# Effects of clove oil anaesthesia on common carp (*Cyprinus carpio* L.)

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**ABSTRACT**: The aim of the study was to investigate acute toxicity of clove oil for common carp and, using values of haematological and biochemical profiles of blood and histological tissue examinations, to assess the effects of the fish exposure to that anaesthetic. Acute toxicity values of clove oil for carp were found 10 minLC50 74.3 mg/l; 10minLC0.1 51.6 mg/l; 10minLC99.9 110.1 mg/l; 96hLC50 18.10 mg/l; 96hLC0.1 15.45 mg/l; and 96hLC99.9 19.80 mg/l. The fish were divided into four groups for haematological and biochemical examinations of blood and histological examinations of tissues. The groups were Control I (before the anaesthetic administration), Experiment I (immediately after 10 min anaesthesia at the concentration of 30 mg/l), Experiment II (24 hrs after 10 min anaesthesia) and Control II (controls examined in parallel with Experiment II). A total of 40 carp were examined. Clove oil anaesthesia had not effect on the haematological profile. The 10-min exposure to clove oil at a concentration of 30 mg/l caused a significant ($P < 0.01$) increase in the concentration of glucose (GLU) and inorganic phosphate (PHOS) immediately after anaesthesia. Clove oil anaesthesia had not effect on other biochemical indices. Histological examination showed capillary ectasia of gill filaments immediately after clove oil anaesthesia. Twenty-four hours after anaesthesia, no ectasia was observed. No histopathological changes were demonstrated in other tissues following anaesthesia. Results of the examinations suggest that the use of clove oil at a concentration of 30 mg/l does not cause irreversible damage in carp.

**Keywords**: acute toxicity; haematological profile; biochemical profile of blood; histological examination of tissues

Anaesthetics are used with increasing frequency in aquaculture, mainly to reduce the stress and to prevent mechanical damage to fish during handling. Their use is particularly common in stripping, marking, biometry, health checks, etc. (Mundey and Wilson, 1997; Ross and Ross, 1999). The use of anaesthetics is also one of foremost requirements of the Protection of Animals Against Torture Act 246/1992 Sb.

At present, clove oil is used in the Czech Republic for short-term immobilization of fish before artificial spawning. The recommended concentration for anaesthetic purposes is 30 mg/l water bath (Svoboda and Kolarova, 1999; Hamackova et al., 2001).

Clove oil is a dark-brown liquid, a distillate of flowers, stalks and leaves of the clove tree *Eugenia aromatica* (Soto and Burhanuddin, 1995). Clove oil is also distilled from stems, leaves and flower buds of *Eugenia caryophyllata*, and its active ingredient, i.e. eugenol (4-allyl-2-methoxyphenol), makes up 70 to 90% by weight (Isaacs, 1983; Briozzo et al.,

Supported by the USB RIFCH (Project No. MSM 6007665809), by the Ministry of Agriculture of the Czech Republic (Project No. QF 3029) and the Grant Agency of the Czech Republic (Project No. 523/03/H076).

1989; Keene et al., 1998). Besides eugenol acetate (> 17%) and cariofilen 5 (> 12%), clove oil also contains a very broad range of terpene compounds that give the oil its characteristic smell and taste (Ross and Ross, 1999; Taylor and Roberts, 1999). Because of its properties, eugenol is used in a variety of different applications, e.g. as an antioxidant (Kremer, 1985; Nagababu and Lakshmaiah, 1992; Pulla Reddy and Lokesh, 1992; Rajakumar and Rao, 1993), antimycotic (Bullerman et al., 1977; Karapinar, 1990) and an antibacterial (Karapinar and Aktug, 1987; Briozzo et al., 1989; Moleyar and Narasimham, 1992), but also as an additive used in certain cigarettes (Voie et al., 1986; Guidotti, 1989). Endo et al. (1972) described the use of clove oil as an anaesthetic in warm-blooded animals.

At present, effects on clove oil on commercially produced fish are studied in a project regarding the application of principles of pharmacovigilancy in aquaculture in the Czech Republic. In the first stage of the project, effects of clove oil on rainbow trout were studied (Velisek et al., 2005). The aim of the present study was to investigate acute toxicity of clove oil in carp and, on the basis of haematological indices, biochemical blood profile values and histological examination of tissues, to assess the changes in the organism of carp induced by the anaesthetic.

## MATERIAL AND METHODS

In the study, clove oil marketed by the Kulich Company (Jan Kulich, Hradec Kralove/Ricany, CR) in 10 ml and 50 ml containers was used.

### Acute toxicity of clove oil

Acute toxicity of clove oil was ascertained by the OECD 203 "Test of acute toxicity for fish". For the 96 h and 10 min LC50 trials, carp (mirror carp M 72) of 15.0 ± 5.0 g (mean ± SD) body weight and 110 ± 21 mm body length were used.

**The 96-h LC50 test**: Experimental fish were exposed to concentrations 5, 10, 13, 15, 18, 20 and 30 mg/l clove oil dissolved in diluting water (pH 7.62; acid neutralization capacity – $ANC_{4.5}$ 1.19 mmol/l; total ammonia 0.04 mg/l; $NO_3^-$ 7.35 mg/l; $NO_2^-$ 0.002 mg/l; $PO_4^{3-}$ 0.02 mg/l; chemical oxygen demand – $COD_{Mn}$ 1.6 mg/l), and controls were placed in diluting water with no tested substance added. Ten carp were used for each concentration and for the control group. The fish and its behaviour, water temperature, pH and oxygen saturation were monitored throughout the tests at individual concentrations and in the control aquarium. Mean lethal concentration (96hLC50) and also 96hLC0.1 and 96hLC99.9 were calculated from mortality rates over the period of 96 hours.

**The 10-min LC50 test**: For 10 min, the fish were exposed to concentrations of 30, 50, 70, 90 and 110 mg/l of clove oil dissolved in diluting water. Ten carp were used for each concentration and for the control group. Diluting water of the same parameters as in previous trials was used. During the 10-min test period, changes in physiological parameters of fish and fish mortality figures were recorded, and after the carp had been moved to clean water, the time of their recovery from anaesthesia was determined. Mean lethal concentrations (10minLC50) and also 10minLC0.1 and 10minC99.9 were calculated from mortality rates over the period of 10 minutes.

Within the tests, the onsets of individual phases of anaesthesia and recovery rates were studied. Evaluations were made in four consecutive phases (Thienpoint and Niemegeers, 1965; Yoshikawa et al., 1988):

1. acceleration and subsequent deceleration of opercular movements, a partial loss of reactivity to external stimuli
2. loss of equilibrium, opercular movements very slow, fish still reactive to strong stimuli
3. total loss of reactivity, fish are lying at the tank bottom and do not respond to handling
4. complete cessation of opercular movements, fish die if left in the bath for too long

Lethal concentration levels (LC50, LC0.1 and LC99.9) were determined by the probit analysis using EKO-TOX 5.1 software.

### Haematological profile after exposure to clove oil

For the haematological profile tests, carp (mirror carp M 72) of 525 ± 43 g (mean ± SD) body weight and 320 ± 30 mm body length were used. A total of 40 fish divided into four groups were examined: Control I (before the anaesthetic administration), Experiment I (immediately after 10 min anaesthesia at the concentration of 30 mg/l), Experiment II (24 hrs after 10 min anaesthesia) and Control II

(controls examined in parallel with Experiment II). Heparinized injection needles were used to take samples of blood from hearts of fish stunned by a blow with a blunt object over the head. To stabilize blood samples, aqueous solution of heparin sodium salt 5 000 U/ml at 0.01 ml per 1 ml blood was used (Svobodova et al., 1991).

The indices used to evaluate the haematological profile included the erythrocyte count (Er), haemoglobin concentration (Hb), haematocrit (PCV), mean erythrocyte volume (MCV), mean colour concentration (MCHC), erythrocyte haemoglobin (MCH), leukocyte count (Leuko) and the differential leukocyte count (leukogram). The procedures were based on Unified methods for haematological examination of fish (Svobodova et al., 1991).

Results of haematological examinations were tested by the variance analysis using the Statgrafics (ANOVA – Tuckey Test) software.

### Biochemical blood plasma profile after exposure to clove oil

For biochemical profile of blood plasma tests, carp (mirror carp M 72) of 525 ± 43 g (mean ± SD) body weight and 320 ± 30 mm body length were used.

Blood plasma was obtained by centrifuging blood samples in a cooled centrifuge (4°C, 837 × g). Biochemical indices determined in blood plasma included glucose (GLU), total protein (TP), albumin (ALB), total globulins (GLOB), ammonia ($NH_3$), triacylglycerols (TAG), aspartate aminotransferase (AST), alanin aminotransferase (ALT), lactate dehydrogenase (LDH), creatin kinase (CK), calcium ($Ca^{2+}$) and inorganic phosphate (PHOS). For the biochemical analysis of blood plasma, the VETTEST 8008 analyzer (IDEXX Laboratories Inc. U.S.A.) manufactured by Medisoft was used.

Results of biochemical examination were tested by the variance analysis using the Statgrafics (ANOVA – Tuckey Test) software.

### Histological examination of tissues

For histological examination of tissues, carp (mirror carp M 72) of 525 ± 43 g (mean ± SD) body weight and 320 ± 30 mm body length were used.

After blood sampling, samples of gills, liver, cranial and caudal kidney, and spleen and skin were taken for histological examinations. The samples taken were immediately fixed in 10% formaldehyde, drained and embedded in paraffin. Sections were made of the paraffin blocks and stained with haematoxylin-eosin.

## RESULTS

**Acute toxicity of clove oil**. During the 96-hour LC50 tests, the mean water temperature was 20.0 to 21.1°C, pH was 7.43–7.71 and water oxygen levels were 80–98% saturation. On the basis of tests of acute toxicity to carp, the 96-hour lethal concentrations of clove oil were determined (96hLC50 18.10 mg/l, 96hLC0.1 15.45 mg/l and 96hLC99.9 19.80 mg/l).

The autopsy performed after the acute toxicity test revealed increased amounts of watery mucous on body surfaces, and the gills were matt dark in

Table 1. Effects of clove oil anaesthesia on haematological indices in carp ($n$ = 10)

| Indices | Control I (before anaesthesia) | Experiment I (immediately after anaesthesia) | Experiment II (24 hrs after anaesthesia) | Control II (after 24 hrs) |
|---|---|---|---|---|
| Er (T/l) | 1.66 ± 0.28[a] | 1.65 ± 0.37[a] | 1.58 ± 0.21[a] | 1.65 ± 0.21[a] |
| Hb (g/l) | 74.25 ± 11.29[a] | 80.0 ± 10.17[a] | 84.22 ± 4.14[a] | 81.0 ± 8.83[a] |
| PCV (l/l) | 0.27 ± 0.04[a] | 0.32 ± 0.05[a] | 0.33 ± 0.05[a] | 0.29 ± 0.03[a] |
| MCV (fl) | 164.61 ± 19.69[a] | 197.21 ± 36.91[a] | 210.57 ± 28.45[a] | 176.80 ± 27.09[a] |
| MCH (pg) | 45.14 ± 4.74[a] | 50.04 ± 8.81[a] | 53.90 ± 6.43[a] | 49.59 ± 6.67[a] |
| MCHC (g/l) | 275.53 ± 21.88[a] | 255.45 ± 27.61[a] | 256.62 ± 12.10[a] | 281.54 ± 18.22[a] |
| Leuko (G/l) | 27.10 ± 9.20[a] | 33.0 ± 10.85[a] | 23.50 ± 7.98[a] | 28.10 ± 9.34[a] |

Groups with different alphabetic superscripts differ significantly at $P < 0.05$ (ANOVA)

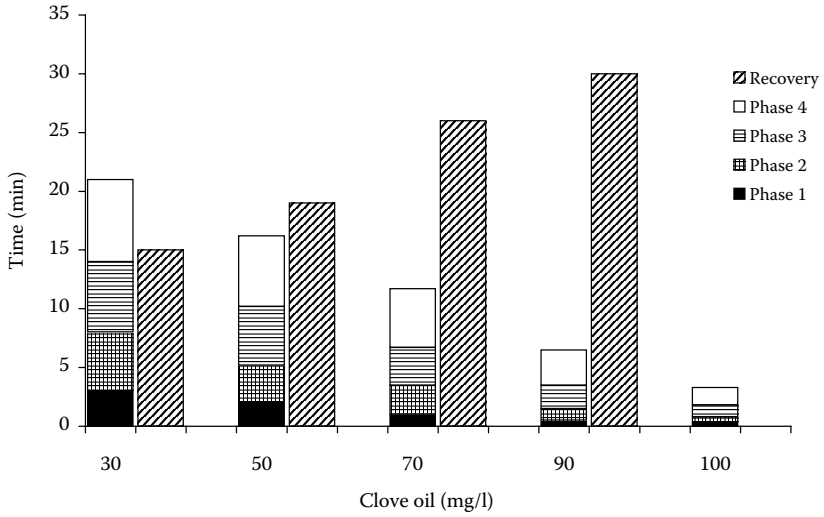


Figure 1. Effects of clove oil concentrations on the onset of individual phases of anaesthesia and recovery in carp

colour. The body cavity contained excess moisture, and an increased injection of visceral vessels was also obtained.

During 10-min LC50 tests, water temperature was 19.9°C, pH was 7.50 and water oxygen level was at 98% saturation. On the basis of tests of acute toxicity to carp, the 10-min lethal concentrations of clove oil were determined (10minLC50 74.3 mg/l, 10minLC0.1 51.6 mg/l and 10minLC99.9 110.1 mg/l).

Effects of clove oil concentrations on the time of onset of anaesthesia, duration of its individual stages and the course of recovery are showed on Figure 1.

**Haematological profile after exposure to clove oil**. Effects of clove oil on the haematological profile of carp are showed in Tables 1 and 2.

The 10-min exposure to the anaesthetic at a concentration of 30 mg/l had no effect on the haematological indices studied (Er, Hb, PCV, MCV, MCHC, MCH, Leuko, leukogram).

**Biochemical blood plasma profile after exposure to clove oil**. Effects of clove oil on the blood plasma biochemical profile of carp are given in Table 3. The 10-min exposure to clove oil at a concentration of 30 mg/l caused a significant ($P < 0.01$) increase in the concentration of glucose and inorganic phosphate immediately after anaesthe-

Table 2. Effects of clove oil anaesthesia on differential leukocyte counts in carp ($n = 10$)

| Indices | | Control I (before anaesthesia) | Experiment I (immediately after anaesthesia) | Experiment II (24 hrs after anaesthesia) | Control II (after 24 hrs) |
|---|---|---|---|---|---|
| Lymphocytes | % | 82.50 ± 7.69[a] | 79.70 ± 5.93[a] | 79.60 ± 7.54[a] | 75.60 ± 10.67[a] |
| | G/l | 22.32 ± 8.15[a] | 26.44 ± 9.73[a] | 18.91 ± 7.07[a] | 29.20 ± 7.98[a] |
| Monocytes | % | 1.50 ± 1.35[a] | 1.30 ± 1.06[a] | 1.50 ± 1.51[a] | 2.40 ± 1.35[a] |
| | G/l | 0.38 ± 0.36[a] | 0.40 ± 0.28[a] | 0.31 ± 0.31[a] | 0.83 ± 0.34[a] |
| Neutrophile granulocytes segment | % | 4.20 ± 4.64[a] | 4.0 ± 2.31[a] | 5.80 ± 4.92[a] | 7.70 ± 4.42[a] |
| | G/l | 1.19 ± 1.28[a] | 1.40 ± 1.01[a] | 1.25 ± 0.89[a] | 3.16 ± 2.34[a] |
| Neutrophile granulocytes bands | % | 3.80 ± 2.74[a] | 3.70 ± 2.71[a] | 3.90 ± 2.69[a] | 5.40 ± 3.31[a] |
| | G/l | 1.12 ± 1.01[a] | 1.40 ± 1.01[a] | 0.98 ± 1.01[a] | 1.98 ± 1.14[a] |
| Developmental phases – myeloid sequence | % | 8.00 ± 2.35[a] | 11.30 ± 3.55[a] | 9.20 ± 2.12[a] | 8.90 ± 4.31[a] |
| | G/l | 2.17 ± 0.64[a] | 3.75 ± 1.17[a] | 2.16 ± 0.50[a] | 2.50 ± 1.21[a] |

Groups with different alphabetic superscripts differ significantly at $P < 0.05$ (ANOVA)

Table 3. Effects of clove oil anaesthesia on biochemical indices of blood plasma in carp ($n$ = 10)

| Indices | Control I (before anaesthesia) | Experiment I (immediately after anaesthesia) | Experiment II (24 hrs after anaesthesia) | Control II (after 24 hrs) |
|---|---|---|---|---|
| GLU (mmol/l) | 6.56 ± 1.23[a] | 8.87 ± 1.02[b] | 6.97 ± 1.41[a] | 6.98 ± 1.18[a] |
| TP (g/l) | 34.20 ± 5.10[a] | 34.70 ± 4.68[a] | 33.90 ± 5.26[a] | 34.30 ± 5.48[a] |
| ALB (g/l) | 7.80 ± 1.20[a] | 7.90 ± 0.89[a] | 7.40 ± 1.11[a] | 8.0 ± 1.02[a] |
| GLOB (g/l) | 26.40 ± 2.48[a] | 26.80 ± 3.10[a] | 26.60 ± 2.21[a] | 26.0 ± 2.68[a] |
| $NH_3$ (µmol/l) | 534.0 ± 2.03[a] | 529.0 ± 2.59[a] | 526.0 ± 3.54[a] | 530.0 ± 2.69[a] |
| TAG (mmol/l) | 1.21 ± 0.11[a] | 1.20 ± 0.24[a] | 1.23 ± 0.16[a] | 1.22 ± 0.13[a] |
| AST (µkat/l) | 1.40 ± 0.19[a] | 1.70 ± 0.23[a] | 1.57 ± 0.14[a] | 1.53 ± 0.18[a] |
| ALT (µkat/l) | 0.23 ± 0.05[a] | 0.25 ± 0.04[a] | 0.23 ± 0.03[a] | 0.22 ± 0.05[a] |
| LDH (µkat/l) | 4.05 ± 0.44[a] | 4.73 ± 0.61[a] | 3.99 ± 0.51[a] | 4.21 ± 0.60[a] |
| CK (µkat/l) | 13.50 ± 0.09[a] | 13.50 ± 0.10[a] | 13.70 ± 0.08[a] | 13.60 ± 0.09[a] |
| $Ca^{2+}$ (mmol/l) | 2.48 ± 0.21[a] | 2.52 ± 0.11[a] | 2.46 ± 0.32[a] | 2.48 ± 0.18[a] |
| PHOS (mmol/l) | 1.26 ± 0.11[a] | 3.41 ± 0.42[b] | 1.34 ± 0.16[a] | 1.41 ± 0.12[a] |

Groups with different alphabetic superscripts differ significantly at $P < 0.01$ (ANOVA)

sia. Their values returned back to normal within 24 hours. The rest of the indices (TP, ALB, GLOB, $NH_3$,TAG, AST, ALT, LDH, CK, and $Ca^{2+}$) were at comparable levels in all groups.

**Histological examination of tissues**. All specimens of common carp showed capillary ectasia of gill filaments immediately after clove oil anaesthesia. Twenty-four hours after anaesthesia, no ectasia was observed. No histopathological changes were demonstrated in other tissues (liver, spleen, cranial and caudal kidneys) following anaesthesia.

## DISCUSSION

Hikasa et al. (1986) recommended 25–100 ppm clove oil as effective anaesthesia for the common carp (*Cyprinus carpio*). It has been demonstrated that onset times of individual stages of clove oil anaesthesia as well as recovery times (Figure 1) were concentration-dependant. The same effect of anaesthetic concentration levels on anaesthesia onset times has been described by Hirata et al. (1970) for the crucian carp (*Carassius carassius*) and by Hamackova et al. (2004) for the tench (*Tinca tinca*).

Waterstrat (1999) reported 100 mg/l clove oil as a safe concentration for anaesthesia of the channel catfish (*Ictalurus punctatus*), adding that exposures longer than 15 min prolonged recovery times and increased mortality. Walsh and Pease (2002) recommended 60–80 mg/l clove oil for anaesthesia of anguillid eels (*Anguilla reinhardti* ) because it is effective, relatively inexpensive, and poses little risk to human health.

To evaluate haematological and biochemical profiles of blood and histopathological changes in tissues of carp, clove oil concentration of 30 mg/l was used in the present study. Haematological and biochemical profiles of blood can provide important information about the internal environment of the organism (Masopust, 2000). Values determined in the present study suggest that internal organs and tissues of carp are not altered by clove oil anaesthesia. That conclusion was also confirmed by the result of histological examination of parenchymatous organs.

In our experiments with carp, a significant increase ($P < 0.01$) in blood plasma glucose immediately after the 10-min clove oil anaesthesia was observed. Increased glucose level returned to normal 24 hours after anaesthesia. Increased blood plasma glucose level after anaesthesia indicates that the procedure caused some stress in the carp. These findings are in accord with results of Holloway et al. (2004) and Velisek et al. (2005) who also detected increase of glucose concentration in rainbow trout (*Oncorhynchus mykiss*) following

clove oil anaesthesia. On the other hand, Iverzen et al. (2003) found no change in the concentration of glucose in Atlantic salmon (*Salmo salar*) following clove oil anaesthesia.

The disadvantage of clove oil is its relatively low therapeutic index, i.e. the ratio between the therapeutic and the toxic concentrations. The generally reported optimum ratio is 1 : 4 or higher (Svobodova and Vykusova, 1991). A comparison between the concentration used in a 10-min anaesthesia of fish (30 mg/l) and the 10minLC50 values found (74.3 mg/l) suggests that the clove oil therapeutic index is 1 : 2.5. According to Taylor and Robers (1999) clove oil is an efficient and relatively safe anaesthetic.

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Received: 05–02–10
Accepted after corrections: 05–05–12

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