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MULTIPLE EXPOSURE OF ASIAN SEA BASS, (*LATES CALCARIFER*, CENTROPOMIDAE) TO CLOVE OIL: A HISTOPATHOLOGICAL STUDY

(With 1 Table and 4 Figures)

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التعرض المتعدد لأسماك القاروص الآسيوي لزيت القرنفل: (دراسة هستوباثولوجية)

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تم دراسة ووصف التغيرات الهستوباثولوجية لأنسجة أسماك القاروص الآسيوي بعد التعرض المتعدد لزيت القرنفل كمادة مخدرة. تعرضت الأسماك إلى تركيزات ٣و٦و٩ جزء في المليون من زيت القرنفل وذلك بإضافة هذه التركيزات في ٣ لتر من مياه البحر. تعرضت مجاميع الأسماك إلى هذه التركيزات لمدة ٤ دقائق ثم نقلت إلى مياه مالحة بدون الزيت لمدة ساعة. تم إعادة هذا التعرض ثلاث مرات متتالية . أما ضوابط التجربة فتم بقائها في المياه المالحة بدون زيت. تم أخذ الخياشيم ، القلب ، الكبد ، الكلية الخلفية من الأسماك المعرضة للتركيزات المختلفة وكذلك ضوابط التجربة ثم تثبيتها في ١٠% فورمالين وتمريرها وتقطيعها وصباغتها بالهيماتوكسلين والأيوسين وفحصها بالميكروسكوب الضوئي. أظهرت الدراسة عدم وجود تغيرات في أنسجة الأسماك المعرضة إلى ٣و٦ جزء في المليون من زيت القرنفل بالمقارنة بضوابط التجربة. أما الأسماك المعرضة إلى ٩ جزء في المليون فكان هناك تنكرز (موت) للخلايا المبطنة للرقائق الثانوية للخياشيم وخاصة في الجزء العلوي من الخيط الخيشومي . كان هناك منطقة محددة نزفية في الطبقة الأولى لقلب الأسماك المعرضة إلى ٩ جزء في المليون والتي قد تعلل زيادة طفيفة في معدل ضربات القلب. لم يستدل على أي تغيرات في الكلبد أو الكلية الخلفية في المعاملات بالمقارنة بضوابط التجربة وهذا يشير إلى أنه لايوجد أثر تراكمي لزيت القرنفل.

SUMMARY

The study described the histopathological examination of Asian sea bass, *Lates calarifer* after being subjected to mulitple exposure of 3, 6 and 9 p.p.m. clove oil. The gills, hearts, livers and posterior kidneys of both

exposed and non-exposed fish to clove oil fixed in 10% formalin, processed and examined by light microscopy. The gills of fish exposed to 3 and 6 p.p.m. did not differ from control, while, fish exposed to 9 p.p.m. clove oil had necrosis of the epiethelium lining the secondary lamellae of the distal region (tips) of the gill filament. Focal subepicardial haemorrhage was observed in the hearts of fish exposed to 9 p.p.m. indicating that a slight increase in heart rate may have occurred. There were no alterations in the livers and posterior kidneys of exposed fish compared to control indicating the rapid elimination of the clove oil.

Key words: Lates calcarifer, Clove oil, Histopathology.

INTRODUCTION

The major role of anaesthetics in fish husbandry is to minimize stress during sorting, tagging, spawning and transportation (NRC 1994). Burhanuddin et al. (1989 showed the potential of clove oil as an anaesthetic in rabbitfish, *Siganus guttatus*. Clove oil is derived from the stem, leaves and buds of *Eugenia caryophyllata* tree and its active ingredient is eugenol (4-allyl-2-methoxyphenol) (Isaacs 1983). Clove oil was evaluated on rainbow trout, *Onchorhynchus mykiss* (Walbaum). The study showed that the 8-96 h Lc_{50} for eugenol was 9 p.p.m. The induction time was found to be faster and at low concenteration compared to MS_{-222} (Keene et al., 1998). The anaesthetic effect of euganol hve been studied also on rabbitfish, gold fish, and Crucian carp (Soto and Burhanuddin, 1955 and Endo et al., 1972). There are many advantages on the use of clove oil in aquaculture including low cost, limited withdrawl time, and its relative safety for both fish and human (Keene et al., 1998 and Soto and Burhanuddin, 1995). Clove oil was used safely at a dose of 10 p.p.m. during sampling and transportation of the camouflage Grouper, *Epinephelus polyphekadion* raised at the hypersaline water of the Fish Farming Center, Kindom Of Saudi Arabia (unpublished data).

The present investigation describes the histopathological alterations in Asian sea bass *calcarifer* subjected to multiple exposures of 3, 6 and 9 p.p.m. clove oil.

MATERIAL and METHODS

Asian sea bass, *Lates calcarifer* reared under hypersaline water conditions and environment (salinity 43% and temperature of 29-30°C) of the Fish Farming Center, Jeddah, Kingdom of Saudi Arabia. Fish were fed to satiation on artificial food (2 mm particle size, 42% protein, and at a rate of 20%). Twenty fish with an average size 42± 9.74 g were randomly assigned to the treatments and control and kept in buckets provided with aereation. The water temperature was 30°C.

Ten ml of clove oil (Clove oil BP, Mayflower close, Ubichem PIC UK) was dissolved in 1 liter boiled fresh water. Eighteen fish were axposed to 3 p.p.m. of clove oil (0.75 ml/31 sea water, n=6), 6 p.p.m. (1.5 ml/31 sea water, n=6), and 9 p.p.m. (3 ml/31 sea water, n=6). Two control fish were kept in seawater without oil. Treated groups were exposed to selected doses of clove oil for 4 minutes then transferred to 201 seawater in buckets with aeration and kept for 1 hour for observation. Treated fish were exposed to three times in the three treatments.

The induction and recovery times per minutes were recorded for each dose and observation for any mortality or abnormal behaviour was made. Treated fish after being subjected to multiple exposures were killed by pithing the brain tissue. The second gill archs, hearts, livers, and posterior kidneys of both treated and control fish were taken immediately, fixed in 10% formalin, dehydrated, embedded in paraffin, sectioned at 4-6u, stained with haematoxylin and eosin and examined by light microscope.

RESULTS

Table (1) showed the induction and recovery times in exposed and non-exposed Asian sea bass, *Lates calcarifer* to different concentrations of clove oil. Data were expressed as the pooled mean ± the standard error. Mortality or abnormal behaviour of fish was not observed in this study.

Table 1: The induction and recovery times (per minutes) in Asian sea exposed to different concentrations of clove oil.

Dose (p.p.m.)	Induction Time	Recovery Time
3(n=6)	0	0
6(n=6)	3.0±1.5	1.0±0.5
9(n=6)	2.8±1.0	3.5±1.5

Histopathology:

Gills:

Control fish kept in sea water showed the normal architecture of the gill tissue, as there were no evidence for lamellar fusion, hyperplasia or epithelial necrosis (Fig. 1). Fish exposed to 3 p.p.m. were the same as control. Fish exposed to 6 p.p.m. had slight epithelial lifting of the distal region (tips) of the gill filament.

There was also no evidence for hyperplasia of the interlamellar epithelium. Fish exposed to 9 p.p.m. showed necrosis of the epithelial lining the secondary lamellae of the distal region (tips) of the gill filament (Fig. 2). There were also no evidence for hyperplasia or lamellar fusion.

Hearts:

Control and fish exposed to 3 and 6 p.p.m. had the normal appearance of myocardial muscle fibers arrangements. There were no evidence for myocardial degeneration or other inflammatory changes (Fig. 3). While, fish exposed to 9 p.p.m. had focal subepicardial haemorrhage (Fig. 4).

Livers and Posterior Kidneys:

There were no detectable changes observed in exposed fish compared to control.

DISCUSSION

In this study, the induction time was almost three minutes for the doses of 6 and 9 p.p.m., while, the recovery time was one and three and half minutes for the doses of 6 and 9 p.p.m. This observation met the first criterion used to evaluate the ideal anaesthetic, which is 3 and 5 minutes for the induction and recovery times, respectively (Markeing and Meyer's, 1985).

The present investigation showed that a dose of 3 and 6 p.p.m. did not elicit any histopathology in terms of lamellar hyperplasia, lamellar fusion, lamellitis or lamellar epithelial necrosis compared to control. While, the lamellar epithelial necrosis observed at a dose of 9 p.p.m. was limited to the distal part of the gill filament, which may not have any significant effect on respiration. Previous reports indicated that the lesions which are limited to the distal end of the filament may not have any significant effect on fish physiology (Booth 1979; Ferguson 1989 and Nowar and Lucas, 1997). The analgestic effects of clove oil result from the inhibition of prostaglandin H synthase (PHS) by euganol

(Dewhirst & Goodoson, 1974; Thompson & Eling, 1989 and Pongprayoon et al., 1991). The major area of entery and excretion of anaesthetics in fish is throught the gills (Hunn and Allen, 1974 and Ferreria et al., 1984). The necrosis observed in the distal region of the gill filaments at a dose of 9 p.p.m. was considered minimal because of the lesion location.

The focal subepicardial haemorrhage observed in the hearts of fish exposed to 9 p.p.m. suggests that a slight increase in the heart rate may have occurred. This result is expected, since the use of some anaesthetics was associated with increased heart rate (Raandall, 1982). The present study showed that no detectable changes were observed in the livers and posterior kidneys of fish subjected to multiple exposure of clove oil and control, as the previous reports have shown that euganol and its conjugates and metabolites are rapidly lost from the circulation and tissues of man. Moreover, euganol was considered neither toxic nor carcinogenic in rats, mice, and chinese hamsters (Maura et al., 1989; Fisher and Debgker, 1990; Fisher et al., 1990 and Phillips, 1990).

CONCLUSIONS

The present study showed that clove oil can act as anaesthetic at levels of 6 and 9 p.p.m. in Asian sea bass, *Lates calcarifer*. Low doses of eugenol produced effects comparable to high doses, so there is a larger safety margin than with MS-222 (Keene et al., 1998). Histopathological alaterations were minimal and limited to the distal parts of the gill filament. Likeise, the clover oil was rapidly eliminated in the exposed fish as evident by the absence of changes in the livers and posterior kidneys.

FIGURE CAPTIONS

Fig. 1: Cntrol fish of Asian sea bass, *Lates calcarifer* showing the normal architecture of the gill tissue. Haematoxylin and Eosin. X 100.

Fig. 2: Asian sea bass, *Lates calcarifer* exposed to 9 p.p.m. clove oil showing necrosis of the lamellar epithelium of the distal region of the gill filament(n). Haematoxyline and Eosin. X400.

Fig. 3: Control fish of Asian sea bass, *Lates calcarifer* showing the normal appearance of myocardial muscle fibers arrangements. Haematoxylin and Eosin. X100.

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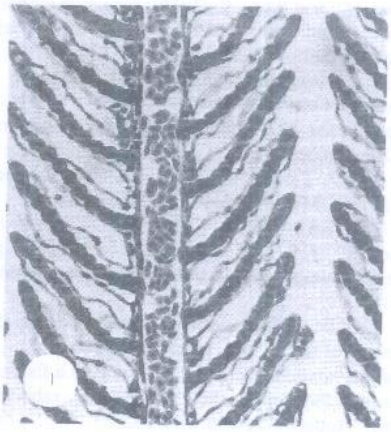

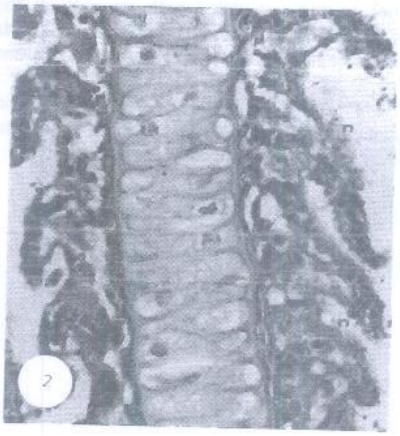

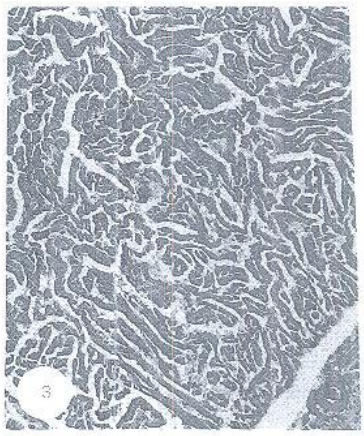
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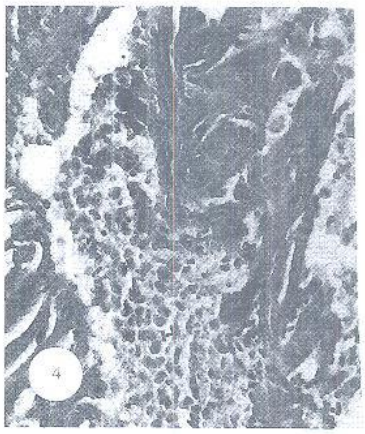
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