

Dept. of Pharmacology  
 Fac. Vet. Med. Zagazig University, Egypt.  
 Head of Dept. Prof. Dr. S.M. Hamza

HIGH EFFICACY OF METHYLENE BLUE POTASSIUM  
 PERMANGANATE AND COPPER SULPHATE  
 IN REATMENT OF ECTOPROTOZOAL INFESTATION  
 IN TILAPIA NILOTICA FISH

(With 2 Tables)

By

A.F. ABDEL ALIM; H. ABDEL FADIL; A.A. SAID;  
 N. EDRESS and K. KHILO

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الكفاءة العلاجية العالية للميثيلين الأزرق، برمنجنات البوتاسيوم وكبريتات النحاس  
 في علاج الأوليات الخارجية في اسماك البلطي

عبد العليم فؤاد ، حسنى عبد الفضيل ، أحمد سعيد ، نجاح ادريس ، خالد كحيلو

لقد اجريت هذه الدراسة لكشف النقاب عن مدى كفاءة بعض مضادات الأوليات في علاج الأوليات الخارجية في اسماك البلطي بجرعة علاجية 4 مجم / لتر. اتضح من هذه الدراسة ان كل من الميثيلين الأزرق ، برمنجنات البوتاسيوم أو كبريتات النحاس له كفاءة علاجية عالية (100%) في علاج الاصابة بالأوليات الخارجية (كيلو دونيلا) في اسماك البلطي. لقد تبين ان الاصابة بالأوليات الخارجية في اسماك البلطي تحدث زيادة معنوية في انزيم جلوتاميك اوكسال استيك ترانس امينيز وانزيم جلوتاميك بيروفك ترانس امينيز وخميرة الفوسفاتيز القلوي والفسفور الغير عضوى بينما حدث نقصا معنويا في الاليومين والكوليسترول والكالسيوم. بعد اسبوع من العلاج حدث تحسن ملحوظ حيث عادت محتويات الامصال في الاسماك للمستوى الطبيعى لجموعة المقارنة. يستخلص من هذه الدراسة ان كل من الميثيلين الأزرق ، برمنجنات البوتاسيوم أو كبريتات النحاس له كفاءة علاجية عالية في علاج الأوليات الخارجية في اسماك البلطي ولقد اتضح ان الاصابة بالأوليات الخارجية في اسماك البلطي لها آثار ضارة على محتويات الأمصال حيث تعود للمستوى الطبيعى بعد اسبوع من العلاج.

SUMMARY

Methylene blue, Pot. permanganate and copper sulphate displayed a high efficacy in treatment of fish ectoprotzoal infections by *Chilodonella hexosticha*. The infected *Tilapia nilotica* fish showed a significant increase in serum GOT, GPT and alkaline phosphatase activities as well as phosphorus level whereas albumin, calcium and cholesterol levels were decreased. The previous changes in serum constituents were completely subsided one week post-treatment. It is concluded that methylene blue, pot. permanganate and copper sulphate have a high efficacy in *Tilapia nilotica* ectoparasites. Moreover, the observed adverse-effects on serum constituents returned to their control levels, one week post-treatment.

Keywords: Efficacy, Methylene blue, potassium permanganate copper sulphate treatment, ectoprotzoal infestation, *Tilapia nilotica* fish.

## INTRPODUCTION

Commercial production of fish is a rapidly growing industry and this results in an increased interest in their parasitic infestation. Investigation of fish for detection of the parasites appeared, therefore, to be of importance for both protection and increasing its population. Among fish parasites, protozoa, are the most dangerous group that probably inflicts more diseases in fish cultures than any other type of parasites (HOFFMAN, 1970). Out of the wide range of ectoparasitic protozoa, Chilodonella hexosticha is known to be of great importance as pathogens of fish. Such protozoa, when present in large numbers greatly impair the epithelium particularly those of the gills while others feed on the cells and mucus resulting in great damage to the host.

SAXON *et al.* (1985) recorded that Chilodonella hexosticha infection resulted in high losses among fish and consequently decreased fish production with reduction of animal protein.

Some antiseptics and disinfectants proved to be highly effective for treating Chilodonella infection in fish (CLEMMENTS and SNEED, 1958).

The present study was carried out, under our environmental conditions, to reinforce the previous literature on the efficacy of these agents in Tilapia nilotica fish infested by Chilodonella hexosticha.

## MATERIALS and METHODS

### Drugs:

Methylene blue, pot. permanganate and copper sulphate were used in the present study and obtained from El-Nasr Co., Egypt.

### Animals:

Ninety Tilapia nilotica fishes, one month old, about 100 gm each, were used in the present investigation.

### Efficacy of methylene blue, pot. permanganate and copper sulphate on protozoal infection:

Forty fishes were divided into four equal groups, each of 10 fishes. The first group was kept as control. The other three groups were experimentally infested with Chilodonella (HOFFMAN, 1970). The infested groups of fish were treated with methylene blue (4 mg/L), potassium permanganate (4 mg/L) as a bath for one hour and the last infested group was treated with copper sulphate (4 mg/L) as bath for 24 hours.

Samples from the skin, gills and muscles, containing cysts were examined microscopically, for presence of protozoa before and after treatment for determining the efficacy (AMLACHER, 1970).

### Serum Biochemical Analysis:

Fifty fishes were divided into five equal groups, each of 10 fishes. The first group was kept as control. The other four groups were experimentally infested with Chilodonella. One of the infested groups was non-treated (second group). The other three infested groups of fish were treated with methylene blue (4 mg/L), potassium permanganate (4 mg/L) as a bath for one hour and the last infested

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group was treated with copper sulphate (4 mg/L) as a bath for 24 hours.

One week post-treatment, blood samples were collected from severed fish tails, left to clot then centrifuged at 2000 r.p.m. for 15 minutes. The separated serum samples were stored at -20°C for biochemical analysis.

Serum samples were analysed for glutamic oxalacetic transaminase (SGOT) and glutamicpyruvic transaminase (SGPT) activities (REITMAN and FRAKEL, 1957); alkaline phosphatase (ALP) and inorganic phosphorus (KILCHING and FRIEBURG, 1951); albumin (KING and WOOTTEN, 1959); calcium (KING and WOOTTEN, 1959); calcium (GLINDER and HKING, 1972) and cholesterol (WALSON, 1960).

**Statistical analysis:**

Student "t" test was carried out according to SNEDECOR (1971).

**RESULTS**

**Efficacy of methylene blue, pot. permanganate and copper sulphate on protozoal infection:**

The recommended methylene blue concentration; 4 mg/L as a bath for one hour, was highly effective (100%) against *Chilodonella*. Pot permanganate 4 mg/L, as a bath for one hour showed an excellent protozoacidal effect (100%). Copper sulphate 4 mg/L as a bath for 24 hours, was highly effective (100%) against *Chilodonella* (Table 1).

**Serum Constituents:**

The infected *Tilapia nilotica* fish showed a significant increase in se-

rum SGOT (P<0.0001), SGPT (P<0.0001), alkaline phosphatase, (P<0.0001) and phosphorus (P<0.01) levels while albumin content (P<0.01), cholesterol level (P<0.01) and calcium concentration (P<0.01) were significantly decreased.

The treatment of infected fish with methylene blue, potassium permanganate or copper sulphate improved the serum constituents toward control level (Table 2).

**DISCUSSION**

The progress of fish production industry is handicapped mostly by the various numerous diseases inflicted by fish ectoparasites.

In the present investigation, it has been shown that methylene blue (4 mg/L) as a bath for one hour and copper sulphate (4 mg/L) as a bath for 24 hours were highly effective (100%) against *Chilodonella* infestation in fish. Similar results have been documented previously with copper sulphate (DEMPSTER, 1955), methylene blue and pot. permanganate (CLEMENTS and SNEED, 1958). The high efficacy of methylene blue, pot. permanganate and copper sulphate reported in this study added a further evidence to the previous justification of the use of these agents in both prevention and control of fish ectoprotzoal infections by *Chilodonella* (SARIG, 1975).

The observed protozoacidal effect of methylene blue in treatment of *Chilodonella* infestation in fish may be resorted to its oxidizing effect (BOOTH and McDONALD, 1982).

The obtained high efficacy of pot. Permanganate against ectoprotzoal infection in fish may be attributed to the release of nacent oxygen. Moreover, the recorded antiprotozoal effect of copper sulphate in fish may be resorted to its disinfectant activity which probably produces its action through inhibition of the essential enzymes necessary for protozoal metabolism.

In the present study, it has been demonstrated that in *Tilapia nilotica* fish infected with *Chilodonella* there is a significant increase inserum SGOT, SGPT and alkaline phosphatase activities as well as phosphorus level with a significant decrease in albumin, cholesterol and calcium levels.

The elevated enzyme activities in the infested fish may be due to slight injury in the tissues and/or internal organs as a result of protozoal infection (JOAN and PANNALL, 1981).

Hypoproteinemia occurs in cases of failure of parenchymal synthesis of serum amino acids, portal cirrhosis, renal disease, pancreatic hypoplasia, diabetes meletus and increased pro-

tein breakdown for gluconeogenesis (BENJAMIN, 1979).

Serum calcium level is usually reduced moderately in association with a fall in plasma protein inrenal diseases, as nephrotic syndrome, high serum inorganic phosphorus of renal failure is sometimes accompanied by marked decrease in serum calcium, a consequence of poor renal production of dihydroxy-corticosteriod (VARLEY *et al.*, 1980).

One week post-treatment, the previous changes in serum SGOT, SGPT, alkaline phosphatase, albumin, cholesterol, calcium and phosphorus levels were improved and returned toward control group.

Thus, it could be concluded from the present study that methylene blue, pot. permanganate, and copper sulphate in a dose of 4 mg/L were 100% effective against *Chilodonella* infestation in *Tilapia nilotica* fish. Moreover, the recorded side-effects on serum biochemical constituents completely disappeared retaining its normal controlvalues, one week post-treatment.

## REFERENCES

- Amlacher, E. (1970): Textbook of fish diseases. T.F.H. Publ. Neptune N.J.p. 75.  
 Benjasmin, M.M. (1979): Outline of Veterinary Clinical Pathology. 3rd Ed. The Iowa State University Press Ames, Iowa, U.S.A.  
 Booth, N.H. and McDonald, L.E. (1982): Veterinary Pharmacology and Therapeutics 5th Ed. Iowa State, University Press U.S.A.  
 Clements, H.P. and Sneed, K.E. (1958): The chemical control of some diseases and parasites of channel catfish. Progr. Fish. Cult. 20(1): 8-15.

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- Dempster, R.P. (1955):* The use of copper sulphate as a cure for fish diseases caused by parasitic dinoflagellates of the genus *Oodinium*, *Zoologica* 40 (12): 133-139.
- Glinder, E.M. and King, J.D. (1972):* Rapid colorimetric determination of calcium in biological fluids with methylene blue. *Am.J. Clin. Path.* 58:376-382.
- Hoffman, G.L. (1970):* Parasites of North American fresh water fishes, Univ. California Press. Berkely and Los Angeles 21.
- Joan, F.Z. and Pannal, P.R. (1981):* Clinical chemistry diagnosis and treatment. 3rd Ed. Lioted-Luke, London.
- Kilchling, H. and Freiburg, B. (1951):* Inorganic phosphorus and alkaline phosphatase in serum. *Clinical Photometric* 3rd Ed., Wiss. Verl. Stuttgart.
- King, E.J. and Wootton, I.O.P. (1959):* Micro methods in medical biochemistry. 3rd Ed. Churchill, London.
- Beitman, S. and Frankel, S. (1957):* A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminase. *Am.J. Clin. Path.* 23: 56-58.
- Sarig, S. (1975):* The status information in fish diseases in Africa and possible means of their control. Symposium on Aquaculture in Africa Ghana CLFA 6P. FAO Roma.
- Saxon, E.C.; Langdon, S. and Humphery, J.D. (1985):* Deaths in Australian fresh water fishes associated with *Chilodonella hexosticha* infection. *Aust. Vet. J.* 62 (12): 409-413.
- Snedecor, G.W. (1971):* Statistical methods 14th Ed. Iowa State College Press, Ames, Iowa.
- Varley, H.; Gowenlock, H.A. and Bell, M. (1980):* Practical clinical biochemistry. Vol. I William Heinemann Medical books 3rd Ed. London.
- Walson, D. (1960):* A simple method for determination of serum cholesterol. *Clin. Chem. Acta*, 5: 637-643.