



## Biochemical and behavioral responses in carp fish exposed to tricaine methane sulfonate (MS-222) as anesthetic drug under transport conditions

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### Abstract

This study aims to determine the effects of Tricaine methane-sulfonate (MS-222) at concentration 150mg/l for one hour as an anesthetic agent to reduce the stress conditions during transfer the fish. Forty fish (*Cyprinus carpio*) were divide in to four groups: the 1<sup>st</sup> is control group (fish without both transfer and anesthesia), the 2<sup>nd</sup> group (fish transfer without anesthesia), the 3<sup>rd</sup> group (fish anesthesia without transfer) and the 4<sup>th</sup> group (fish transfer with anesthesia). The induction time reached to 3 minutes while the recovery time take at lasted for 7 to 10 minutes. Furthermore, the cortisol was significantly decreased in fish serum in both anesthetized groups that were transferred or that were left in the pond without transmission in compare to control groups (both transfer and without transfer fish). The glucose level rose significantly ( $P < 0.05$ ) in the serum of fish in both 3<sup>rd</sup> and 4<sup>th</sup> groups in compare to control groups. There was no difference in hematological parameters (hemoglobin and packed cell volume), blood sodium ion concentration, and enzyme activity represented by alanine amino transferase in all groups while level of creatinine phosphate was decrease significantly in both groups 2<sup>nd</sup> and 3<sup>rd</sup> in compare to 1<sup>st</sup> group. It can be concluded that tricaine methane-sulfonate stimulates the recovery and shortens the time of induction and reduces the stress condition caused by fish transport.

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### Introduction

Fish in wild and captive environments are exposed to stressful conditions, which are defined as groups of various factors that affect fish and are unable to maintain normal physiological status (1). Stress in fish can be caused by biological causes such as microorganisms or their toxins, changes in the physicochemical properties of the water environment, and physical causes such as fishing, overgrowth, and nets, which arise the stress during fish transport (2-4). Many stress factors are associated with the transport of fish like poor water quality, reservoir identification, and handling. Stress responses can be primary responses to blood release hormones and the circulatory system that lead to secondary responses, including gill blood

flow (ionic osmotic disorders), metabolic rate and heart rate (5). Various methods have been used to reduce stress and mortality during transportation by pumping air, adding ice and liquefied oxygen, non-toxic salt, or a low concentration of calcium chloride (6). Anesthesia is an essential method in aquaculture that is used to relieve stress during the handling and transportation of fish. This anesthetic drug is characterized by rapid induction and recovery; it is also not toxic for fish or human's consumption (7,8). Tricaine methane-sulfonate (MS-222) is a benzocaine derivative, a white crystalline powder that dissolves in water. It can be in the form of a powder or a liquid; it is unstable under direct light, and it should be stored in dark solutions. It is absorbed through the gills and skin and then biologically converted into the liver, kidneys, and gills and its metabolites were

excreted in the urine and bile, it take 21 day for withdrawal from fish body (9,10). The MS-222 concentration ranges from 15 to 330 mg/l. many factors affect the difference and the effectiveness of MS-222. The most important factor is water temperature, a higher temperatures mean higher efficiency (with regards to induction and recovery time) for MS-222 (11). Moreover, small fish are more sensitive to MS-222 than larger fish, in general it may have related to high metabolic activity than large fish. A lower concentration is also more effective in anesthesia (12). The physiological state of the fish as maturity, age, sex and fish species play a role in the effectiveness of MS-222 (13). Moreover, the anesthetic concentration varies depending on the the alkalinity of water, pH, hardness, and seasonality (14). For instance, some studies show the effects of MS-222 stress by increasing cortisol release in fish and leukopenia; there may be an increase in the concentration of hemoglobin and abnormal erythrocytes in *Cyprinus carpio* (15-17).

The present study aims to determine the time of induction and recovery in anesthetized fish using MS-222 to assess changes in hormonal, biochemical, and hematological factors with ion concentration in fish serum.

## Materials and methods

Fish (*Cyprinus carpio*) (18) were collected from one of the commercial fishponds. The average weight of fish was  $125 \pm 10$  g. The fish were kept in a cement tank to ensure regular freshwater supply, and they were fed with

commercial pellets for seven days. The fish were starved for 24 hours before the experiment and placed in the aquarium two hours before the experiment.

Forty fish were divided into four groups, each group included ten fish that were placed in a glass aquarium with 40\*40\*30 cm dimensions. The first group was considered a control group fish left in the water without any treatment. The second group underwent a one-hour transfer and was considered a positive control group, while the third group received MS-222 150 mg/l (19) for one hour and was left in the aquarium. The fourth group received MS-222 and was subjected to a one-hour transfer. The induction time and anesthesia stage were determined according to behavioral responses such as respiratory rate, abnormal swimming, and fish interaction with muscle tension and swing depended in table 1 (20). Fish were transported to freshwater without MS-222 to determine the healing time.

A blood sample was drawn from a caudal vein from the fish in the four groups one hour after treatment and transport for biochemical tests (Cortisol, Glucose, Alanine aminotransferase ALT, Creatinine phosphate and sodium ion concentration) usage spectrophotometers method and blood study (Hb concentration and PCV).

## Statistical Analysis

CRD-based design experiment and Duncan test were used to determine the relevance among treatments, SAS statistical programs, 2002.

Table 1: Stage of anesthesia in *Cyprinus carpio* exposure to MS-222 150 mg/l for one hour

Stage	Description	Behavior Signs
A0	Normal	Active swimming, normal equilibrium, normal muscle tone to external stimuli
A1	Light sedation	Reduced swimming, slight loss reactivity to visual and tactile stimuli and reduced respiratory rate
A2	Light narcosis	Slight loss in equilibrium
A3	Deep narcosis	Loss in equilibrium decreased muscle tone

## Results

The results showed that using tricaine methane-sulfonate for fish 150 mg/liter as anesthetic dosage for one hour at a low respiratory rate of the fish resulted in a reduction in the frequency of opening the gills in both anesthetized groups of fish that underwent and did not undergo transportation. The study calculated the anesthesia time, which was 1 to 7 minutes and started at zero since the fish were swimming well and had normal muscle tension. The fish showed a low breathing frequency with a lack of response to external influences and slow swimming with loss of balance, muscle relaxation, and tail swaying with stability on the bottom after one minute of the starting drug effect (Table 1).

At the end of the test period, the fish were transferred to anesthetic-free water to calculate the wake-up time, which was 7 to 10 minutes, since after one minute the fish showed a slow movement of the gill lids, and after two minutes the

fish started to swim slowly. It took some time to return to swim at a normal rate. A roll-up time of 3 to 5 minutes was observed. The fish swim normally and balanced after seven to ten minutes as shown in (Table 2). The serum cortisol concentration level in fish was significantly clear in both groups treated with the drug with transport and without transport (498.88 and 547.60) mg/ml compare to the control group. The results of the present study refer to the first clinical signs. There was a decrease in respiratory rate (opercula frequency OF) in both anesthetized groups the transported and non-transported. The MS-222 with 150 mg/l took 1-7 min to get in the system of the fish and exhibit variable character for anesthetic stages beginning by normal behavior to the surgical stage. The MS-222 took the fish about 7 minutes to lose sensation and sink to the bottom of the aquarium (Table 2). The time of the experiment was one hour, so after these time fish were transported to freshwater and the time and stages of fish recovery were determined.

The time begins with R1 characterize by return slow movement of opercula at 1 min and in R2 slow swimming at 2min, partial recovery of equilibrium at R3 at 3-5 min until fish appear to be normal swimming at 7-10 min (Table 2). The study results showed that there is a significant decline in serum cortisol concentration in both groups of anesthetized fish with and without transportation. The cortisol level reached to 498.88mg/ml and 547.60 mg/ml in 1-hour compare to both control groups. The serum glucose levels in anesthetized fish that were transferred reached to 211.07 mg/ml and 192.80 mg/ml in not transferred anesthetized fish. There is no variation between them. However, both were

significantly elevated as compared to the positive control group as shown in (Table 3).

The biochemical result revealed that there were no effects of MS-222 on the concentration of ALT on all groups. Additionally, the concentration of creatinine phosphate enzyme in the serum of anesthetized and transferred fish with MS-222 for 1 hour does not vary from other groups with statistical variation between all groups. Furthermore, there were no effects of MS-222 on both hematological parameters represented by Hb concentration and PCV and sodium ion concentration in the serum in all groups as can be seen in (Table 3).

Table 2: Stages of recovery

Stage	Behavior signs	Time
R1	Reappearance of the opercula movement	1 min
R2	Reappearance of slow swimming activity, loss equilibrium	2 min
R3	Abnormal swimming, Partial recovery of equilibrium, weak muscle tone but loss tail stimuli	3-5 min
R4	Reappearance of equilibrium, response to visual and tactile stimuli, still abnormal response	7 min
R5	Normal swimming activity and normal behavior activity	7-10 min

Table 3: Effects of MS-222 on Biochemical and hematologic parameters and sodium-ion concentrations in fish

Parameters	Control (Mean $\pm$ Std.)		Anesthesia (Mean $\pm$ Std.)	
	Control	with transported	without transported	with transported
Cortisol (g/ml)	719.18 $\pm$ 50.36a	726.04 $\pm$ 49.17a	547.60 $\pm$ 49.73b	498.88 $\pm$ 38.21b
Glucose (mg/l)	140.14 $\pm$ 21.44ab	104.00 $\pm$ 8.70b	192.80 $\pm$ 21.06a	211.07 $\pm$ 31.23a
ALT(U/L)	45.54 $\pm$ 6.60a	53.98 $\pm$ 12.69a	52.15 $\pm$ 13.69a	49.83 $\pm$ 2.48a
CK (U/L)	1.30 $\pm$ 0.16a	0.46 $\pm$ 0.14b	0.52 $\pm$ 0.11b	0.88 $\pm$ 0.18ab
Na (mmol/l)	36.97 $\pm$ 10.71a	22.02 $\pm$ 5.50a	32.33 $\pm$ 11.90a	65.96 $\pm$ 22.92a
Hb (g/dl)	12.20 $\pm$ 0.47a	13.08 $\pm$ 0.58a	13.98 $\pm$ 1.11a	13.51 $\pm$ 0.78a
PCV (%)	38.29 $\pm$ 1.27a	37.20 $\pm$ a1.71	38.60 $\pm$ 2.11a	39.81 $\pm$ 1.24a

Varied letters mean there is a significant variation at  $P < 0.05$ .

## Discussion

Fish exposed to various stressors that cause negative effects on fish performance and survival. According to Griffiths, the most important causes of stress include size, weighting, overcrowding, disturbances of water quality, and fish transportation (21). Moreover, stress causes physiological injuries, immunosuppressant's, and death (22). According to the type of stress variable methods have been used in aquaculture to reduce stress as food additive probiotic (23), anesthetic methods, decrease overcrowding and keep the water quantity and quality. The effective concentration of MS-222 was 150mg/l. The induction and recovery were shorter and lasted for 7 minutes. These results agree with the results of Park *et al.* (24) who mentioned the optimum anesthetic concentration. The anesthesia should be induced with 3 minutes with recovery occurring within 19 minutes (24). When the fish transported to clean water the recovery began at 1 -2 minutes characterized by slow movement of opercula and then returned to normal swimming after 7 -10 minutes. According to Kiessling, these

rapid recoveries are attributed to the rapid elimination of MS-222 (25), which is not different from what we found in our results.

Tricaine induced aversive behavioral responses. These results are parallel to the results of Readman *et al.* (26) in that the anesthetic stages in *Cyprinus carpio* begin at the decline of opercula movement and loss of balance at a later stage, and fish settle at the bottom of the aquarium. All these anesthetic stages require 3 minutes. These results agree with the result of Park *et al.* (24). The characteristics of these stages of anesthesia may be related to the rapid absorption of MS-222 through the gills which inhibit the neural signal transmission from the periphery to the central nervous system (27).

During stress conditions, the neuroendocrine system is stimulated so there is an initial response characterized by the release of corticosteroid, which affects other physiological and some biochemical parameters such as glucose, hemoglobin, hematocrit, and enzyme activity (28). The cortisol level is a well-established indicator to fish stress response, and in our result, there was a significant elevation

of cortisol in the fish serum in the control group that underwent the transportation to clean water. Stress hormones increase cardiac output and ventilation leading to an increase in bronchial blood flow (11). While the cortisol levels in the anesthetized transported fish declined significantly as compared to the control group. Moreover, the glucose levels elevated in the fish serum in both anesthetized groups with and without transportation as compared to the control group. These results go along with the results of Bahrekazemi *et al.* (29) and can be attributed to the side effects of MS-222 that cause hypoventilation and affected ATP/Na<sup>+</sup>-K<sup>+</sup>ase activity so the glucose levels increased as well as the concentration of creatinine phosphate which are the product of carbohydrate metabolism used to supply energy (30). The results of this study reveal that there is no effect of MS-222 on liver cells shown as there is no significant variation in the level of ALT in fish serum in all groups. However, the creatinine phosphate activity declined in both anesthetized groups which contradict the results of Congleton (30) who reported increased activity of creatinine phosphate in the blood of chinook salmon. The variation of these results may be related to the genetics, type, and the age of fish or species, and the dose of MS-222 in addition to the physical and chemical characteristics of water.

The results of the present study exhibited no significance difference in hematological parameters in all groups, this pointed with other workers Wisteska *et al.* (31) who reported a presence of minor statistical variation in hematology characters in common carp. Moreover, Stockman *et al.* (32) observed no changes in PCV levels in *Cyprinus carpio* treated with MS-222 (50-190 mg/l). These may be due to stress hormone (cortisol), which does not vary from the control group. Consequently, it did not affect to initiate a secondary response to stress such as degeneration of protein, and disturbances in osmolality (chlorine, sodium and potassium and other ions inflation of RBC, and the breakdown of fat and glycogen) (33).

## Conclusion

This study concluded that MS-222 induces rapid induction and recovery time and can reduce stressful conditions resulted from fish transportation.

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## Conflict of Interest

No conflict interests

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## الاستجابة السلوكية والكيموحيوية في اسماك الكارب والمعرضة للتراكين ميثان سلفونيت كعقار مخدر وتحت ظروف النقل

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### الخلاصة

هدفت هذه الدراسة لتحديد تأثير التراكين ميثان سلفونيت وبتريكز ١٥٠ ملغم/لتر ولمدة ساعة واحدة كمادة مخدرة للتقليل من إجهاد النقل في الأسماك. قسمت ٤٠ سمكة كارب الى أربع مجموعات: المجموعة الأولى اعتبرت مجموعة سيطرة (تركبت الأسماك بدون نقل وبدون تخدير)، المجموعة الثانية (تم نقل الأسماك بدون تخدير)، المجموعة الثالثة (تم تخدير الأسماك وتركبت بدون نقل) اما المجموعة الرابعة (تم تخدير الأسماك مع النقل). استغرق احداث التخدير ٣ دقائق في حين استغرق وقت الاستفاقة من ٧ الى ١٠ دقائق. إضافة إلى الانخفاض المعنوي في مستوى الكورتيزول في مصل دم الأسماك المخدرة ولكلا المجموعتين المنقولة وغير المنقولة مقارنة مع مجموعة السيطرة ارتفع معنويا (>٠,٠٥). مستوى الكلوكوز في مصل دم اسماك المجموعتين الثالثة والرابعة مقارنة مع مجموعة السيطرة. لم يكن هنالك فروق معنوية في المعايير الدموية (الهيموغلوبين وحجم خلايا الدم المرصوصة) وأيون الصوديوم وكذلك في فعالية الإنزيمات والمتمثلة بالإنزيم الناقل للحامض الأمينى الألائين وفي كل المجموعات، في حين كان مستوى أنزيم الكرياتينين فوسفيت منخفض معنويا في كلا المجموعتين الثانية والثالثة مقارنة مع مجموعة السيطرة. استنتج من هذه الدراسة بأن المادة تراكين ميثان سلفونيت تستغرق وقت قصير في استحداث التخدير والاستفاقة والتقليل من إجهاد النقل في الأسماك.