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The Anaesthetic Effect of Nutmeg Powder, *Myrisitca fragrans* on Young Common Carp, *Cyprinus carpio*

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Abstract: Anaesthetic effect of nutmeg powder, *Myrisitca fragrans* Houtt. on young common carp (*Cyprinus carpio* L.) were studied in the laboratory. Three concentrations of nutmeg powder (300, 400 and 500 mg/l) were investigated. The best results were achieved with a concentration of 500 mg/l as the anaesthesia time was 13.83 minutes and the recovery time was 31.3 minutes. The studied characteristics are times of anaesthesia and recovery according to anaesthesia and recovery stages and biochemical indices of blood serum (ALP, AST, ALT, LDH and CK). Results showed that anaesthesia time decreased with increasing concentrations of nutmeg powder, and there were significant differences ($P<0.05$) in anaesthesia time between the different concentrations. Results revealed also direct relationship between recovery time and concentrations of the anaesthetic, with significant differences ($P<0.05$). Results of the current study indicated that differences in nutmeg powder concentrations effects had no significant differences ($P>0.05$) on the biochemical indices (ALP, AST, ALT, LDH and CK) measured immediately after anaesthesia. Also, there were no significant differences ($P>0.05$) between these biochemical indices for anaesthetic fishes compared with control fishes. Based on the present results, nutmeg can be recommended as a very suitable, economically advantageous and easy accessible anaesthetic for fishes. It has no any side effects on fishes and doesn't represent ecological or hygienic risks.

Keywords: Anaesthetic, Recovery, Nutmeg, Common carp.

INTRODUCTION

During aquaculture and stocking activities, fishes are faced with several potential stressors, in particular, transportation, capture and

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handling procedures, a highly crowded and confined farming environment, possible air exposure and variation in water quality are all factors that may increase the stress level of organisms (Zahl et al., 2012), and have significant effects on fish physiology and survival (Harmon, 2009).

Fish stress and mortality can cause significant losses of resources and productivity in both capture and culture systems (Davis, 2010). Anaesthetics are used with increasing frequency in aquaculture, mainly to reduce the stress and to prevent mechanical damage to fishes during handling. Their use is particularly common in stripping, marking, biometry, health checks, etc. (Ross & Ross, 1999).

Different anaesthetic agents such as MS222, quinaldine, benzocaine, 2-phenoxyethanol, methomidate, isoeugenol and propofol were used to anaesthetize fishes in aquaculture. Most researchers have tried to find the best and most efficient anaesthetic agent with the least side effects (Kiessling et al., 2009). Organic farming of agricultural and horticultural crops is being used as a popular venture in the direction of sustained and eco-friendly food production activity. Organic farming looks for alternatives to those chemicals that are currently being used in aquaculture, and the anaesthetics are one such important input, as a result, different natural anaesthetics were investigated to compare their effectiveness with chemical products (Taylor & Roberts, 1999).

Dry nutmeg *Myrisitca fragrans* powder is a common medicinal plant available in all medicinal plant stores, and is not expensive. To our knowledge, no report research was conducted on effect of the nutmeg powder extract towards anaesthetics in fishes. Various researches discovered the therapeutic values of nutmeg powder; mace and seed of nutmeg possess anti-inflammatory effect (Murcia et al., 2004), anti-cancer effect (Olajide et al., 1999), anti-glycation (Kazeem et al., 2012), anti-diarrhea (Grover et al., 2002), antioxidant and antimicrobial effect (Kazeem et al., 2012; Suthagar et al., 2012; Ashish et al., 2013). The aim of the present study was to evaluate the anaesthetic effects of nutmeg powder on some biochemical characteristics of blood serum of young common carp *C. carpio*.

MATERIALS AND METHODS

The experiment was conducted on young common carp (average length 8.05 ± 1.18 cm and average weight 8.01 ± 1.21 gm). They were brought from the fish farm of Marine Science Center, University of Basrah, and kept in aquariums with dimensions of 50 x 30 x 30 cm. Experimental fishes were acclimated for a minimum of two weeks prior

to the onset of experiments. During acclimation period, fishes were fed with commercial pellets. Aquariums were cleaned by siphoning the feces and non-consumed feed. Fishes used in the experiment (two specimens for each run) were unfed for 24 hours prior to the experimental trial as suggested by Brown (2011). Three concentrations (300, 400 and 500 mg/l) of nutmeg powder (Fig. 1) were prepared according to Al-Jashami et al. (2002) with little modification according to Al-Niaeem (2006). Three replicates in each concentration were used. Aquariums of dimensions of 24 x 14 x 10 cm were used for anesthetization and recovery aquariums were with dimensions of 50 x 30 x 30 cm. Water temperature in aquariums during the experimental trials was 23° C, pH was 7.6 and salinity was 3.5 mg/l. Anaesthesia time (total loss of equilibrium, no body movements, no reaction to external stimuli and decrease in opercula rate) and recovery time (recovery of equilibrium, body movements and response to external stimuli) were measured to the nearest minutes.

Fishes were weighed and transferred to a recovery aquarium that had been filled with aerated freshwater at the same time of preparation the anaesthetic baths. In the recovery aquarium, the fishes were monitored continuously to determine time to full equilibrium. The studied characteristics were times of anaesthesia and recovery according to anaesthesia and recovery stages and biochemical indices of blood serum. The blood samples from each fish of different groups were collected by suction of the caudal peduncle. Blood plasma was then separated by centrifugation for three minutes at 1500 rpm (Yang & Chen, 2003). The levels of serum enzymes were assayed according to the instructions provided with the corresponding enzymatic kits. These enzymes were alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), lactic dehydrogenase (LDH) and creatine kinase (CK).

By application of SPSS (version 18), the data were subjected to one-way analysis of variance (ANOVA) to determine the difference between the means and the significant differences were tested by LSD Test.

Figure 1: Nutmeg, *Myrisitca fragrans*.

RESULTS

Table (1) shows the anaesthesia and recovery time of young common carps. Anaesthesia time decreased with increasing concentrations of nutmeg powder. At a concentration of 500 mg/l, the time to reach a complete anaesthesia was 13.83 min. which is significantly different ($P<0.05$) from the other concentrations (300 and 400 mg/l). There was a clear direct relationship between recovery time and concentrations of the anaesthetic, with significant decrease ($P<0.05$). The longest recovery time was 31.3 minutes at a concentration of 500 mg/l, while the shorter recovery time was 11.38 at a concentration of 300 mg/l nutmeg powder.

Table 1: Effect of nutmeg powder concentrations on anaesthesia and recovery time (min.) in common carps (Mean ± SD).

Concentration (mg/l)	Anaesthesia time (min.)	Recovery time (min.)
300	55.30 ± 1.21^{a}	11.38 ± 0.75^{a}
400	34.83 ± 0.75^{b}	21.67 ± 1.21^{b}
500	13.83 ± 0.40^{c}	31.30 ± 1.21^{c}

Different letters in the same column are significantly different ($P<0.05$).

Table (2) shows the effects of nutmeg powder concentration for anaesthesia on the blood serum biochemical indices of common carp. The 55.3 min., 34.83 min. and 13.83 min. exposure to nutmeg powder at

a different concentration had no significant (P>0.05) effects on the biochemical indices (ALP, AST, ALT, LDH and CK) measured immediately after anaesthesia. Also, there were no significant (P>0.05) differences between these biochemical indices for anaesthetic fishes compared with controlled fishes.

The highest ALP concentration was 69.75 Ul/l for control fishes and the lowest was 61.5 Ul/l for fishes anaesthetic by 500 mg/l of nutmeg powder. The highest ALT concentration was 6.3 Ul/l for control fishes and the lowest was 5.55 Ul/l for fishes anaesthetic by 400 mg/l of nutmeg powder. The highest AST concentration was 52.75 Ul/l for control fishes and the lowest was 44.35 Ul/l for fishes anaesthetic by 500 mg/l of nutmeg powder. The highest CK concentration was 44.87 Ul/l for control fishes and the lowest was 40.45 Ul/l for fishes anaesthetic by 400 mg/l of nutmeg powder. The highest LDH concentration was 84.5 Ul/l for fishes anaesthetic by 500 mg/l of nutmeg powder and the lowest was 78.9 Ul/l for fishes anaesthetic by 400 mg/l of nutmeg powder.

Table 2: Effect of nutmeg powder concentrations on blood serum biochemical enzymes of common carp anaesthesia (Mean ± SD).

Parameter (Ul/l)	Control	300 mg/l	400 mg/l	500 mg/l
ALP	69.75 ± 4.59^{a}	65.25 ± 3.88^{a}	64.75 ± 3.18^{a}	61.5 ± 2.54^{a}
ALT	6.30 ± 0.56^{b}	5.70 ± 0.42^{b}	5.55 ± 0.35^{b}	5.95 ± 0.07^{b}
AST	52.75 ± 3.74^{c}	47.25 ± 3.88^{c}	44.4 ± 1.27^{c}	44.35 ± 4.03^{c}
CK	44.87 ± 4.84^{d}	42.77 ± 3.64^{d}	40.45 ± 4.51^{d}	44.33 ± 3.88^{d}
LDH	80.9 ± 3.67^{e}	83.30 ± 3.82^{e}	78.9 ± 4.55^{e}	84.5 ± 3.34^{e}

Different letters in same row are significantly different (P<0.05).

DISCUSSION

The results of the present study showed that anaesthesia time decreased with increasing concentrations of nutmeg powder, and there were significant differences (P<0.05) in anaesthesia time between different concentrations (300, 400 and 500 mg/l). Also, the results revealed direct relationship between recovery time and concentrations of the anaesthetic, with significant differences (P<0.05). When using anaesthetics, it is expected that there will be a strong negative correlation between the applied concentration and the time required to induce anaesthesia to the desired stage, as observed previously for several fish species (Weber et al., 2009; Pawar et al., 2011; Kamble et al., 2014). Long exposure to anaesthetic led to more anaesthetic absorption

by fish which, in turn, lengthened the recovery time. On the other hand, Weyl et al. (1996) pointed out that compared with anaesthesia duration, anaesthetic concentration plays more important role on the recovery time. It is believed that anaesthetic is taken up by the fish through a concentration gradient at the gill interface. Therefore, when equilibrium level established between the gill and anaesthetic solution, no further anaesthetic will be taken up by the fish, and during recovery, the anaesthetic agent is leaked through such gradient.

Results of the current study indicated that the 55.3 min., 34.83 min. and 13.83 min. exposure to nutmeg powder at different concentrations had no significant differences ($P>0.05$) on the biochemical indices (ALP, AST, ALT, LDH and CK) measured immediately after anaesthesia. Also, there were no significant differences ($P>0.05$) between these biochemical indices for anaesthetic fishes compared with controlled fishes. Biochemical indices of blood can provide important information about the internal environment of the organism (Masopust, 2000). Values determined in the present study suggest that internal physiological rolls of organs and tissues of carp are not altered by nutmeg anaesthesia.

Based on results of the present study, nutmeg can be recommended as a very suitable and easy accessible anaesthetics for fishes. Moreover, as an advantage, it refers to natural substance which has no any side effects on fishes and does not represent any ecological or hygienic risks.

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