Ass. Univ. Bull. Environ. Res. Vol. 3 No. 1, March 2000

AUCES

TOXIC EFFECTS OF THE FUNGICIDE MALACHITE GREEN ON CATFISH (*CLARIAS GARIEPINUS*)

Khaled A. Abdou[1] and Zakaria. M. Zaky[2]

1-Dept. of Forensic Med. and Toxicology, Faculty of Vet. Med.,(Beni-Suef), Cairo University
2-Dept. of Forensic Med. and Toxicology, Faculty of Vet. Med., Assiut University.

ABSTRACT :

The sub-clinical toxic effect of the fungicide malachite green in fish especially on the reproductive system was investigated. One hundred and twenty male and female catfish were divided into two groups (n=60). The first group was exposed to 0.1 mg malachite green per liter in water for three weeks while the second group was kept as control. Then the exposed fish were transferred to non treated clean water for another two weeks. At the 3rd, 4th and 5th weeks from the onset of the experiment, blood, liver, kidney, testis and ovaries samples were collected from twenty fish (ten from each sex) of each group for biochemical and histopathological examination. Gonadosomatic index (GSI), cortisol, estradiol 17 β, testosterone, AST, ALT, total protein, creatinine and urea were estimated. The results revealed a significant increase in cortisol at the 3rd and 4th weeks and AST, ALT, creatinine and urea at the 3rd, 4th and 5th weeks post-exposure. A significant decrease in estradiol 17 β at the 3 rd week, testosterone at the 3 rd and 4 th weeks and total protein level at the 3rd, 4th and 5th weeks post-exposure were recorded. Histopathological changes were examined and described.

INTRODUCTION :

Fishes are commonly infected by various species of aquatic fungi as fungal infections or saprolegniasis which usually considered secondary to bacterial or viral infections or may be act as primary pathogens and often follow physical damage to the surface of fish caused by handling and intensive culture conditions [1,2&3]. Fungal infections of hatchery reared fish can usually be controlled with malachite green [4&5].

In spite of its powerful antifungal activities, malachite green induced a potential teratogenic effects [6]. Malachite green have been shown to decrease the opercular ventilation rate per minute and to cause respiratory distress to the

Oreochromis niloticus **fingerlings [7]. Malachite green fungicide decreased the total protein concentration in plasma of common carp fish treated with 0.5 mg malachite green/litre and cause mild dystrophic changes in parenchymatous tissues [8]. They concluded that these changes indicated an increased sensitivity to hypoxia and impaired protein synthesis in the treated fish.**

Severe degenerative changes were shown in the different parenchymatous tissues in fish [9&10]. Malachite green increased the mortality rate in *Cyprinus carpio, Clarias gariepinus* and *Heteroclarias fry* [11&12].

Concerning the urgent use of malachite green as an antifungal especially in the hatchery-reared fish. The present study was carried out to investigate the sub clinical effect in catfish (*Clarias gariepinus*) especially on the reproductive system.

MATERIAL AND METHODS :

1- Chemical:

Malachite green pure was obtained from A.R., Koch-light laboratories Ltd. Colnb-rook Bucks England.

2- Fish:

A total number of one hundred and twenty apparently healthy mature catfish (Clarias *gariepinus*) with an average body weight 160±10 g and body length of 23 ±5 cm, were brought from Bahr Yossef channel, Beni-Suef. Fish were kept in full glass aquaria supplied with aerated chlorine free water seven days prior to the experiment.

3- Experimental design and dosing:

Fish were divided into two equal groups each of 60. The first group was exposed to malachite green at the concentration of 0.1 mg/L water for 60 minutes daily regularly for three weeks then followed by a withdrawal time of two weeks in clean water as treated one. The second group was considered as control. The experiment lasted for 5 weeks and 20 fish were sacrificed at the end of the exposure period and weekly during the withdrawal one. Blood samples were collected to obtain serum which was kept frozen at -20 °C till assaying for Cortisol, Estradiol- 17β and Testosterone hormones [13,14&15] while gonadosomatic index (GSI) was determined according to [16].

Serum transaminases (AST&ALT), protein, urea and creatinine were determined colorimetrically according to [17,18, 19 & 20] respectively.

Liver, kidney, testis and ovaries were fixed in 10% formalin for histopathological examination according to Carleton [21]. Statistical analysis of the obtained data were performed according to the methods described by Snedecor [22].

RESULTS :

Data representing gonadosomatic index (GSI) in female and male catfish exposed to malachite green for three weeks which immediatly transfered to clean water for another two weeks (Table 1&2) indicated that GSI was not influenced at any time of the experimental period.

Cortisol hormone level (ng/dl) was significantly increased (P≤0.001) at the end of the third week (16.89±0.16) and one week after the transfer of the fish to the clean water (10.32 ± 0.21) compared with the control fish (8.60±0.13 and 8.55±0.04) respectively (Table 1&2).

Serum Estradiol 17β (ng/ml) was significantly decreased (P≤0.05) at the end of the third week (1.67±0.21) versus (2.37±0.16) in control group while there were no significant changes after the transfer of the fish to clean water (Table 1&2).

Testosterone level was decreased in the 3rd and 4th week of the experiment in the experimental groups (P≤0.02) (8.24±0.61 and 8.9±0.24) compared with the control (10.40±0.40 and 10.10±0.32) respectively (Table,1&2).

Serum AST and ALT (u/ml) were significantly increased (P≤0.001) at the end of the third week, one and two weeks after the transfer of the fish into clean water (16.21±0.42 and 9.49±0.19), (12.6±0.17 and 8.59±0.32) and (10.44±0.47 and 5.67±0.12) compared with the control (6.17±0.09 and 4.68±0.19), (6.20±0.03 and 4.58±0.08) and (6.17±0.03 and 4.05±0.67) respectively. While serum total protein (mg/100 ml) was significantly reduced (P≤0.01) at the end of the third week and one week after the transfer of the fish to clean water (0.79±0.38 and 0.85±0.43) and reduced (P≤0.05) two weeks after the transfer of the fish to clean water (1.39±0.31) compared with control (4.08±0.90, 4.10±0.03 and 4.11±0.03) respe-ctively (Table 1&2).

Serum creatinine (mg/dl) showed significant increase (P≤0.001 and 0.01) at the end of the third week, one and two weeks after the transfer of fish to clean water (2.07±0.46; 1.9±0.42 and 1.83±0.39) as compared with the control (0.52±0.01; 0.49±0.01 and 0.50±0.02) respectively. A significant increase in the serum urea (mg/dl) at (P≤0.001) allover the experiment (62.06±1.40; 45.25±0.69 and 36.19±1.46) was recorded in comparison with the control (25.98±0.58; 25.59±0.17 and 25.89±0.01) respectively (Table 1&2).

Histopathological changes:

The result of histopathological findings revealed a diffuse proliferation of kupffer cells in-between the laminae of the hepatocytes with sever dilatation of central veins and sinusoids. The portal area was infiltrated by inflammatory cells, extra-vasated red blood cells with fibroblastic proliferation inaddition to dilatation of portal vein. Cystic dilatation of some bile ducts was observed (Fig. 1). Diffuse extravasated red blood cells were observed in-between the degenerated renal tubules and hyperemic glomeruli. The intertubular blood vessels were severely dilated and engorged with blood (Fig. 2) while there was no histopathological findings observed in the ovarian and testicular tissues.

Table (1): Effect of malachite green exposure on GSI%, Cortisol, Estradiol- 17β, Testosterone, AST, ALT, total protein, creatinine and urea after 3 weeks in catfish (C*larias gariepinus*)

Parameters	Treated group	Control group
GSI (%) Females	11.59±0.12	11.93±0.13
GSI (%) Males	0.70±0.036	0.68±0.021
Cortisol (ng/dl)	16.89±0.16****	8.60±0.13
Estradiol 17 β (ng/ml)	1.67±0.21*	2.37±0.16
Testosterone (ng/dl)	8.24±0.61**	10.40±0.40
AST (u/ml)	16.21± 0.42	6.17±0.09
ALT (u/ml)	9.49±0.19****	4.68±0.19
Total protein (mg/100ml)	0.79±0.38***	4.08± 0.90
Creatinine (mg/dl)	2.07±0.46***	0.52±0.01
Urea (mg/dl)	62.06±1.40****	25.98±0.58

Values represents X ± SE

Significant * P ≤0.05 ** P≤ 0.02 *** P≤ 0.01 **** P ≤0.001

Table (2): GSI%, Cortisol, Estradiol- 17β, Testosterone, AST, ALT, total protein, creatinine and urea in the treated catfish (C*larias gariepinus*) after rearing in clean water for 2 weeks

Parameters	Weeks	Treated group	Control group
GSI (%) Females	4th	11.52±0.15	11.66±0.11
	5th	11.95±0.11	11.49±0.10
GSI (%) Males	4th	0.75±0.020	0.75 ±0.016
	5th	0.69±0.19	0.68±0.015
Cortisol (ng/dl)	4th	10.32±0.21****	8.55±0.04
	5th	8.36±0.18	8.66±0.14
Estradiol 17 β (ng/ml)	4th	1.74±0.28	2.37±0.05
	5th	2.05±0.13	2.31±0.02
Testosterone (ng/dl)	4th	8.90±0.24 **	10.10±0.32
	5th	8.78±0.14	9.61±0.35
AST (u/ml)	4th	12.60±0.17****	6.20± 0.03
	5th	10.44±0.47****	6.17 ± 0.03
ALT (u/ml)	4th	8.59±0.32****	4.58± 0.08
	5th	5.67±0.12****	4.05±0.67
Total protein (mg/100ml)	4th	0.85±0.43***	4.10±0.03
	5th	1.39±0.31*	4.11±0.03
Total protein (mg/100ml)	4th	1.9±0.42**	0.49±0.01
	5th	1.83±0.39**	0.50±0.02
Urea (mg/dl)	4th	45.25±0.69****	25.59±0.17
	5th	36.19±1.46****	25.89±0.01

Values represents X ± SE

Significant * P ≤0.05 ** P≤ 0.02 *** P≤ 0.01 **** P ≤0.001

Fig. (1): Liver of female catfish exposed for malachite green for three weeks showing leucocytic inflammatory cells infiltration, extravasated red blood cells, fibroblastic proliferation and dilated portal vein in the portal area (H&E, X40).

Fig. (2): Kidney of female catfish exposed for malachite green for three weeks showing diffuse extravasated red blood cells in-between the degenerated renal tubules and hyperemic glomeruli (H&E, X160).

DISCUSSION :

Exposure of aquatic lifes to sub-lethal concentration of toxic chemicals usually with growth and reproductive capabilities. The given results indicated that the GSI in both sexes did not significantly alter after exposure to the malachite green. The insignificant changes in the GSI may be attributed to the decrease in the body and gonadal weights by the same ratio. These results partially agree with authors [16, 23].

The first obvious result recorded in this investigation was the striking increase in cortisol level in fish exposed to malachite green in the 3 rd and 4 th weeks post-exposure. Cortisol level in blood can be affected by several factors like type of pollutants [24], environmental conditions [25], duration of exposure [26] and dose of pollutant [27]. In teleosts, cortisol is the best indicator of stress [28] also it plays an important role in the immune function and reproduction [29&30].

Estradiol- 17β significantly decreased only after three weeks of malachite green exposure. Campbell [28] reported that exposure of fish to chronic stress in the months prior to spawning resulted in reduced plasma sex steroids and vitellogenin. The significant decrease in Estradiol-17 β level can be attributed to the inhibition of the enzyme Δ5 3B-HSD which is responsible for biosynthesis of sex steroids in the ovarian tissues [31&32]. The reduction in feed intake and utilization accompanied the chronic stress may resulted in decrease in the body proteins [33&34] which consequently resulted in reduced pituitary gonadotropin level which in turn led to decrease in GSI, oocyte diameter and estradiol level. Pituitary gonadotropins had double effects on vitellogenesis as it stimulated in corporation of vitellogenin into the yolky oocytes by eliciting micropinocytic activity at oocyte surface [35] and it also stimulated estrogen secretion by the granulosa layer of the developing oocytes which in turn stimulated vitellogenin secretion by the liver [36]. In the teleosts, estradiol-17β is known to induce the synthesis and secretion of vitellogenic protein by the liver [37&38]. The deter-mined decrease in the testosterone in the 3 rd and 4 th weeks of the experiment can be also attributed to the enzyme Δ5 3B-HSD [39], activation of the hepatic cytochrome oxides (P450) [40] and blocking the release of gonadotropin from pituitary gland [41]. These observations were in accordance with those of previous studies carried out on catfish (Clarias *gariepinus*) using another chemicals [23&42]. On the other hand contradicting results have been also reported by Abou El-sooud and Abass [43]. These variation may be attributed to the concentration used and time of exposure.

There was a marked increase in AST, ALT, creatinine, and urea while significant decrease in total protein was recorded in the exposed fish (Table 2). The significant increase in AST and ALT values may referred to the toxic destructive action of malachite green on liver cells which are normally increase in liver dysfunction cases [44]. The significant decrease in serum total protein may be attributed to the effect of malachite green on the liver, as the liver is the organ responsible for protein synthesis [45&46] which is confirmed by the histopathological changes in the liver tissues as diffuse proliferation of kupffer

cells in-between the laminae of the hepatocytes with sever dilatation of central veins and sinusoids and infiltration of the portal area by inflammatory cells, extravasated red blood cells with fibroblastic proliferation inaddition to dilatation of portal vein as shown in Figure, 1. Also Person [34] found that the reduction in feed intake and utilization which accompanied the chronic stress resulted in decrease in proteins in the body.

The significant increase of urea and creatinine (the end product of protein catabolism) in serum which considered an indicator of renal function indicates the degenerative changes in kidney due to malachite green toxicity which is confirmed by the histopathological findings as diffuse extravasated red blood cells in-between the degenerated renal tubules and hyperemic glomeruli in the kidney tissues (Fig. 2).

Concerning with the results of serum biochemical and histopathological changes our results closely resembling those of [8,9&10].

The imbalance in the Estradiol- 17 β and testosterone hormones was not enough to induce histopathological changes in the ovarian and testicular tissues in the exposed fish.

Generally the use of malachite green with large concentration for long time of exposure may creates a great threat for the fish reproduction. We advice to use malachite green in small concentration for short periods as malachite green adverse effects should be taken into account when it is prescribed for treatment of fungal and protozoal diseases. In addition to the previously recorded that, exposure of catfish (*Clarias gariepinus*) to different chemicals resulted in prominent affection of the reproductive health [23,42,47&48], the result of this study warning about the non obvious hazardous effects of malachite green in the aquatic field.

REFERENCES :

1-Robert, R. J. and Shepherd,C. J. (1974): Handbook of trout and salmon diseases. Fishing News (Books), West Byfleet, Surrey, England.

2-Neish, G.A. (1977): Observations on saprolegniasis of adult socky salmon, Oncorhynchus nerka (Walbaum). J. Fish Biol. 10: 513-522.

3-Richards, R.H. and A.D. Pickering. (1978): Frequency and distribution patterns of Saprolegnia infection in wild and hatchery-reared brown trout Salmo trutta L. and char Salvelinus alpinus L. J. Fish Dis. 1:69-82.

4-Meyer, F.P. and Hoffman,G.L. (1976): Parasites and diseases of warm water fishes. U.S. Fish Wildl. Serv., Resour. Publ. 127. 20 pp.

5-Bailey,T.A. (1983): Method for in vitro screening of aquatic fungicides. J. Fish Dis. 6: 91-100.

6-Meyer,F.P. and Jorgenson, T.A. (1983): Teratological and other effects of malachite green on development of rainbow trout and rabbits. Trans. Am. Fish. Soc. 112: 818-824.

7-Omoregie, E.; Ofojekwu, P.C. ; Anosike, J. C. and Adeleye, A.O. (1998): Acute toxicity of malachite green to the nile tilapia, Oreochromis niloticus (L) Journal of aquaculture in the tropics.13: 4, 233-237.

8-Svobodova, Z.; Groch, L.; Flajshans, M.; Vykusova, B. and Machova, J.(1997): The effect of long-term therapeutic bath of malachite green on common carp (Cyprinus carpio L.). Acta-veterinara-Brno. 66: 2, 111-116.

9-Gerundo, N.; Alderman, D.J.; Clifton-Hadley, R. S. and Feist, S.W. (1991): Pathological effects of repeated doses of malachite green (A preliminary study). Journal of fish diseases. 14: 5, 521-532.

10-Srivastava, A.K.; Sinha, R.; Singh, N. D. and Srivastava, S.J. (1999): Histopathological changes in a freshwater catfish (Heteropneustes fossilis) following exposure to malachite green. Proceedings of the National Academy of Sciences India. Section B, Biological Sciences. 68: 1, 23-27.

11-Theron, J.; Prinsloo, J. F.; Schoonbee, H.J. (1991): Investigations into the effects of concentration and duration of exposure to formalin and malachite green on the survival of the larvae and juveniles of the common carp (Cyprinus carpio L.) and the sharptooth catfish (Clarias gariepinus) (Burchell). Onderstepoort Journal of Veterinary Research. 58: 4, 245-251.

12-Okafor, N. and Taege, M. (1996): Toxicity of four commonly used chemotherapeutic compounds on hybrid fry of African catfish Heteroclarias(Heterobranchus longifilis x Clarias gariepinus). Journal of Aquaculture in the Tropics. 11: 2, 113-118.

13-Travis, J.C. (1976): Plasma Cortisol. R: RIA for Physicians VI: No. 8.

14-Xing, S.; Cekan, S. and Diczfalusy, U. (1983): Validation of RIA for oestradiol by isotope dilution mass spectrophotometry and by a test of radiochemical purity. Clin. Chemica. Acta, 135: 189-201.

15-Wilson, D.; Forster,D.W. and Affe, B.M.(1992): Williames textbook of endocrinology, Philadelphia saunders. 923-926.

16-Kirubagaran, R. and Joy, K.P. (1992): Toxic effects of mercury on testicular activity in the fresh water teleosts, Clarias batrachus (L.). J. fish Biol. 41: 305-315.

17-Reitman, S. and Frankel, S. (1957): A colorimetric method for determination of serum glutamic oxaloacetic and glytamic pyruvic transaminases. Am. J.Clin. Path. 28:56.

18-King, E. J. and Wooten, I.D.P. (1959): Microanalyst in medical biochemistry. Churchill LTD London, p.58.

19-Fawcett, J.K. and Scott, J.E. (1960): Enzymatic determination of urea nitrogen (urease modified Berthelot reaction). Journal of Clinical Pathology, 13, 156-158.

20-Husdan, H. and Rapoport, A. (1968): Estimation of creatinine by Jaffe reaction A comparison of three methods. Clinical Chemistry, 14, 222-238.

21-Carleton, M.A.; Drury, R.A.B.; Willington, E.A. and Cameron, H. (1967): Carletons histological techniques. 4th Ed. Oxford Univ. Press, New York.

22-Snedecor, G.W. (1971): Statistical methods 14 th ed. the Iowa states cellage press Amer., Iowa.

23-Abd El Halim, K.I. (1997): Effect of cadmium and mercuric chlorides pollution onhormonal and gonadal functions in catfish (Clarias lazera). MVSc thesis,

Fac. Vet. Med. Beni-Suef, Cairo University.

24-Assem, H. (1984): Potential use of physiological changes as indices of stress in the teleosts, Cyprinus carpio. Bull. Inst. Ocean. Fish. ARE. 10: 111-121.

25-Wafaa, E.M. and Gamal, S. (1996): Effect of copper and zinc pollution on growth and reproductive performance of golden tilapia (Tilapia aurea) under different levels of water hardness. Zag. Vet. J. 24 (1): 65-75.

26-Hontela, A.J.; Joseph, B.; Celina, A. and Goston,C. (1992): Impaired cortisol stress response in fish from environments polluted by PAHs, PCB and mercury. Arch. Environ. Cotam. Toxicol., 22: 278-283.

27-Bleau, H. Daniel, C.; Chevaliarig and Hontela, A. (1996): Effects of cadmium on vitellogenin metabolism in estradiol induced flounder (platichthys flesus). Aquat. Toxicol. 17 (3): 253-262.

28-Campbell, P.; Pottinger, T. and Sumpter, J. (1994): Preliminary evidence that chronic confinement stress reduced the quality of gametes produced by brown and rainbow trout. Aquaculture, 120:151-169.

29-Frederick, W.C. (1983): Hormonal control of oocyte final maturation and ovulation in Fishes in Fish physiology. Hoar, W.S., Randall D.J. and Donaldson, E.M. (ed). vol. 9, reprod-uction, part B, Academic press, p.:117-169.

30-Pickering, A.P. (1993): Endocrine-induced pathology in stressed salmonid fish. Fish. Res. 17: 179-187.

31-Matty, A.J.(1985): Gonadal hormones. In Fish Endocrinology. A.J. Matty, ed. 1 st Ed., pp:138-173.

32-Schoonen, W.V.; J.G.D. Lambert, A.M. and Viveen, W.J.(1987): Steroidogenesis in pre and post spawned ovaries of feral African catfish (Clarias gariepinus). Aquaculture. 63: 129-142.

33-Fagerlund, R.M.; Lance, V.A.; Joanen, T. and McNease, L. (1991): Stress related effects of hatchery rearing density on Coho salmon. Trans. Am. Fish. Soc., 110: 644-649.

34-Person, J.L.; Galland, R.; Le Roux, A. and Chartois, H.(1997): Chronic ammonia toxicity in juvenile turbot (Scopthalmus maximus). Aquaculture, 154: 155-171.

35-Wallace, R.A. and Selman, K. (1981): Cellular and dynamic aspects of oocyte growth in teleosts. American Zoology, 21: 325-343.

36-Ng, T.B. and Ilder, D.R. (1983): Yolk formation and differentiation in teleost fishes. Fish Physiol., 9: 373-404.

37-Vaillant, C.; Leguellec, C. and Ped Kel, F. (1988): Vitellogenin gene expression in primary culture of rainbow trout hepatocyte. Gen.Comp. Endocrinol. 70: 284-290.

38-Pereira, J.J.; Mercaldo-allen R.; Kuropat, C.; Luedke, D. and Sennefelder, G. (1993): Effect of cadmium accumulation on serum vitellogenin levels and hepatosomatic and gonadosomatic indices of winter flounder (Pleuronectes americanus). Arch. Environ. Contam. Toxicol, 24: 427-431.

39-Kirubagaran, R. and Joy, K.P.(1988): Inhibition of testicular (3B- hydroxy Δ5 3B- HSD) activity in catfish Clarias batrachus by mercurials. Ind. J. Exp. Biol. 26: 907-908.

40-Kime, D.E. (1984): The effect of cadmium on steroidogenesis by testis of rainbow trout (Salmo gairdneri) Toxicol. Lett. 22,83.

41-Chan, K.K. (1984): Chronic effects of methyl mercury on the reproduction of teleost fish, Oryzias Lapites Ph.D. thesis Dept. of zoology university of British Columbia vancouver, B.C. Canada.

42-Saad, M.F. and Samira S. Rezeka (1998): reproductive performance of female catfish exposed to chronic pollution with ammonia and nitrite. 8th Sci. Con. Fac. Vet. Med. Assiut University, Egypt.

43-Abo-elsooud, K. and Abass, H.I. (1998): Effect of malachite green on the efficacy and pharmacokinetics of oxytetracycline with special reference to their biochemical and hormonal changes in experim-entally infected Clarias lazera. Vet. Med. J., Giza. Vol.46, No. 4 A: 555-567.

44-Mary, N.A. (1994): Toxicological studies on some water pollutants. MVSc thesis, Fac. of Vet. Med. Cairo University.

45-Sorenson, E.M.B.; Smith, N.K.R. and Ramirez-Mitchell, R. (1982): Electron probe x-ray microanalysis of arsenic inclusions in fish.Arch. Environ. Contam. Toxicol., (11) 469.

46-Gwozdzinski, K. (1992): Structural changes of proteins in fish red blood cells after copper and mercury treatment. J. Arch. Envir. contam. and toxic., 23 (4) 426-430.

47-FAO (1995): Water quality and fish health. EIFAC. technical paper: 22-27.

48-Ghousia, B.; Shantha, V.; Begum, G. and Vijayaraghavan, S. (1995): Chronic effects of dimethoate on the reproductive potential of the freshwater teleost, Clarias batrachus. Pesticides Science. 44: 3, 233-236.

التأثير السام للمبيد الفطرى الملاخيت الأخضر على أسماك القرموط

خالد عباس حلمى عبده [١] وزكريا مختار زكى [٢]

١- قسم الطب الشرعى والسموم - كلية الطب البيطرى - جامعة القاهرة (فرع بنى سويف) .
٢- قسم الطب الشرعى والسموم - كلية الطب البيطرى - جامعة أسيوط .

مما لاشك فيه أن الحفاظ على الثروة السمكية أصبح من الأمور الهامة والحيوية ، وخاصة بعد أن أثبتت الدراسات المختلفة الانخفاض الشديد فى المحصول السمكى على مستوى العالم ، ففى هذه الدراسة استخدم مائة وعشرون سمكة قرموط ، قسمت إلى مجموعتين المجموعة الأولى عددها ٦٠ سمكة من ذكور وإناث القرموط للمبيد الفطرى الملاخيت الأخضر (١٠/١ مجم/لتر ماء) لمدة ثلاثة أسابيع ثم لماء نظيف لمدة أسبوعين ، واستخدمت المجموعة الثانية وعددها ٦٠ سمكة من ذكور وإناث القرموط) كمجموعه ضابطة . أخذت عينات من الدم ، الكبد ، المبايض والخصيتين من عشرين سمكة من كل من الأسماك المعرضة ، وكذلك من المجموعة الضابطة.

أثبتت النتائج وجود زيادة معنوية فى هرمون الكورتيزول فى الأسبوع الثالث والرابع ونقص معنوى لهرمون الأستراديول فى الأسبوع الثالث من التجربة وانخفاض معنوى فى هرمون التستيستيرون ، كما لوحظ وجود ارتفاع فى مستوى أنزيمات الترانس أميناز والكرياتينين واليوريا فى حين لوحظ انخفاض معنوى فى بروتين المصل فى جميع أوقات التجربة . كما تم تسجيل التغيرات الهستوباثولوجية فى الكبد والكلى والمبايض والخصيتين.