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PHYSIOLOGICAL IMPACTS OF USING CLOVE POWDER AND OIL AS FISH ANESTHETIC ON YOUNG COMMON CARP (*Cyprinus carpio* L.)

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Key words: Anesthesia, Common carp, Clove powder, Clove oil

ABSTRACT

The aim of the present study is to assess the physiological impact of clove powder and oil as anesthetics on young common carp (averaged 60 g in weight). Three concentrations of clove powder (200, 300 and 400 mg/L) and clove oil (1, 1.5 and 2 ml/L) were used with three replicates of 3 fish in 30 L glass aquaria each. Time of anesthesia and recovery in addition to physiological indices (ventilation rate, plasma sugar and cortisol levels, RBC and WBC count) were monitored. Carp exposed to doses of clove powder showed clear adverse relation between induction time and concentration of the anesthetic, (452 sec. in 200 mg/ L and 137sec. in 400 mg/L). Recovery time was negatively correlated to induction time and was directly proportional with increasing doses of clove powder (290 sec. at 400mg/L and 199 sec. at 200mg/L). The ventilation rate increased significantly in all stages of anesthesia and recovery compared to control (17.5 /15 sec.). RBC decreased at higher concentrations to 0.78 and 0.34 $x10^{12}$ cells/L compared to control ($0.93x10^{12}$ cell/L) during anesthesia and recovery stages. WBC count declined in both anesthesia and recovery stages, coinciding with the elevation of sugar and cortisol which act as an immunosuppressive. Fish exposed to 1 and 1.5 and 2 ml/L of clove oil took 275, 208 and 93 sec. respectively, to enter complete anesthesia. The longest time to the full recovery (239.25 sec.) was seen at high concentration and decreased to 229 sec. in light concentration. There was a clear negative correlation between anesthesia and recovery times. RBCs count decreased significantly to ($0.88x10^{12}$cells/L) at1.5 ml/L. It increased during recovery compared to control ($0.92x10^{12}$cells/L). WBC count in anesthetized fishes with 1, 1.5 and 2 ml/L show significant increase to (218, 198 and $232x10^{9}$cells/L) respectively, when compared to control group

(128x10^{9}cells/L). They increased to (191, 162 and 207 x10^9 cells/L) during recovery. Significant increase in the concentrations of cortisol was seen during anesthesia and recovery compared with control. No increase in sugar level was detected during anesthesia with only slight increase during recovery. Results were discussed in terms of physiological status of fish during sedation and recovery for both materials.

INTRODUCTION

Practices that require fish handling are a common source of stress in aquaculture operation, research activities and monitoring projects (21), to solve this problem, fish handlers have employed the use of anesthetics, added to water, to immobilize fish, reduce stress levels, and prevent mortality. Anesthetic stages in fish usually depend on the dose and the length of exposure (9). It can be detected via changes in swimming activity, balance, respiratory frequency as well as reactions to external stimuli (42).

Over the years, a large number of chemicals and agents have been applied to fish with a high safety level such as MS-222, benzocaine, Isoeugenol, methomidate, 2-phenoxyethanol and quinaldine (10). Nowadays, there are strict controls on chemicals used in aquaculture, particularly with regard to their safety, environmental impact and health risk and efficacy.. This inadequacy of chemical anesthetics has resulted in a renewed interest to develop green (plant-derived) chemical anesthetics with low environmental impact and health risk (Ramanayaka and Atapatu, 2006). Cloves are the unopened flower buds of the evergreen tree (*Eugenia caryophyllus*, Family: Myrtaceae). Its main active ingredients include eugenol (76.8-88.58%), eugenyl acetate (1.2-5.62%) and β-caryophyllene (1.39-17.4%) (6). Clove oil is considered an appropriate anesthetic for fish because of its low costs, simple obtaining, and considerable anesthetic efficiency without any toxic effect (42; 27). On *C. carpio* (35) studied the anaesthetizing effect of clove oil, with the purpose of investigating the toxic effect. According to (8), a suite of research has been conducted that characterizes the dose responses to clove oil for a variety of cultured fishes.

There are little studies about fish anesthesia in Iraq, The study of (3) used clove powder as a new anesthetic for common carp. Fry, fingerling and mature fish undergone different concentrations of the anesthetic ranged 120-230 mg/L. Three other experiments

were conducted by (4) to study some properties of clove powder as an anesthetic for two fish species. The aim of the present study is to assess the physiological impact of clove oil on young common carp.

MATERIALS AND METHODS

The experiment was conducted on young common carp (*C. carpio* L.) weighing 60g on average. They were brought from a local hatchery and stocked in glass aquaria (100cm x 50cm x 60 cm) containing approximately 150 liters of water. Experimental fish were acclimated for a minimum of two weeks prior to the outset of experiments, ensuring that they had sufficiently recovered from possible capture-related or transport stress. During acclimation period, fish were fed with commercial pellets. Tanks were cleaned by siphoning the feces and non-consumed feed. Fish used in the experiment were unfed for 24 hours prior to the experimental trial as suggested by (5). Three concentrations of clove powder are: 200, 300 and 400 mg/L and for clove oil are: 1, 1.5 and 2 ml /L.

Each anesthetic bath was individually prepared in 30-L glass tank in advance. The anesthesia-induction stages suggested by (23) and stages of anesthesia and recovery by (19) were adopted in this experiment: Stage I anesthesia: (partial loss of equilibrium, some body movements and reduced reaction to external stimuli). Stage II anesthesia: (total loss of equilibrium, no body movements, no reaction to external stimuli). Stage III anesthesia: As in stage II with decrease in opercular rate. Recovery time: (Recovery of equilibrium, body movements and response to external stimuli).

Induction and recovery times were measured with a video recorder to the nearest second. Following the induction into stage III each fish was weighed and transferred to a recovery aquaria that had been filled with aerated freshwater at the same time of preparation the anesthetic baths. In the recovery tub the fish were monitored continuously to determine time to full equilibrium. Recovered fish were promptly returned to their holding system and monitored for survival at 24 and 72 hours post-experiment (19). Fish were held for a period of two months following the experiments to assess long-term mortality. The studied Characteristics are:

1. Times of anesthesia and recovery according to anesthesia and recovery stages

2. Ventilation which was calculated by opercular movement per 15 sec.

3. Blood plasma components (sugar and cortisol hormone).

4. Blood Indices (Red Blood Cell RBC) and (White Blood Cell WBC).

The blood samples from each fish of the different groups were collected by suction of the caudal peduncle. Whole blood samples were collected in heparinized vials for determination of RBC & WBC counts using hematological analyzer BC-2800 Blood plasma was then separated by centrifugation for 3-5 minutes at 4500g and used for measuring blood sugar and cortisol hormone by another hematological analyzing device (ACCENT 200 and COBAS C 111).

Water temperature and water quality parameters in all aquaria were monitored during experimentation using digital meters and kits. The following values were recorded:

Water Temperature (°C)	28.0 ±1
Dissolved Oxygen (mg/L)	6.5 ±1
Hardness (ppm)	330 ±10
pH	7.2 ±0.5
TDS (ppm)	230 ±10

RESULTS AND DISCUSSION

1. Clove Powder

Time of anesthesia and recovery

Table (1) shows the induction time of young *C. carpio* which decreased with increasing concentrations of clove powder. At 400 mg/L, the time to reach a complete anesthesia (stage III) was 137.11 sec. which is significantly different ($P<0.05$) from the other dosages (200 and 300 mg/L). There was a clear adverse relation between induction time and concentration of the anesthetic, with the longest induction times (452.85 sec.) for fish in the group exposed to the lowest dose of clove powder (200 mg/ L) and the shortest (137.11 sec.) for fish exposed to 400 mg/L. Also, recovery time was negatively correlated to induction time as observed in Table (1) as well. When using anesthetics, it is expected that there will be a strong negative correlation between the applied concentration and the time required to induce anesthesia to the desired stage, as observed previously for several fish species (38 , 25, 22).

The recovery time was directly proportional with increasing doses of clove powder. The longest recovery time (290 sec.) was observed at 400mg/L and the shortest time to reach total recovery stage (199 sec.) was detected at 200mg/L as shown in Table (1). Long exposure to anesthetic led to more anesthetic absorption by fish which, in turn, lengthened the recovery time. On the other hand, (39) pointed out that compared with anesthesia duration, anesthetic concentration plays more important role on the recovery time. It is believed that anesthetic, is taken up by the fish through a concentration gradient at the gill interface. Therefore, when equilibrium level established between the gill and anesthetic solution, no further anesthetic will taken up by the fish, and during recovery, the anesthetic agent is leaked through such gradient. The ideal concentration must be the lowest dose concentration which enables a transition to general anesthesia in 3 min (180 s) and a full recovery in 10 min (600 s) (27) as seen in the effective dosage (300 mg/L) that induces anesthesia in *C. carpio* in this study.

Table (1): Effect of clove powder on anesthesia and recovery time (sec.) at three stages in common carp (Mean ± SD)

	Anesthesia Time (sec.)			Recovery Time (sec.)		
	I	II	III	I	II	III
Stages Conc.						
200 mg/L	71±0.1a	399±0.1a	452±0.1a	37±0.4b	99±0.4a	199±0.1b
300 mg/L	61±0.4ab	129±0.5b	186±0.3b	57±0.4ab	108±0.3a	255±0.2a
400 mg/L	49±0.2c	96±0.3c	137±0.2c	80±0.4a	111±0.2a	290±0.2a

Ventilation Rate

Table (2): Effect of clove powder on ventilation rate of common carp (Operculum movement /15 sec.) at three stages of anesthesia and recovery (Mean ± SD)

Conc.	Anesthesia stages			Recovery stages		
Stages	I	II	III	I	II	III
Contrl	17.5 ±0.1b	17.5 ±0.1b	17.5 ±0.1b	17.5 ±0.1c	17.5 ±0.1c	17.5 ±0.1b
200	28 ±0.06a	20 ± 0.09b	19.28±0.05b	22.85 ±0.16b	22.57 ±0.12b	21.57 ±0.1a
300	26.66 ±0.1a	23.33 ±0.1a	22.55±0.11a	27.62 ±0.08a	26.62 ±0.08a	23.75 ±0.13a
400	28.22 ±0.04a	24.33 ±0.1a	23.33±0.06a	27.88 ±0.05a	27.11 ±0.06a	23 ± 0.06a

The ventilation rate of common carp at the different stages of anesthesia exposed to clove powder was presented in Table (2), as the concentrations of clove powder increased the ventilation rate in stage I, stage II and stage III control (17.5 /15 sec.), and this agrees with results of study done by (12). It may be due to the rapid change in the environment of the fish as reported by (4).

Hematological parameters

Data of hematological parameters are shown in Table (3). There was an increase in RBC count to ($1.21x10^{12}$cells/L) compared to control group (0.93×10^{12}cells/L) when fish inducted to of 200 mg/L of clove powder, but in other two doses, RBC level decreased significantly ($P<0.05$) compared with control group. During recovery, however, no significant different was seen in RBC level between all dosages and control group. (31) observed that the anesthetic effect of clove powder on roach (*Rutilus rutilus*) after 7 min anesthesia, at the concentration of 175, 225, 275 and 350 mg/L immediately and 24 h post anesthesia had no effect on hematological parameters, and (13) had same results when they used different dosages of Propofol as anesthetic agent for Gold fish (*Carassius auratus*). In a study by (2) significant variations were observed at higher concentrations (150 and 200 mg/l) of the clove extracts. The alterations in blood variables may be due to cytotoxicity of clove extracts at higher concentrations. These caused a significant decrease in RBC of the fish at higher concentrations of the extracts. This reduction may be attributed to haemolysis which results in haemodilution.

From the immunological point of view, WBC count was measured to evaluate the effect of clove powder on carp immune system. It showed a decline in both anesthesia and recovery stages. This may coincide with the elevation of cortisol which act as an immunosuppressive, so it could suppress humoral factors and lead to declining the circulating WBC along with elevating cortisol as stated by (11). This is in agreement with present results in which the lowest WBC count (101.7×10^{9}cells/L) and the highest level of cortisol (1750 nmol/L) were seen in fish exposed to 300 mg/L of clove powder (Table 3) and (Table 4).

Table (3): Effect of clove powder on red blood cells and white blood cells level of common carp anesthesia and recovery (Mean ± SD)

	Red blood cells (RBC) (x 10^{12} cells/L)		White blood cells (WBC) (x10^{9} cells/L)	
	Anesthesia	Recovery	Anesthesia	Recovery
Con.				
Control	0.93 ± 0.01b	0.93 ± 0.3a	128.0 ± 1.4a	128.0 ± 1.5a
200	1.21 ± 0.4a	0.61 ± 0.02a	119.8 ± 0.55c	99.7 ± 1.18d
300	0.78 ± 0.1c	1.00 ± 1.01a	101.7 ± 0.43d	125.5 ± 1.5c
400	0.34 ± 0.3d	0.64 ± 0.2a	120.7 ± 0.9b	128.3 ± 1.4a

Plasma components

The cortisol hormone and sugar levels measured in the blood of the fish are presented in Table (4). All the anaesthetized groups at all the stages of anesthesia and recovery had significantly increased the concentrations of cortisol and sugar levels compared with those of the control group ($P<0.05$). Cortisol and sugar levels show the highest level (1750 nmol/L and 6.9 mmol/L), respectively, in 300 mg/L concentration of clove powder compare to other two dosages 200, 400 mg/L and control group in anesthesia. In recovery the cortisol and sugar show the highest level (1484 nmol/L and 7.1 mmol/L), respectively, in 400 mg/L concentration of clove powder compare to other two dosages 200, 300 mg/L and control group. During anesthesia and recovery, plasma cortisol was increased compared with the control levels, and clove powder anesthesia seemed to be an additional stressor for the fish.

Plasma cortisol and sugar are physiological indicators of stress in fishes and their interactive effects on metabolism during recovery from stress have recently become a subject of more intense study (36,38). Earlier studies have shown that anesthetics could be a potential stressor aggravating plasma cortisol levels (34, 42). Present findings agree with those of (16) who also detected an increase in sugar concentration in rainbow trout (*O. mykiss*) following clove oil anesthesia. Rapid increases in plasma sugar are mediated

by the release of catecholamines, which increase (presumably in response to the hypoxia caused by cessation of respiration) in the plasma of anaesthetized fish (36). Differences in sugar and cortisol levels recorded in this study between different doses (300 and 400 mg/L) may indicate that the response of plasma sugar and cortisol to anesthetics depends on other factors apart from the kind of anesthetic used as suggested by ((24).

Table (4): Effect of clove powder on some plasma components in common carp during anesthesia and recovery (Mean ± SD)

Con.	Cortisol (nmol/L)		Sugar (mmol/L)	
	Anesthesia	Recovery	Anesthesia	Recovery
Control	20.1 ± 5.1d	20.1 ± 5.1d	4.9 ± 0.2b	4.9 ± 0.2c
200 mg/L	319.3 ± 7c	274 ± 6.4c	6.3 ± 0.16a	7 ± 0.14a
300 mg/L	1750 ± 6.6a	707 ± 6.1b	6.9 ± 0.14a	6.9 ± 0.7b
400 mg/L	558.4 ± 7.1b	1484 ± 6.7a	4.6 ± 0.22b	7.1 ± 0.17a

Clove oil

Time of anesthesia and recovery

Table (5) shows mean induction and recovery times of common carp subjected to three different doses of clove oil. The time to reach stage III anesthesia was reduced to 275.5 and 208 sec. with increasing clove oil concentration to 1 and 1.5 ml/L of clove oil respectively. Whereas fish exposed to 2 ml/L of clove oil took 93.16 sec. to enter the same stage of anesthesia. The induction time significantly ($P<0.05$) decreased with increasing concentration of clove oil. This agrees with the findings of, (27) and (22) who examined efficacy of clove oil on common carp. Ideal clove oil concentrations ranged from 10-50 mg/L for a wide variety of fish species. On the other hand, species like the eel (*A. reinhardtii*) (37) and tambaqui, (*Colossoma macropomum*) (28) require higher concentrations, which vary between 65 and 80 mg/L for the induction of surgical anesthesia. These differences could be due to the different biological and environmental factors along with the level of active ingredient of clove oil.

The longest time to the total recovery (239.25 sec.) was observed in fish exposed to 2ml/L of clove oil. The shortest time (228.75 sec.) was detected at the dose of 1.5 ml/L. Differences in total recovery time between groups were not significant (*P> 0.05*). These results agree with previous studies (15, 41), who found no significant correlation between clove oil concentration and recovery time in common carp. No direct relationship between clove oil concentration and recovery times in adult sockeye salmon (*Oncorhynchus nerka*) has been found by (40). The results of this study suggest that the clove oil could be used as an appropriate anesthetic drug for common carp and it could be recommended as a suitable alternative for chemical anesthetics. The most applicable dose for deep anesthesia of carp is 2 ml/L for induction and recovery and it is recommended as the most appropriate amounts for an effective sedative and anesthetic agent in economic terms for aquaculture activities, such as handling, catching with net, transporting to another tank.

Table (5): Effect of clove oil on anesthesia and recovery time (sec.) in common carp during three stages of anesthesia and recovery (Mean ± SD)

	Anesthesia stages Time (sec.)			Recovery stages Time (sec.)		
Stages Con.	I	II	III	I	II	III
1	63.5±028a	112.5±0.21a	275.5±0.1a	119.5±0.34a	198.75±0.13a	229±0.11a
1.5	54.16±0.64a	127.5±0.38a	208±0.34a	124.25±0.15a	188±0.25a	228.75±0.13a
2	44.16±0.22b	60.33±0.08b	93.16±0.1b	130.25±0.21a	239.25±0.16a	239.25±0.16a

Ventilation rate

The ventilation rate at the different stages of anesthesia of *C. carpio* exposed to clove oil is presented in Table (6). As the concentrations of clove oil increased the ventilation rate at light sedation increased, then in deep sedation and total loss of reflex and equilibrium decreased, also in first stage of recovery ventilation rate was increased

while in other two stages of recovery decreased. All were significantly different (*P<0.05*) compared to the control (18 oper. mov./15sec.). These decreasing in ventilation rates are agree with results of (19), who suggested pattern of ventilation rate in fish during anesthesia clearly in his study. In present study there is no significant different in ventilation rate during recovery stages between different dosages of anesthetic agent this results are agree with results of previous study done by (12).

Table (6): Effect of clove oil on ventilation rate of common carp (Operculum movement/15 sec.) in three stages of anesthesia and recovery (Mean ± SD)

	Anesthesia stages Operculum movement /15 sec.			Recovery stages Operculum movement /15 sec.		
Stages Con.	I	II	III	I	II	III
Control	18±0.08$_c$	18±0.08$_c$	18±0.08$_c$	18±0.08$_c$	18±0.08$_b$	18±0.08$_b$
1	30.8±0.06$_a$	26.3±0.04$_a$	24.8±0.05$_a$	24.2±0.1$_b$	25±0.06$_a$	24.7±0.02$_a$
1.5	27.1±0.13$_b$	23.5±0.05$_b$	22.6±0.04$_b$	24.5±0.03$_{ab}$	23.2±0.02$_a$	23.5±0.02$_a$
2	28.5±0.11$_{ab}$	24.6±0.05$_b$	23.6±0.06$_{ab}$	28±0.1$_a$	26±0.11$_a$	24±0.03$_a$

Hematological parameters

Effects of clove oil on the hematological RBC and WBC count of common carp are shown in Table (7). RBCs level was decreased significantly (0.88 x10^{12}cells/L) when fish inducted to concentration of 1.5 ml/L of clove oil compared to other concentrations and control groups. In recovery all three concentrations show increase in RBCs level compared to control group (0.92 x10^{12} cells/L). These findings agree with study of (1). 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 (32). Anesthetic treated fishes with concentrations of 1, 1.5 and 2 ml/L show significant increase in WBC level (218, 198 and 232 x 10^9 cells/L), respectively, when compared to control group (128 x 10^9 cells/L) as observed in Table (7). In recovery WBC

were increased to (191, 162 and 207 x 10^9 cells/L) for the three doses respectively, compared to control group. These results are in agreement with those of (33) in which the effects of clove oil and 2-phenoxyethanol on cultured rainbow trout (*O. 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 (29). No changes in leucocytes count followed anaesthesia in common carp, rainbow trout, European catfish and Siberian sturgeon (34, 35 and 14).

Table (7): Effect of clove oil on Red blood cells and White blood cells level of common carp in anesthesia and recovery (Mean ± SD)

Parameters	Red blood cells (RBC) level(x10^{12}cell/L)		White blood cells (WBC) level(x10^9cell/L))	
	Anesthesia	Recovery	Anesthesia	Recovery
Con.				
Control	0.92 ± 0.39a	0.92 ± 0.39c	128.7 ± 9d	128.7 ± 9d
1 ml/L	0.95 ± 0.11a	1.02 ± 1.6b	218.1 ± 7.1b	191.5 ± 8b
1.5 ml/L	0.88 ± 0.1b	1.02 ± 0.1b	198.7 ± 8.4c	162.3 ± 6.35c
2 ml/L	0.93 ± 0.11a	1.12 ± 0.9a	232.4 ± 7a	207.3 ± 4.05a

Plasma components

Effects of clove oil on the blood plasma biochemical profile of common carp are given in Table (8). Fish exposure to clove oil at a different concentration caused significant ($P<0.05$) increase in the concentrations of cortisol in anesthesia and recovery compared with control groups (19.7 nmol/L). The highest level of cortisol in anesthesia and recovery (1543, 1223 nmol/L), respectively, was show when fish exposure to (1.5 ml/L) of clove oil.

Table.(8): Effect of clove oil on some plasma component of common carp in anesthesia and recovery (Mean ± SD)

Parameters	Cortisol (nmol/L)		Sugar (mmol/L)	
Con.	Anesthesia	Recovery	Anesthesia	Recovery
Control	19.7 ± 3.1d	19.7 ± 3.1d	4.63 ± 0.11a	4.63 ± 0.11c
1 ml/L	942.6 ± 7.4c	688.6 ± 11.7b	4.4 ± 0.23a	6.2 ± 0.16a
1.5 ml/L	1543 ± 6.6a	1223 ± 12.3a	4.8 ± 0.21a	5.6 ± 0.18b
2 ml/L	945 ± 6.7b	551 ± 10.6c	4.1 ± 0.27a	4.4 ± 0.23c

During anesthesia stage, all concentrations of clove oil have no significant ($P>0.05$) effect on plasma sugar level of carp compared to control group (4.63 mmol/L). During recovery the sugar level was increased significantly ($P<0.05$) compared to control group except in dose 2 ml/L. Our results are controversial to those reported by (20) and (30) who have reported an increase in plasma sugar in recovered fish giving an indication of some stress in the experimental fish In other studies, clove oil did not induce changes in plasma sugar as seen in trout and goldfish (7, 36), which is fail in agreement with the results of the present investigation. No change in the concentration of blood plasma sugar was found by (18) in Atlantic salmon (*S. salar*) following clove oil anesthesia.

Data of Table (8) shows an increase in plasma cortisol level in carp subjected to doses of clove oil both during anesthesia and recovery stages. In accordance with present results, (16) have detected the increase of cortisol level in rainbow trout (*O. mykiss*) following clove oil anesthesia. The report of (32) proved that clove oil does not block the cortisol response to stress; as happens with other anesthetics. Clove oil was found to limit the activity of cortisol, but not completely block its effect in *Brycon cephalus* (17). Although the mechanism is not well known, (18) suggested that clove oil blocks transmission of impulses to the Hypothalamus-Pituitary Interregnal axis (HPI). Clove oil

induced a stress response in the carp even during recovery, this was indicated by the increase in plasma sugar and cortisol concentration. There was a correlated rise of plasma cortisol and blood sugar. This well-known pattern of hyperglycemia after stress has been shown to result from catecholamine and corticosteroids released into the blood and have been reported in other research (24).

الآثار الفسلجية لاستخدام مسحوق وزيت براعم القرنفل في تخدير صغار أسماك الكارب الشائع *Cyprinus carpio* L.

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الخلاصه

تهدف الدراسة الحالية لتقدير الآثار الفسلجية لمسحوق وزيت براعم القرنفل كمادة مخدرة على صغار أسماك الكارب (بوزن 60 غم كمعدل). استخدم المسحوق بتراكيز 200 و 300 و 400 ملغم/ لتر والزيت بتراكيز 1 و 1.5 و 2 مل /لتر وبثلاث مكررات لكل معاملة تحوي الواحدة منها على 3 أسماك في حوض زجاجي سعة 30 لتر. تم قياس وقت التخدير ووقت استعادة الوعي فضلا عن المؤشرات الفسلجية مثل معدل التهوية ومستوى السكر والكورتيزول في البلازما وعدد كريات الدم الحمراء والخلايا البيضاء.

أظهرت أسماك الكارب المعرضة لمسحوق القرنفل علاقة عكسية بين وقت التخدير وتركيز المخدر 452 ثانية في 200 ملغم/ لتر و137 ثانية في 400 ملغم/ لتر. أما وقت الاستعادة فقد تناسب عكسيا مع وقت التخدير وكان مرتبط طرديا مع تركيز المخدر(290 ثانية في 400 ملغم/ لتر و 199 ثانية في 200 ملغم/ لتر). ازداد معدل التهوية بشكل معنوي في كل مراحل التخدير والاستعادة مقارنة بمعاملة السيطرة. عدد خلايا الدم الحمراء قلت عند التعرض الى التراكيز العلياووصلت الى(0.78 و 0.34 x 10 ^{12} خلية/لتر) مقارنة بمعاملة السيطرة (0.93 x 10 ^{12} خلية/لتر) خلال التخدير والاستعادة. كذلك قلت مستويات خلايا الدم البيضاء مترافقة مع زيادة في مستويات السكر والكورتيزول في البلازما حيث يعمل الكورتيزول كمثبط مناعي.

استغرقت الأسماك التي تعرضت لتراكيز 1 و 1.5 و 2 مل/لترمن زيت القرنفل لمدة 275 و 208 و 93 ثانية للوصول الى حالة التخدير الكامل على التوالي. أما وقت الأستعادة الأطول (239 ثانية) فقد ترافق مع التركيز العالي وانخفض مع التركيز الأوطأ, كما ارتبط وقت الأستعادة عكسيا مع وقت التخدير. انخفض عدد كريات الدم الحمراء معنويا الى 0.88 x 10 ^{12} خلية / لتر عند التعرض الى 1.5 مل/لتر بينما زادت في مرحلة الاستعادة مقارنة بمعاملة السيطرة (0.92 x 10 ^{12}خلية/لتر.). عدد خلايا الدم البيضاء ازداد في الأسماك المخدرة بالتراكيز 1 و 1.5 و 2 مل /

لتر الى (218 و 198 و 232 x 10 ^{9} خلية /لتر على التوالي بالمقارنة مع معاملة السيطرة (128 x 10 ^{9} خلية / لتر). وازدادت الى (191 و 162 و 207 x 10 ^{9} خلية / لتر) في مرحلة الأستعادة. كما لوحظت زيادة معنوية في مستوى الكورتيزول في البلازما خلال مراحل التخدير والاستعادة بزيت القرنفل مقارنة بمعاملة السيطرة. لم تلاحظ زيادة في مستوى سكر البلازما خلال مرحلة التخدير بالزيت بينما شهدت مرحلة الاستعادة زيادة طفيفة. نوقشت نتائج الدراسة على أساس الحالة الفسلجية للسمكة خلال مراحل التخدير واستعادة الوعي عند تعرضها لهاتين المادتين.

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