Plasma enzymatic, biochemical and hormonal responses to clove oil, 2-phenoxy ethanol, and MS-222 exposed to Caspian brown trout (*Salmo trutta caspius*, kessleri)

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Abstract

The effects of three anesthetics, clove oil (CO) (30 mgl^{-1}), 2-phenoxy ethanol (2-PE) (0.3 mlL^{-1}), and MS-222 (100 mgl^{-1}) were examined on blood enzymes including lactate dehydrogenase (LDH), creatine kinase (CK), alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin, glucose, and cortisol levels in the Caspian brown trout. Blood parameters were evaluated in the control (no anesthetics), and three other treatments 10 min and 24 h after the end of anesthesia. Significant increases were seen in blood albumin, ALT, AST, glucose, and cortisol levels in the treatment 24 h following anesthesia by MS-222 compared to the control ($p < 0.05$). Significant increase in blood glucose was also observed in the treatment 10 min after anesthesia with CO. Post-anesthesia cortisol levels significantly increased in 2-PE treatment after 10 min, in CO treatment after 10 min and 24 h, and in MS-222 treatment following 24 h compared to control. CK activity was significantly decreased only in 2-PE treatment in comparison to control. Except for the treatments of 24 h after anesthesia with MS-222 and 10 min after anesthesia by 2-PE, ALT enzyme activity did not show significant differences in all treatments compared to control ($p > 0.05$). As results, MS-222 is less stressful than both 2-PE and CO in short term. The long term stressing effect of 2-PE was detected to be lesser than both MS-222 and CO. So, based on the enzymatic and hormonal responses, MS-222 can be effectively used to reduce severe instantaneous stress such as surgery and spawning.

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Introduction

Population development particularly in the last decades, has been increased at an average annual rate of 3.2 percent in the period 1961– 2013 resulting in increasing average of consumption per capita whole the World (Soltani, Kakoolaki & Keisami 1998, SamCookiyaei, Afsharnasab, Razavilar, Motalebi, Kakoolaki, Asadpor & Yahyazade 2012). Transportation of fish within and/or out of their natural environments is often associated with many problems. They struggle during capture and transport leading to large effects on their physiology. As a result, it often seems necessary to immobilize the fish before taking any simple operation. For this purpose, anesthesia accounts for a valuable tool in fisheries management (Ross & Ross 2008). Common anesthetics in aquaculture include tricaine methane sulfonate (MS-222), benzocaine, Kuinaldine, Methomydate, clove oil extract, and 2-phenoxy ethanol (Velisek & Svobodova 2004a). The use of anesthetics can cause complications for fish, which may not be desirable for both the culturists and farming purposes. Research has been conducted on stress-induced anesthetics in captive, transportable fish (Stein 1998; Velisek, Svobodova & Piackova 2007; Velisek, Stara, Silovska & Turek 2011). Assessment of cardiovascular effects, blood pressure in dorsal aorta, cortisol response, and some blood enzymes could provide indicators that these substances may be stressful (Soltani, Ghaffari, Khazraeinia & Bokaei 2003). The use of anesthetics in aquaculture procedures is inevitable, though; appropriate anesthetics should be selected associated with lowest risk, injury, and stress. This, in particular, becomes more important in the case of species that are economically and/or environmentally influential (Ross & Ross 2008).

Caspian brown trout is one of the rare species of Caspian Lake which migrates to related rivers for reproduction. Fish spend initial stages of their lives including parr and smolt in freshwater river. Changes in river's environment due to various factors as well as overfishing endangered population of this species, so the spawning is artificially propagated for restocking in a research center on cold-water fish in Tonekabon, northern Iran (Mojazi Amiri, Bahrekazemi, Pousti & Vilaki 2005). In order to raise the Caspian brown trout, it seems necessary to need appropriate anesthetics causing lowest stress when manipulating, stripping, and vaccination of broodstock, and for juveniles at the time of sorting, transportation, etc.

Effect of different anesthetics on the blood paremeters of rainbow trout (*Oncorhynchus mykiss* Walbaum) has been investigated by Holloway, Keene, Noakes & Moccia 2004; Ucar & Atamanalp 2010, Velisek *et al.* 2011. In other salmonids such as Brown trout (*Salmo trutta fario* L.*)* (Ucar & Atamanalp 2010) and Chinook salmon (*Oncorhynchus*

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tshawytscha L.) (Congelton 2006) effects of some anesthetics on blood enzymes, blood glucose, and albumin has been studied.

The aim of this study was to compare stressful effects of three anesthetics commonly used in aquaculture (clove oil, 2-phenoxy ethanol, and MS-222) on the Caspian trout in order to select an anesthetic with the lowest risk to be used in aquaculture and restocking systems.

Materials and Methods

Anesthesia tests on the Caspian trout using clove oil (CO), 2-phenoxy ethanol (2-PE), and MS-222 was conducted in a Fish Research Centre, Tonekabon, northern Iran. A total of 160 farmed Caspian trout (25.18 ± 4.01 g; 14.19 ± 1.62 cm; 1.0 year old) were initially adopted to the experimental conditions. The fish were then randomly distributed in four fiberglass tanks (four treatments, one control group and three experimental treatments). All of the physicochemical conditions of water (such as temperature, oxygen content, pH) was maintained at optimal levels during the experiment (Table 1).

Table 1 Physicochemical conditions of water throughout the experimental period

Parameter	Level
Temperature (^oC)	12.6 ± 1
Dissolved oxygen (mgl^{-1})	6.5 ± 0.5
EC (µs)	602 ± 9
pH	7.4 ± 0.4

To prepare the anesthetic solution, first the amounts of anesthetics were added to the water using a calibrated pipette according to Table 2, then the solution was stirred and homogenated completely . To conduct anesthesia tests, fish feeding was stopped 24 h before the start of the experiment. After anesthesia, the fish were monitored for 24 h to ensure that no side effects of the anesthetics happen during this time (Stoskopf 1993).Blood samples (four per treatment) were taken from the caudal peduncle by plastic syringes (2 mm) in two steps: 10 min and 24 h after recovery of fish. Blood enzyme activities, albumin, and glucose were assayed by an autoanalyzer (RA1000, STARNA, USA)

Table 2 The doses of anesthetics used in this study (Ackerman, Morgan & Iwama 2005)

Anesthetic	Recommended dose
MS-222	100 mgL^{-1}
Clove oil (CO)	30 mgL^{-1}
2-phenoxy ethanol (2-PE)	0.3 mlL^{-1}

Serum cortisol level was measured through radioimmunoassay method (Patrono & Peskar 1987). The enzymes creatine kinase (CK) and lactate dehydrogenase (LDH) were estimated by photometric method at 340 nm (Stein 1998; Thomas 1998). Albumin was assayed by colorimetric method (Doumas 1971) using the kit BCG (Bromo Corozel Green) to create a complex with albumin and produce measurable color at 620-640 nm. The enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured by photometric method (Thomas 1998). Glucose was estimated by spectrophotometer (DR 6000, HACH, USA) at 500-540 nm (Barham 1972).

The statistical design used in this study was completely randomized design with three anesthetic treatments and a control group each with 4 replicates. Data were analyzed by SPSS software (version 21) at a confidence level of 95 percent.

Differences between the data obtained from the treatments were determined by one-way ANOVA and the means were compared with Duncan's test. Paired t-test was applied to compare the numbers of blood sampling after 10 min and 24 h post-anesthesia.

Results

Based on the results of this study, blood albumin in the fish from 10 min after anesthesia showed a significant reduction ($p<0.05$) only in 2-PE treatment (1.05 ± 0.08 $mgml^{-1}$) compared to the control group (1.26 ± 0.16 $mgml^{-1}$). The amount of blood albumin in the fish 24 h after anesthesia was significantly increased ($p<0.05$) only in the treatment MS-222 (1.46 ± 0.04 $mgml^{-1}$) than the control group. The other treatments did not reveal significant differences in comparison with control group (Table 3). In contrast, no significant changes were observed at the levels albumin in samples from 24 h post-anesthesia compared to 10 min treatment ($p<0.05$) (Table 4).

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Table 3 Comparison of significant differences in studied parameters among the treatments and control

	Control	2-phenoxy ethanol (10min)	Clove oil (10min)	MS-222 (10min)	2-phenoxy ethanol (24h)	Clove oil (24h)	MS-222 (24h)
Albumin ($mgml^{-1}$)	0.16^{b}±1.26	0.08^{a}±1.05	0.073^{b}±1.25	0.15^{b}±1.24	0.17^{B}±1.25	0.04^{B}±1.24	0.04^{C}±1.46
CK (IUL^{-1})	618.33^{b}±3960	449.11^{a}±2150	316.44^{b}±3973.33	856.52^{b}±4083.33	515.98^{A}±2003.33	520.03^{B}±4446.67	122.88^{B}±4030
LDH (IUL^{-1})	147.30^{bc}±2280	364.28^{abc}±1910	125.03^{a}±1636.67	128.97^{abc}±2113.33	198.58^{ABC}±1923.33	245.76^{AB}±1710	259.39^{C}±2450
ALT (IUL^{-1})	4.51^{b}±35.67	1.53^{a}±21.67	3.63^{b}±31.00	5.51^{b}±35.33	2.52^{B}±35.67	4.59^{B}±31.00	1.53^{C}±51.67
AST (IUL^{-1})	59.35^{bc}±660	86.64^{ab}±568	33.86^{abc}±606.33	24.51^{a}±554	51.29^{C}±675.66	41.62^{BC}±658.33	59.94^{D}±828.33
Glucose ($mgdl^{-1}$)	14.57^{a}±145.67	6.43^{ab}±170.33	9.87^{b}±183.33	4.39^{ab}±168	22.94^{A}±149.67	15.69^{A}±143.67	13.08^{C}±230
Cortisol ($ngml^{-1}$)	38.11^{a}±290.33	66.56^{c}±473.33	78.23^{bc}±402.33	21.46^{a}±290.33	54.37^{ABC}±374	71.04^{C}±469	41.52^{C}±424.33

*Different letters in each row indicate significant difference between treatments at $P<0.05$

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The amount of CK enzyme in the control was not statistically different from those found in both 10 min after anesthesia with M-S-222 and CO; this enzyme activity, however, showed a significant decrease in 2-PE treatment (2150±449.11 IuL^{-1}) as opposed to control (Table 3). The results of 24 h from anesthesia in all treatments were similar to those for 10 min after anesthesia. No significant differences were found in the levels of CK enzyme between samples from 10 min and 24 h after anesthesia in all three treatments (Table 4).

The amount of LDH in the control was not significantly different from those in 10 min after anesthesia with MS-222 and 2-PH treatments. Nevertheless, the amount of this enzyme showed a significant decrease in samples from 10 min after anesthesia with CO compared to the control. Serum levels of this enzyme in 24 h from the anesthesia treatment did not show a significant difference compared to control (Table 3). In comparison, the fish displayed a marked increase in the level of LDH enzyme after 24 h of anesthesia with MS-222 as opposed to those examined after 10 min anesthesia with MS-222 (Table 4).

The volume of ALT in control fish showed no significant differences with values in 10 min of anesthesia by each of MS-222 and CO, while the activity of this enzyme significantly decreased in fish 10 min after anesthesia with 2-PE compared to control. This enzyme activity significantly rose in the samples treated with MS-222 following 24 h in comparison with control, but the enzyme values from CO and 2-PE treatments were not significantly different from control (Table 3). Additionally, 24 h after anesthesia with MS-222 and 2-PE, significant elevations were recorded in the ALT levels compared to those observed in 10 min after anesthesia treatment. ALT activity in CO treatment was not significantly different between the values observed in the two sampling times (Table 4).

AST levels in the control was almost similar to values obtained from the fish after 10 minutes of anesthesia with CO and 2-PE, but it declined in samples of 10 min after anesthesia with MS-222 , which was significantly lower than the control. In addition, the enzyme activity markedly elevated at 24 h after anesthesia with MS-222 compared to the control, but the values for the fish in CO and 2-PE treatments did not show significant differences with the control (Table 3). The amounts of this enzyme measured in the two sampling times for all three treatments displayed significant increases in 24 h post-anesthesia as opposed to those estimated in 10 min after anesthesia treatments (Table 4).

The results of serum glucose measurement showed an amount of 145.67±14.57 $mgdl^{-1}$ in the control group. The increases in glucose in the fish from 10 min after anesthesia with MS-222 and 2-PE were not significant. Anesthesia with CO, on the other hand, resulted in significantly higher glucose level after 10 min than the control group. In samples from 24 h after anesthesia, only the anesthetic MS-222

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yielded a significant increase in glucose levels compared to the control whereas in the samples anesthetized with CO and 2-PE, glucose levels returned to the baseline values (Table 3). Comparing the amounts of glucose from two sampling times showed that fish treated with MS-222 presented a significant increase in glucose level following 24 h of anesthesia compared to 10 min post-anesthesia. In the CO treatment, a significant glucose reduction was observed after 24 h of operation compared to 10 min after anesthesia. In the fish from 2-PE treatment, the average amount of glucose reduced after 24 h of anesthesia in relation to the first stage of sampling (10 min after anesthesia), but the difference was not statistically significant (Table 4). The result of serum cortisol assay in the control fish indicated 290.33 ± 38.11 $ngml^{-1}$, which was not significantly different from that recorded in 10 min of anesthesia by MS-222 .However, cortisol levels elevated significantly in samples from 10 min after anesthesia with CO and 2-PE compared to the control levels. The samples from 24 h after anesthesia with MS-222 (424.33±41.52 $ngml^{-1}$) and CO (469±71.04 $ngml^{-1}$) exhibited significant rises in the amount of cortisol compared to control. In the fish anesthetized by 2-PE, the amount of cortisol decreased, but it was not significantly different from the control ($p>0.05$) (Table 3). Comparing the two sampling times revealed that the amount of cortisol markedly rose in MS-222 and CO treatments, and significantly increased in 2-PE treatment after 24 h in comparison with hormone values obtained from 10 min treatment after being contacted with three anesthetics examined (Table 4).

Discussion

Any kind of general adaptation syndrome to stress is affected by two groups of hormones: catecholamine and the hypothalamic-pituitary inter-renal (HPI) axis hormones, especially cortisol (Barton 2002). The effect of a rise in cortisol is all-encompassing and often hazardous if prolonged leading to increased activity of enzymes, interruption in growth, reduced carbohydrate intake, increased glucose production from tissue protein, liver glycogen breakdown, changes in membrane permeability, increased ATPase activity, and increased number of white blood cells. Following prolonged exposure to chronic stressors, cortisol levels may return to normal levels (though stress still exists) (Ross & Ross, 2008). Stress may increase the susceptibility to diseases, growth and reproductive defects, all of which seem to be caused by cortisol secretion (Pickering 2003).

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Table 4 Paired comparison among the measured parameters at different treatments of 10 min and 24 h post-anesthesia

		Treatment	
		10 min	24 h
Albumin($mgml^{-1}$)	2-PE	1.05±0.08	1.25±0.17
	CO	1.25±0.073	1.24±0.04
	MS-222	1.24±0.15	1.46±0.04
CK (IUL^{-1})	2-PE	2150±449.11	2003.33±515.98
	CO	3973.33±316.44	4446.67±520.03
	MS-222	4083.33±856.52	4030±122.88
LDH (IUL^{-1})	2-PE	1910±364.28	1923.33±198.58
	CO	1636.67±125.03	1710±245.76
	MS-222	2113.33±128.97	2450±259.39*
ALT (IUL^{-1})	2-PE	21.67±1.53	35.67±2.52*
	CO	31.00±3.63	31.00±4.59
	MS-222	35.33±5.51	51.67±1.53*
AST (IUL^{-1})	2-PE	568±86.64	675.66±51.29*
	CO	606.33±33.86	658.33±41.62*
	MS-222	554±24.51	828.33±59.94*
Glucose ($mgdl^{-1}$)	2-PE	170.33±6.43	149.67±22.94
	CO	183.33±9.87	143.67±15.69*
	MS-222	168±4.39	230±13.08*
Cortisol ($ngml^{-1}$)	2-PE	473.33±66.56	374±54.37*
	CO	402.33±78.23	469±71.04*
	MS-222	290.33±21.46	424.33±41.52*

* Values marked with * are statistically different ($p<0.05$).

The results of this study showed that at 10 min after anesthesia, the amount of albumin significantly decreased in the blood of fish treated with 2-PE compared to the control group. Following 24 h of anesthesia, the amount of albumin significantly increased only in MS-222 treatment in comparison with the control fish. The elevated value of this protein after 24 h of anesthesia with MS-222 can be due to the albumin ability in transferring exotic chemicals. The decrease in the amount of albumin after 10 min anesthesia with 2-PE may be due to its effects on the liver and catabolism of albumin (Doumas 1971). Velisek & Svobodova (2004_a) observed a significant albumin increase in rainbow trout after 24 h exposure to the same dose of 2-PE; such an effect was not observed in the Caspian trout. It can be because of the species and the age of fish. Also, the study of Mavaddati & Habibian (2011) on the anesthetic

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effects of 2-PE and CO upon rainbow trout revealed no significant changes in the blood albumin after 15 min and 24 h of anesthesia.

Our results showed that at 10 min after anesthesia, blood glucose levels in fish treated with CO increased significantly compared to the control and returned to the baseline values after 24 h, which corresponds to the results of Velisek *et al.* (2011) in rainbow trout. At 24 h post-anesthesia, the amount of glucose increased markedly only in MS-222 treatment as opposed to control. Velisek & Svobodova (2004b) found no changes in blood glucose levels in common carp (*Cyprinus carpio* L.) anesthetized with the same dose of 2-PE. Cortisol is known as the most important indicator of stress in organisms. This study recorded significant cortisol rises in fish following 10 min anesthetizing with each of CO and 2-PE in comparison to control. Similarly, the fish anesthetized by MS-222 and CO displayed elevated cortisol values after 24 h of anesthesia. In this regard, Small (2003) examined the effects of CO in channel catfish (*Ictalurus punctatus* Rafinesque) and found that this substance was very effective in inhibiting the secretion of cortisol in this species, which is contrary to the results of the present study. Wagner, Singer & Mckinley (2003) showed a significant increase in blood cortisol levels in rainbow trout treated with clove oil after an hour of anesthesia compared to MS-222, which accords with the results of this study.

Some hematological effects of stress that can be mentioned include high concentration of blood, an increase in plasma osmolarity because of the effect of stress hormones on the degeneration of proteins, fats and glycogen, increase in chlorine, sodium, potassium and other ions amounts and inflation of red blood cells (Ross & Ross 2008). Some other effects may be investigated indirectly, which are mediated by the impact of stressors on body tissues and the resultant enzymatic secretions into the blood such as LDH, CK, AST and ALT (Velisek *et al*. 2011). The results of this study showed that in both times of 10 min and 24 h after anesthesia, the enzyme CK levels was significantly lower in fish anesthetized with 2-PE than the control group. According to Henry (2001), a significant reduction in the amount of CK can be a result of a sharp increase in basal metabolism. Accordingly, it can be concluded that 2-PE greatly increased the metabolism of Caspian trout at least 24 h after anesthesia. Meanwhile, Congleton (2006) reported increased activity of CK in the blood of Chinook salmon (*Onchorhynchus tshawytscha* Walbaum) exposed to acute doses of MS-222, which is completely contrary to the findings of this study. Evaluation of LDH at 10 min after anesthesia with COE showed a significant decline compared to the control. LDH levels in the fish anesthetized with MS-222 markedly rose after 24 h in relation to those following 10 min of anesthesia. LDH serves as a potential indicator to determine the toxicity of the chemicals. Congleton (2006) noted elevated blood LDH activity in Chinook salmon exposed to acute

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doses of MS-222. Also, Velisek & Svobodova (2004 a) detected no significant differences in the blood LDH of rainbow trout treated with 2-PE and those in control. However, Soltani *et al.* (2003), noticed a considerable reduction of this enzyme in the blood of common carp treated with CO, which is in accordance with that found after 10 min of anesthesia with the same material in the Caspian trout. Mavaddati & Habibian (2011) did not observe any significant changes in the level of this enzyme in the blood of rainbow trout treated with 2-PE, CO, and MS-222. Additionally, Velisek *et al.* (2011) reported no significant changes in the level of this enzyme in the blood of rainbow trout treated with CO, 2-PE, and MS-222, all of which are against the results of present research. The results obtained from LDH activity in the examined sera might indicate non-toxicity and lack of injury from the tested anesthetics on the organs and internal body tissues of Caspian trout.

Our results also showed that 10 min after anesthesia in fish treated with 2-PE, a significant decrease occurred in the amount of ALT enzyme as opposed to the control. Compared with the control, there was a significant increase in the levels of this enzyme after 24 h of anesthesia with MS-222. ALT enzyme activity in plasma rises as a result of damage to the liver membrane (Porchas, Cordova & Enriquez 2009). According to Henry (2001), a significant reduction in ALT enzyme levels in the blood can increase dopamine release. It can, therefore, be concluded that the stress caused by 2-PE at 10 min after anesthesia should have led to the release of high dopamine levels rendering reduced ALT in the control. However, the significant increase in the activity of ALT after 24 h of anesthesia with MS-222 could be explained through the possibility of damaging the liver tissue by this chemical. Velisek & Svobodova (2004a) studied the enzyme activity in rainbow trout exposed to 2-PE and did not realize any significant difference, which disagrees with the results of this research. In accordance with the results of this study, Congleton (2006) reported a significant increase in serum ALT activity of Chinook salmon after exposure to MS-222.

Significant decrease and increase, respectively, were recorded in the levels of the enzyme AST after 10 min and 24 h of anesthetizing the fish with MS-222. Increase in plasma AST activity is a result of mitochondria breakdown leading to inflation of liver tissue. In addition to an elevation when the liver's membrane is damaged, AST also rises with heart and muscle damages (Porchas *et al.* 2009). Velisek & Svobodova (2004a) observed a significant decrease in the level of AST activity in rainbow trout immediately after anesthesia with 2-PE. On the other hand, Congleton (2006) perceived a significant increase in serum activity of this enzyme in Chinook salmon treated with MS-222 corroborating our results on the fish with the same anesthetic after 24 h of exposure. Soltani *et al.* (2003) noticed no significant

differences in the level of this enzyme in common carp treated with CO, which is according to the results of this study. Velisek *et al.* (2011) showed a significant increase in AST enzyme levels in the blood of rainbow trout exposed to CO (10 min and 24 h after anesthesia) and 2-PE (after 10 min) contrary to the results of the present study. Genetic, type and age of the species, type and dose of anesthetic and other water physicochemical conditions can be the possible reasons for this difference.

The results of current research suggest that the plant origin of an anesthetic cannot solely make it a suitable choice for biological applications. Although the examined doses of the three anesthetics used caused no irreversible fish damages and mortalities, the results suggest that MS-222 rendered lesser stressful effects in short term than the other two anesthetics, hence, it can be used where the aim is to eliminate instantaneous stresses such as those induced by surgery and stripping. Nonetheless, where the fish is farmed for aquaculture purposes, the use of MS-222 is not recommended as this anesthetic revealed liver tissue damage through elevation of ALT and AST enzymes in Caspian trout juvenile after a long-term (24 h) anesthesia. In contrast, 2-PE proved to have lesser stressful effects than MS-222 and CO in long-term (24 h).

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پاسخ آنزیمی، بیوشیمیایی و هورمونی ماهی آزاد دریای خزر(*Salmo trutta caspius*) در مواجه با روغن میخک، ۲- فنوکسی اتانول و ام-اس-۲۲۲

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چکیده

تاثیر ۳ ماده بیهوشی روغن میخک($30\ mgl^{-1}$)، ۲-فنوکسی اتانول($0.3\ mll^{-1}$) و ام-اس-۲۲۲($100\ mgl^{-1}$) بر فعالیت آنزیم‌های خونی شامل لاکتات‌دهیدروژناز، کراتین‌کیناز،آلانین‌آمینوترانسفراز،آسپارتات‌آمینوترانسفراز،آلبومین،گلوکزوهورمون کورتیزول خون ماهی آزاد دریای خزر مورد بررسی قرار گرفت. پارامترهای خونی در گروه کنترل که هیچ بیهوش کننده ای را دریافت نکرد و سه تیمار دیگر، ۱۰ دقیقه و ۲۴ ساعت پس از اتمام بیهوشی اندازه گیری شد.افزایش معنی‌دار در میزان آلبومین، آلانین‌آمینوترانسفراز، آسپارتات‌آمینوترانسفراز، گلوکز و هورمون کورتیزول خون، در تیمار ۲۴ ساعت پس از بیهوشی با ام -اس-۲۲۲ نسبت به شاهد مشاهده شد ($p<۰/۰۵$). همچنین افزایش معنی‌دار در میزان گلوکز خون در تیمار ۱۰ دقیقه پس از بیهوشی با عصاره گل میخک مشاهده شد.هورمون کورتیزول نیز افزایش معنی‌دار نسبت به نمونه شاهد در تیمارهای ۱۰ دقیقه پس از بیهوشی با ۲- فنوکسی اتانول و ۱۰ دقیقه و ۲۴ ساعت پس از بیهوشی با عصاره گل میخک و ۲۴ ساعت پس از بیهوشی با ام -اس- ۲۲۲ نشان داد. فعالیت آنزیم کراتین‌کیناز تنها در تیمار مربوط به ۲-فنوکسی اتانول نسبت به نمونه شاهد کاهش معنی‌دار نشان داد. فعالیت آنزیم آلانین‌آمینوترانسفراز در همه تیمارها به جز ۲۴ ساعت پس از بیهوشی با ام-اس-۲۲۲ و ۱۰ دقیقه پس از بیهوشی با ۲-فنوکسی اتانول، تفاوت معنی‌داری را نسبت به نمونه شاهد نشان نداد ($p>۰/۰۵$).در نتیجه ام-اس-۲۲۲ اثر استرس‌زایی کمتری نسبت به روغن گل میخک و ۲-فنوکسی اتانول در کوتاه مدت دارد. در بلندمدت نیز ۲-فنوکسی اتانول اثر استرس‌زایی کمتری را نسبت به ام-اس-۲۲۲ و عصاره گل میخک نشان داد. بنابر این بر اساس پاسخ های آنزیمی و هورمونی ، ام- اس-۲۲۲ می‌تواند در شرایطی که کاهش استرس شدید لحظه‌ای اهمیت می‌یابد مانند جراحی‌ها و تخم‌کشی مولدین موثر باشد.

کلمات کلیدی: ماهی آزاد دریای خزر، ۲- فنوکسی اتانول، روغن میخک، ام- اس- ۲۲۲، آنزیم های خونی

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