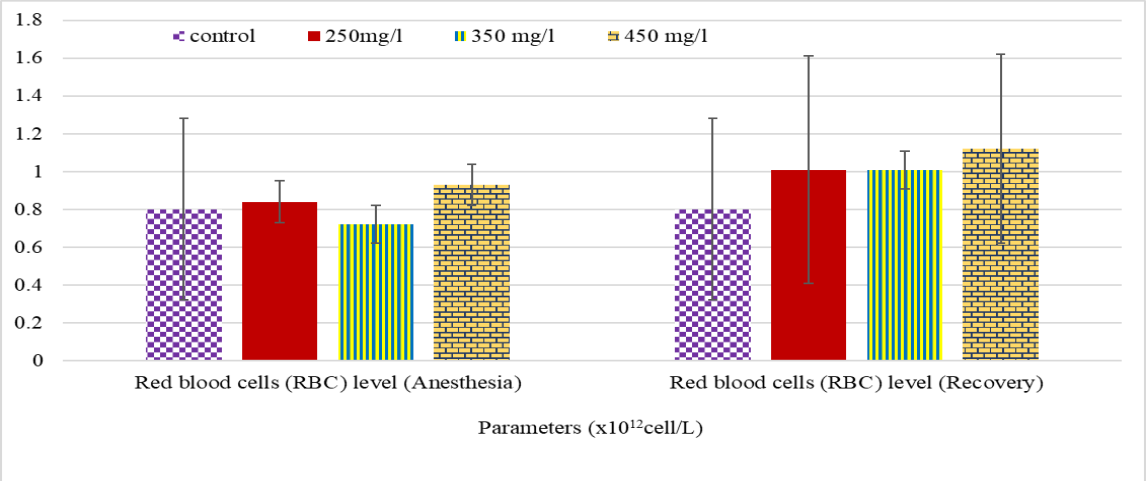

Figure 1. Effect of Valerian on Red blood cells level of common carp in anesthesia and recovery.

3.2.2. White Blood Cells

The study results showed that the lowest value of (WBC) was 12.02 x10^9 cell/L in the control treatment on the other hand there is no significant difference of White blood cells count between them after recovery and during the anesthesia period (T1,T2and T3) Where the number of white blood cells(180,190.5and ,200.2) x10^9 cell/L respectively during the anesthesia and (180,190 and 200) x10^9 cell/L respectively after recovery ($p < 0.05$). Figure 2.

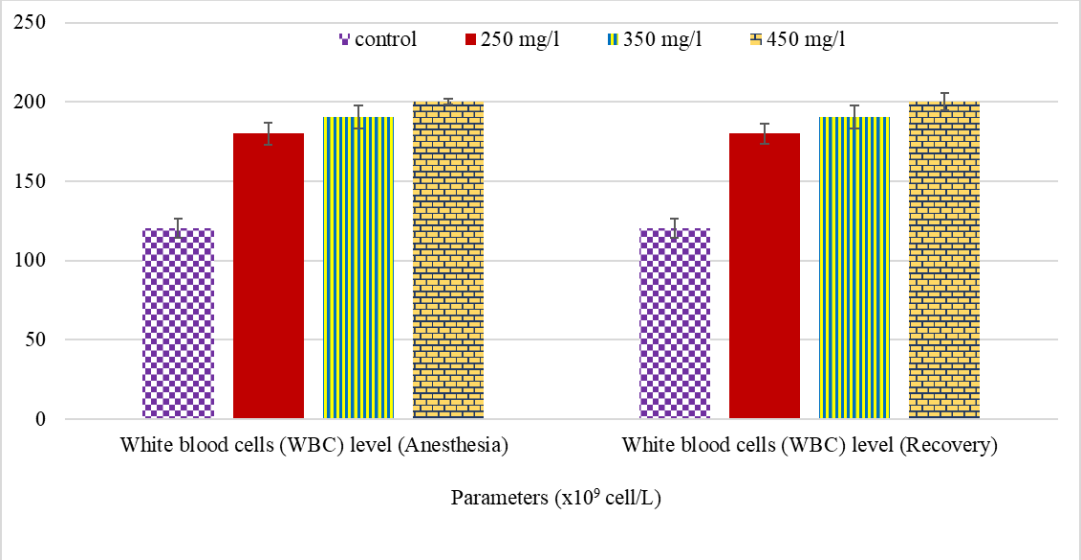

Figure 2. Effect of Valerian on White blood cell level of common carp in anesthesia and recovery.

3.3. Fish Live Enzymes and Blood Serum

Figure three shows that there were no significant differences in blood plasma enzymes between the control and the rest of the treatments (T1, T2, and T3), where the values of creatine kinase (CK) were (41.2, 40.53, 40.56, 41.37) Ul/l for each of the control, T1, T2, and T3 treatments, respectively.

The lactate dehydrogenase values (LDH) were 80.05 Ul/l, 80.41 Ul/l, 82.21 Ul/l, and 83.23 Ul/l for the control treatment and T1 T2 T3. And the value of ALT was (6.3 6.3 6.5 6.7) Ul/l for each of the control, T1, T2, and T3, respectively. Also, the results of the current study showed that there were no significant differences in the AST values, as they were as follows: 44.5 Ul/l for the control, 46.32 Ul/l for T1, 46.45 Ul/l for T2, and 44.62 Ul/l for T3.

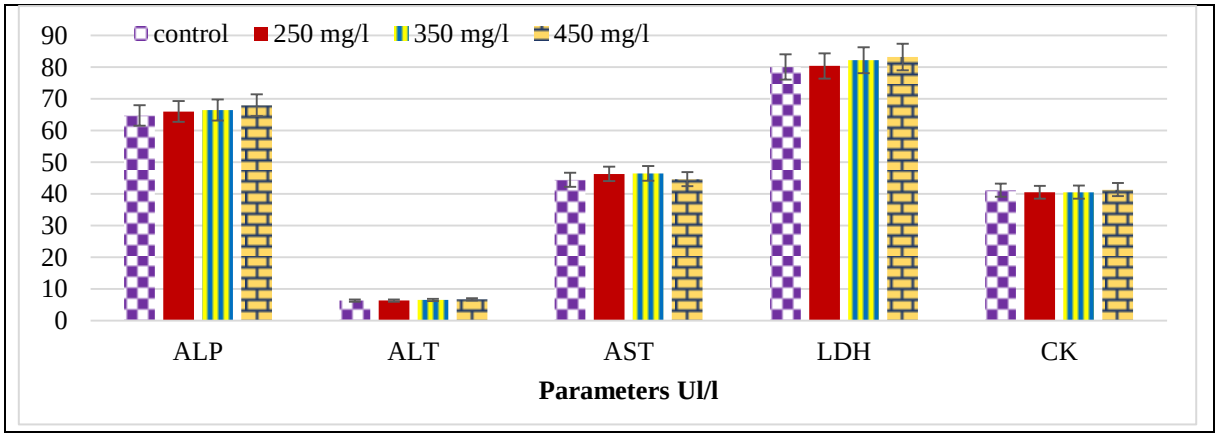

Figure 3. Effect of Valerian on blood serum enzymes level of common carp.

4. Discussion

Anesthesia is commonly used to reduce stress and physical injury in aquaculture activities [22] Different anesthetics have different effects on fish species with different conditions in terms of age, size, sex and environmental conditions [23].In the current study, *Valeriana officinalis* powder was used as an herbal anesthetic for common carp.This study was to determine the optimal concentration for anesthesia and to evaluate the anesthesia time Total and partial, the time required for partial and total recovery, the efficacy of the anesthetic and its safety for fish by measuring some physiological characteristics of common carp fish. Based on previous studies, it has been reported that valerian (*Valeriana officinalis*) has a debilitating effect on the central nervous system and that the clinical symptoms of this extract are attributed to the effects of plant active ingredients on GABA receptor activity [24]according to the results presented in Table 1 It turns out that with increasing concentration the duration of access to partial and total anesthesia decreases, and this may be due to the effect of the active substances in the valerian plant represented by valeric acid and valeric acid, which lead to calming pain and have anti-anxiety and sleep-inducing effects [25]. stated [26] that increasing the concentration of the drug increases the depressant effect on the central nervous system and increases the severity of the excitatory action. This was confirmed by the results of the current study, and in the same context [14] showed that recovery time decreased with increasing concentration in a study of the anesthetic effect of *Valeriana officinalis* on rainbow trout and common carp fry. On the other hand, in a study by [20]on the induction of anesthesia in catfish using valerian extract and comparing it with clove extract. They stated that despite the increase in concentration, the total and partial recovery time was increased, but this increase was not significant, and they stated that this may indicate that the active substances were damaged as a result of the extraction method, and they recommended other extraction methods such as the hydro and alcoholic extract. In current study, it was noted that with increasing the concentration of valerian, the recovery time from anesthesia decreased, and this may be due to the lack of exposure time to valerian [27].

Anesthesia symptoms appeared after 10 minutes and the total anesthesia 16 minutes when the fish were exposed to a concentration of 450 mg /liter, while the recovery period was the longest in T1 (which the fish were exposed to a concentration of 250 mg / liter), where the partial recovery was 22 minutes and the total recovery was 29 minutes. Concerning the relationship between the concentration of valerian and the time of recovery from anesthesia, conflicting results were published, but most of them noted the inverse relationship between the concentration of the anesthetic substance, and this was confirmed by [24]. [20]. Regarding the recovery time with increasing the concentration of valerian in a study conducted on rainbow trout and small common carp, on the other hand, the recovery time decreased in Tambako fish with increasing the concentration of the anesthetic [27] which are similar to the results obtained in the current study with respect to they indicated that this decrease in recovery time may be due to the shorter exposure time, which occurs when exposure to a higher concentration of valerian (due to faster anesthesia at higher concentrations). no significant difference was observed in (RBC) and (WBC) ($p > 0.05$). The blood parameters provide the required information on the

physiological state of the fish [28]. [29] observed that the anesthetic effect of clove powder on *(Rutilus rutilus)* 7 minutes after anesthesia, at a concentration of 175, 225, 275 and 350 mg/L immediately and 24 hours after anesthesia had no effect on blood parameters, and [28] reported the same results when they used different doses of Propofol as an anesthetic agent for goldfish (*Carassius auratus*). While [30] observed anesthesia at concentrations of 200, 300 and 400 mg/L.

The same trend in lower WBC . was reported by [31] who studied the effect of two levels of clove oil concentrations (0.75 and 150 mg/L) on goldfish (*C. auratus*). Significant decrease in WBC in fish subjected to anesthesia and in the same vein reported by [32] that anesthesia with clove extract caused a decrease in white blood cells (WBC) in *Acipenser persicus*.It appears from the results of the experiment that the fish were not exposed to severe stress according to the results of blood enzymes that did not show a significant difference between them before anesthesia and after recovery from anesthesia, and this is what he mentioned [33]When anesthesia results in severe stress, it affects the tissues of the body and causes stress, which in turn leads to a defect in enzyme secretions in the blood such as LDH, CK, AST, and ALT, and this was confirmed by [34]in the results of a study conducted on brown trout showed that in both periods 10 minutes and 24 hours after anesthesia, CK enzyme levels were significantly lower in fish anesthetized with 2-PE compared to the control group. When the fish is exposed to stress, it leads to an imbalance in the blood plasma enzymes. According to [21], the significant decrease in the amount of CK could be the result of a sharp increase in basal metabolism to provide energy for stress resistance and energy compensation. LDH works as an indicator to determine the toxicity of chemicals, so a change in its value confirms the toxicity of the substance to which fish are exposed and the absence of significant differences in its value, as the results of the current experiment showed, perhaps for two reasons. The first is that Valerian is a natural substance, so the fish body does not deal with it psychologically as a toxic substance. Necessary to anesthetize fish when using valerian plant. [35] observed an increase in serum LDH in Chinook salmon exposed to acute doses of MS-222. However, while. [36] Did not observe any significant changes of this enzyme in the blood of rainbow trout treated with clove powder. According to [37], a significant decrease in liver enzymes can lead to an increase in dopamine release. Increased AST activity in plasma is a consequence of mitochondrial breakdown leading to hyperplasia of liver tissue. In addition to the rise when the liver membrane is damaged, the enzyme AST also rises with heart and muscle damage [38]. No significant difference was observed in the value of liver enzymes in the current study and for all concentrations used, which indicates the safety of valerian plant on common carp fish. Moreover, as an advantage, it refers to a natural substance that does not have any side effects on fish and does not present any health risks there are no differences in the effect of anesthetics on serum enzymes in all studied variables with control treatments).

Among the problems caused by the use of clove essential oil, especially in small spaces without ventilation, are headaches, nausea, and fatigue caused by the smell of this substance [39]. Insoluble or low solubility in water [40] is another common drawback of this anesthetic. Concentration, compared to clove powder. According to the criteria mentioned for evaluating a suitable anesthetic in aquaculture, valerian unlike clove extract which is insoluble and floats in water, dissolves easily in water and does not pollute the water during testing, or cause decreased eye vision in fish It does not settle on the gills of fish, which causes breathing disorders, etc.

Conclusions

Given the results obtained in this study, and given the great performance of the valerian plant, it is recommended to use it in aquatic anesthesia. It is worth noting that more studies are needed to determine the appropriate concentration and evaluate the effects of the extract on Physiology and biological activities in other species and sizes of fish.

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