

Effect of Propolis, Florfenicol, And Their Combination On Catfish (*Clarias Lazera*) Experimentally Infected With *Aeromonas hydrophila*

Sawsan M A El-Sheikh, Hosny A E Ibrahim, Refaat K Mohamed and
Dalia I M Ibrahim

Department of Pharmacology, Faculty of Veterinary Medicine, Zagazig University,
Department of Pharmacology, Animal Health Research Institute, Zagazig Branch

ABSTRACT

The aim of the present study was to compare some biochemical and haematological effects of propolis, florfenicol and their combination against experimentally infected Nile catfish (*Clarias Lazera*) with *Aeromonas hydrophila*. One hundred and twenty five Nile catfish were randomly classified into five equal groups each of 25 fish. Fish in group 1 were fed on basal diet (negative control). Fish in group 2 were inoculated intraperitoneally (I/P) with 0.2 ml of 24 hr broth cultures of *A. hydrophila* (2.5×10^8 / ml) and kept without medication (positive control). Fish in group 3 were experimentally infected similarly, and were given the basal diet, containing propolis-ethanolic-extract (10 gm /kg diet). Group 4 were experimentally infected similarly, and treated with florfenicol (10 mg /kg body weight in feed). Group 5 were experimentally infected similarly, and treated with therapeutic dose of propolis plus florfenicol for 10 successive days. Two blood samples were taken from each fish on 1st, 7th and 14th days post treatment. The findings of this study demonstrated that administration of propolis plus florfenicol improved the haematological and biochemical parameters of infected fish with *A. hydrophila* compared with administration of propolis or florfenicol alone.

INTRODUCTION

Fish is the cheapest source of animal protein and is, therefore, important in the diets of the lowest income groups with highly nutritive value. Most of the countries nowadays pay a great attention to improve and develop their inlet water resources to satisfy their requirements of animal protein (1).

Fish diseases, especially bacterial infections, are a major problem facing fish farming industry, which is currently growing fast with an annual increase of approximately 12% (2). *Aeromonas hydrophila* is one of the most important agents of the outbreaks in fresh water fish, in which skin ulcers, hemorrhage and necrosis of the visceral organs are the major symptoms (3).

Propolis (bee glue) is a resinous product that produced by honeybees. It contains a variety of chemical compounds, such as polyphenols (flavonoid aglycones, phenolic acids and their esters, phenolic aldehydes, alcohols, and ketones), sesquiterpene quinones, coumarins, steroids, amino acids, and inorganic compounds (4). The antibacterial and antifungal activities are the most popular and most extensively investigated biological activities of propolis. It has many different pharmacological activities, such as anticancer, anti-inflammatory, antibiotic, antioxidative, antiviral, antifungal, anaesthetic, immunostimulant and cytostatic effects (5).

Florfenicol is a fluorinated analogue of thiamphenicol and its structure also resembles that of chloramphenicol. Thiamphenicol and

chloramphenicol have been used as broad spectrum veterinary antibiotics. Florfenicol is a synthetic broad spectrum antibiotic potentially effective in controlling a number of bacterial infections in fish (6).

Several hazards and side effects have been associated with the excessive use of antibacterial drugs for fish, such as immunosuppression, nephrotoxicity, growth retardation, the development of resistant bacterial strains, environmental problems, such as drug residues in fish farm sediments, and drug residue in fish products (7).

Therefore, this study was proposed to compare the effects of propolis, florfenicol and their combination to find out whether propolis could be an alternative or an adjunctive treatment for experimentally infected *Clarias lazera* with *Aeromonas hydrophila* pathogen. Some haematological and biochemical changes in fish after treatment were investigated.

MATERIAL AND METHODS

Experiment was performed on one hundred and twenty five Nile catfish (*Clarias lazera*). The range of weight and length were 55-77 gm and 23 -30 cm, respectively. They were kept in a well aerated glass aquaria measuring 100 x 50 x 50 cm to be acclimatized on dechlorinated tap water for 15 days. Each aquarium was supplied with two air pumps. Water temperature was fixed at $27^{\circ}\text{C} \pm 2$, pH was 7-8.5. Fish were fed on commercial pelleted ration at a rate of 2% body weight once daily (the food of fish contain the drugs that were added to the fish ration before pelleted).

Fish were randomly classified into five equal groups, each 25 fish. Group 1 was fed on basal diet (negative control). Group 2 was

inoculated intraperitoneally (I/P) with 0.2 ml of 24 hr broth cultures of *A. hydrophila* (2.5×10^8 / ml) and kept without medication (positive control). Fish in group 3 were experimentally infected similarly, and were given the basal diet, containing propolis-ethanolic-extract (10 gm /kg diet). Group 4 were experimentally infected similarly, and treated with florfenicol (10 mg /kg body weight in feed). Group 5 were experimentally infected similarly, and treated with therapeutic dose of propolis plus florfenicol for 10 successive days. Blood samples were collected by the caudal artery method which is considered the suitable way (8). Two blood samples were taken from each fish on 1st, 7th and 14th days post treatment. First sample of blood was collected in a test tube mixed with EDTA for haematological measures. Second blood sample was collected in plain centrifuge tube, clotted and serum was separated by centrifugation at 3000 r.p.m. for 20 minutes. Clear serum was separated carefully and stored in a screw capped sterile bottles at $-20^{\circ}\text{C} \pm 1^{\circ}\text{C}$ until used for biochemical analysis.

Haematological studies

Total erythrocytic and leucocytic counts were counted using method described by Natt and Herrick (9). Hemoglobin was determined colorimetrically, according to the method described by Wintrobe (10). The packed cell volume (PCV %) was determined using the microhaemocrit method according to Cohen (11)

Liver function tests

AST and ALT were estimated according to Reitman and Frankel (12) Total proteins were estimated according to Grant et al. (13). Serum albumin was determined calorimetrically according to the method of Doumas et al. (14).

Kidney function tests

Determination of serum urea level was performed according to the method of Patton and Crouch (15) Estimation of serum creatinine is accomplished by photometric colorimetric test for kinetic measurements

without deproteinization according to Henry (16).

Statistical Analysis

Analysis of variance (ANOVA) was carried out following the method described for one – way classification for comparing the different groups and different times with each other, using SAS (17). Means within the same column bearing different superscripts are significant at $P < 0.05$.

RESULTS

Effect on haematological parameters

Administration of propolis, florfenicol, and their combination for treatment of *Aeromonas hydrophila* infected fish displayed a significant increase at ($p < 0.05$) in RBCs, Hb and PCV % compared with infected non treated group all over the experimental period as shown in Table 1,2 &3 respectively.

Table 1. Effect of propolis (10 gm /kg diet) and florfenicol (10mg/kg b.wt) and their combination administered in feed for 10 successive days on erythrocytic count (M±S.E) (n=5)

Group	Erythrocyte count ($10^6 / \text{mm}^3$)		
	Time post treatment		
	1 st day	7 th day	14 th day
Non infected non treated (control) (G1)	2.35 ± 0.03 a	2.23 ± 0.15 a	2.23 ± 0.15 a
Infected non treated (G2)	1.53 ± 0.09 d	1.44 ± 0.08 d	1.44 ± 0.08 c
Infected & treated with propolis (G3)	1.85 ± 0.03 c	1.73 ± 0.03 c	2.18 ± 0.04 a
Infected & treated with Florfenicol(G4)	1.85 ± 0.04 c	1.80 ± 0.06 c	2.17 ± 0.03 a
Infected & treated with propolis and florfenicol (G5)	1.93 ± 0.04 c	1.85 ± 0.04 bc	2.21 ± 0.05 a

Different letters at the same column means that there was a significant changes at $p < 0.05$

Table 2. Effect of propolis (10 gm /kg diet) and florfenicol (10mg/kg b.wt) and their combination administered in feed for 10 successive days on haemoglobin concentration (M±S.E) (n=5)

Group	Haemoglobin concentration (gm/ dl)		
	Time post treatment		
	1 st day	7 th day	14 th day
Non infected non treated (control) (G1)	11.67 ± 0.33 a	12.00 ± 0.58 a	12.00 ± 0.58 ab
Infected non treated (G2)	8.33 ± 0.33 c	8.67 ± 0.33 c	8.67 ± 0.33 d
Infected & treated with propolis (G3)	10.33 ± 0.33 b	11.00 ± 0.58 ab	11.33 ± 0.33 bc
Infected & treated with Florfenicol(G4)	10.17 ± 0.17 b	10.83 ± 0.60 ab	10.67 ± 0.33 c
Infected & treated with propolis and florfenicol (G5)	10.52 ± 0.29 b	11.17 ± 0.44 a	12.17 ± 0.44 ab

Table 3. Effect of propolis (10 gm /kg diet) and florfenicol (10 mg/ kg b.wt) and their combination administered in feed for 10 consecutive days on the packed cell volume (PCV %) (M±S.E) (n=5)

Group	PCV %		
	Time post treatment		
	1 st day	7 th day	14 th day
Non infected non treated (control) (G1)	27.33 ± 0.33 a	26.67 ± 0.33 ab	26.67 ± 0.33 a
Infected non treated (G2)	14.67 ± 0.33 c	13.67 ± 0.33 e	16.00 ± 0.58 b
Infected & treated with propolis (G3)	18.67 ± 0.67 b	18.67 ± 0.33 c	25.33 ± 0.89 a
Infected & treated with Florfenicol(G4)	17.67 ± 0.33 b	16.67 ± 0.88 d	26.00 ± 0.58 a
Infected & treated with propolis and florfenicol (G5)	19.00 ± 0.58 b	17.00 ± 0.58 cd	25.67 ± 0.67 a

Different letters at the same column means that there was a significant changes at $p < 0.05$

Total leucocytic count in fish experimentally infected with *Aeromonas hydrophila* and non treated displayed a significant increase at ($p < 0.05$) on 1st, 7th and 14th days post treatment compared with control group as observed in Table (4). Fish treated with propolis displayed a significant decrease on 1st post treatment followed by non significant change on 7th and 14th days post treatment compared with infected non treated group. Treatment of *Aeromonas hydrophila*

infected fish with florfenicol showed non significant difference in total leucocytic count on 1st day post treatment followed by significant decrease at ($p < 0.05$) on 7th and 14th days post treatment compared with Group (2). The effect of both (propolis and florfenicol) in treatment of *Aeromonas hydrophila* infected fish showed significant decrease at ($p < 0.05$) in total leucocytic count compared with Group (2) all over the experimental period

Table 4. Effect of propolis (10 gm /kg diet) and florfenicol (10 mg/ kg b.wt) and their combination administered in feed for 10 consecutive days on total leucocytic count (M±S.E) (n=5)

Group	Total Leucocytic count ($10^3 / \text{mm}^3$)		
	Time post treatment		
	1 st day	7 th day	14 th day
Non infected non treated (control) (G1)	24 ± 0.58 c	23.38 ± 0.76 b	23.38 ± 0.76 b
Infected non treated (G2)	27 ± 0.58 a	27.47 ± 0.25 a	26.13 ± 0.40 a
Infected & treated with propolis (G3)	25.50 ± 0.29 b	27.45 ± 0.33 a	25.67 ± 0.40 a
Infected & treated with Florfenicol (G4)	27.12 ± 0.44 a	22.05 ± 1.16 bc	22.54 ± 0.24 b
Infected & treated with propolis and florfenicol (G5)	25.83 ± 0.44 ab	23.00 ± 0.58 b	22.00 ± 0.58 b

Different letters at the same column means that there was a significant changes at $p < 0.05$

Effects on biochemical parameters
Liver function parameters

Administration of propolis, florfenicol,
and their combination for treatment of

Aeromonas hydrophila infected fish displayed a significant decrease at ($p < 0.05$) in ALT and AST compared with infected non treated group as shown in Table (5&6) respectively.

Table 5. Effect of propolis (10 gm /kg diet) and florfenicol (10 mg/ kg b.wt) and their combination administered in feed for 10 consecutive days on alanine aminotransferase (ALT) (M±S.E) (n=5)

Group	ALT (U/ L)		
	Time post treatment		
	1 st day	7 th day	14 th day
Non infected non treated (control) (G1)	10.67± 1.45 d	9.33 ± 1.86 d	9.67 ± 1.20 b
Infected non treated (G2)	31.67 ± 2.91 a	35.00± 0.58 a	32.67 ± 1.76 a
Infected & treated with propolis (G3)	22.66 ± 2.67 bc	23.00± 2.64 b	31.33 ± 1.86 a
Infected & treated with Florfenicol(G4)	30.00 ± 1.15 a	13.67 ± 2.67 cd	12.33 ± 1.20 b
Infected & treated with propolis and florfenicol (G5)	24.00 ± .58 bc	13.33 ±0.88 cd	10.33 ± 0.88 b

Different letters at the same column means that there was a significant changes at $p < 0.05$

Table 6. Effect of propolis (10 gm /kg diet) and florfenicol (10 mg/ kg b.wt) and their combination administered in feed for 10 consecutive days on Aspartate aminotransferase (AST) (M±S.E) (n=5)

Group	AST (U/ L)		
	Time post treatment		
	1 st day	7 th day	14 th day
Non infected non treated (control) (G1)	175.67± 18.48 de	153.33 ± 13.98 b	149.67 ± 13.86 b
Infected non treated (G2)	367.67± 9.61 a	296.67 ± 37.73 a	305.00 ± 36.00 a
Infected & treated with propolis (G3)	163.67±4.48 de	157.33 ± 9.52 b	156.33 ± 13.57 b
Infected & treated with Florfenicol(G4)	362 ±6.08 a	142.67 ± 1.67 b	143.67 ± 13.42 b
Infected & treated with propolis and florfenicol (G5)	182.33±3.92 cd	146.67 ± 4.40 b	151.33 ± 15.98 b

Different letters at the same column means that there was a significant changes at $p < 0.05$

Fish experimentally infected with *Aeromonas hydrophila* and non treated displayed a significant decrease at in albumin and total protein (gm/dl) on 1st, 7th and 14th days post treatment compared to non infected non treated group. Administration of propolis,

florfenicol, and their combination for treatment of *Aeromonas hydrophila* infected fish displayed a significant increase at ($p < 0.05$) in albumin and total protein compared with infected non treated group as shown in Table (7&8) respectively.

Table 7. Effect of propolis (10 gm /kg diet) and florfenicol (10 mg/ kg b.wt) and their combination administered in feed for 10 consecutive days on albumin (gm/dl) (M±S.E) (n=5)

Group	Albumin (gm/ dl)		
	Time post treatment		
	1 st day	7 th day	14 th day
Non infected non treated (control) (G1)	1.55 ± .24 abc	1.57 ± 0.09 a	1.60 ± 0.06 a
Infected non treated (G2)	1.26 ± .21 abcd	1.10 ± 0.06 b	0.97 ± 0.03 b
Infected & treated with propolis (G3)	1.51 ± 0.01 abc	1.54 ± 0.09 a	1.67 ± 0.18 a
Infected & treated with Florfenicol(G4)	1.25 ± .21 abcd	1.53 ± 0.18 a	1.47 ± 0.09 a
Infected & treated with propolis and florfenicol (G5)	1.50 ± 0.02 abc	1.50 ± 0.06 a	1.58 ± 0.08 a

Table 8. Effect of propolis (10 gm /kg diet) and florfenicol (10 mg/ kg b.wt) and their combination administered in feed for 10 consecutive days on Total proteins (gm/dl) (M±S.E) (n=5)

Group	Total protein (gm/ dl)		
	Time post treatment		
	1 st day	7 th day	14 th day
Non infected non treated (control) (G1)	3.37 ± 0.18 ab	3.80 ± 0.06 a	3.87 ± 0.03 a
Infected non treated (G2)	1.93 ± 0.09 c	2.17 ± 0.30 b	2 ± 0.31 b
Infected & treated with propolis (G3)	3.43 ± 0.41 a	3.43 ± 0.18 a	3.97 ± 0.18 a
Infected & treated with Florfenicol(G4)	1.91 ± 0.06 c	3.83 ± 0.07 a	4 ± 0.38 a
Infected & treated with propolis and florfenicol (G5)	3.10 ± 0.06 ab	3.63 ± 0.09 a	3.80 ± 0.06 a

Different letters at the same column means that there was a significant changes at $p < 0.05$

Effects on kidney function parameters

Urea and creatinine levels in infected non treated group were significantly increased at ($p < 0.05$) on 1st, 7th and 14th days post treatment compared with non infected non treated group. Treatment of *Aeromonas*

hydrophila infected fish with propolis, Florfenicol, and their combination showed significant decrease at ($p < 0.05$) in urea and creatinine level compared with infected non treated group all over the experimental period as shown in Table (9&10) respectively.

Table 9. Effect of propolis (10 gm /kg diet) and florfenicol (10 mg/ kg b.wt) and their combination administered in feed for 10 consecutive days on urea level (mg/dl) (M±S.E) (n=5)

Group	Urea (mg / dl)		
	Time post treatment		
	1 st day	7 th day	14 th day
Non infected non treated (control) (G1)	9.33 ± 0.67 cd	9.33 ± 0.67 b	8.83 ± 0.44 b
Infected non treated (G2)	16.67 ± 1.20 a	17.00 ± 0.58 a	16.00 ± 0.58 a
Infected & treated with propolis (G3)	14.67 ± 2.40 ab	10.67 ± 1.76 b	9.00 ± 0.58 b
Infected & treated with Florfenicol(G4)	12.33 ± 0.33 bc	10.67 ± 3.18 b	10.00 ± 2.00 b
Infected & treated with propolis and florfenicol (G5)	12.00 ± 0.58 bcd	10.00 ± 0.58 b	8.67 ± 0.89 b

Different letters at the same column means that there was a significant changes at $p < 0.05$

Table 10. Effect of propolis (10 gm /kg diet) and florfenicol (10 mg/ kg b.wt) and their combination administered in feed for 10 consecutive days on Creatinine level (mg/dl) (M±S.E) (n=5)

Group	Creatinine (mg / dl)		
	Time post treatment		
	1 st day	7 th day	14 th day
Non infected non treated (control) (G1)	0.53 ± 0.33 c	0.56 ± 0.03 de	0.56 ± 0.03 b
Infected non treated (G2)	0.86 ± .03 a	0.96 ± 0.07 a	0.93 ± 0.03 a
Infected & treated with propolis (G3)	0.77 ± .03 ab	0.69 ± 0.06 bc	0.70 ± 0.05 b
Infected & treated with Florfenicol(G4)	0.60 ± 0.06 c	0.79 ± 0.05 b	0.70 ± 0.06 b
Infected & treated with propolis and florfenicol (G5)	0.63 ± 0.03 bc	0.67 ± 0.02 bcd	0.66 ± 0.06 b

Different letters at the same column means that there was a significant changes at $p < 0.05$

DISCUSSION

In the current study, the effect of propolis, florfenicol and their combination against experimentally infected *Clarias lazera* inoculated intraperitoneally with *Aeromonas hydrophila* pathogen was investigated and their effect on some haematological and biochemical parameters were also investigated.

Concerning the haematological results, it is clear that infected fish with *Aeromonas hydrophila* (G2) resulted in a significant decrease in total erythrocytic count, haemoglobin concentration and packed cell volume while there is a significant increase in total leucocytic count on 1st, 7th and 14th days post treatment. Coles (18) related the increase in total leucocytic count due to an antigenic stimulation by bacterial infection. The obtained results were in accordance with that obtained by Ahmed (19) and Amer et al. (20) who reported that *Clarias lazera* infected with *A. hydrophila* induced significant decrease in total erythrocytic count, haemoglobin concentration and packed cell volume.

On the other hand, the group treated with propolis improved the haematological parameters when compared with infected group, that may be attributed to the chemical structure of propolis including polyphenols (flavonoid aglycones, phenolic acids and their esters, phenolic aldehydes, alcohols, and ketones), sesquiterpene quinones, coumarins, steroids, amino acids, protein, vitamins (A, B1, B2, B3 and biotin), minerals (iron, zinc, copper, cobalt) and inorganic compounds (3). Propolis improves formation of haemoglobin and erythrocyte formation as it contains protein, iron and copper (21,22). Also Bratter et al. (22) added that propolis improved digestive utilization of iron, increase erythrocytic count and it has immunostimulant effect.

In the present study, the infected fish treated with florfenicol displayed a significant increase in RBCs count, Hb concentration and PCV % compared with infected non treated group and returned nearly toward normal level on 14th day post treatment. The obtained results were in agreement with Abd El-Rahman (23)

who found that treatment with florfenicol improved the adverse effects of *P. multocida* infection on haematological parameters as evidenced by improvement of macrocytic anaemia after 15 and 21 days compared to the infected non treated group.

Treatment of infected catfish with combination of propolis and florfenicol displayed a significant increase in RBCs count, Hb concentration and PCV % when compared with infected group and returned toward normal level on 14th day post treatment. The obtained results were parallel with Yonar et al. (24) who found that propolis improved the adverse effect of Oxytetracycline (OTC) administration in rainbow trout on haematological parameters. As the erythrocyte GSH-Px activity was significantly reduced by (OTC) simultaneous treatment with propolis caused a significant increase in erythrocyte GSH-Px activity when compared with the OTC group. Compared to the OTC group, a statistically significant increase was observed in the groups treated with propolis. So they concluded that simultaneous treatment with propolis provided a protective effect against the oxidative stress and immunosuppression induced by OTC; and propolis could be used as an antioxidant and immunostimulant in fish.

Regarding the biochemical parameters, the infection of fish with *Aeromonas hydrophila* resulted in elevation in some biochemical parameters manifested by a significant increase in AST, ALT, urea and creatinine while a significant decrease in albumin and total proteins compared with control group. These results were in agreement with those reported by Ahmed (19) and Amer et al. (20) that they recorded an increase in the serum enzymatic activities in infected fish with *Aeromonas hydrophila*. This increase in enzyme activities was attributed to the liver damage which caused by the effect of the infectious agent toxins which is followed by the escape of these enzymes into serum in high levels (25). Furthermore, an elevation of serum urea and creatinine in *Oreochromis niloticus* infected by *Aeromonas hydrophila* was recorded by Ghareeb (26). The hypoproteinemia

in the fish experimentally infected with *A. hydrophila* was attributed to hepatocyte disorders resulting in an increase of catabolic rate (27) at in albumin and total protein (gm/dl) on 1st, 7th and 14th days post-treatment. Propolis, florfenicol and their combination administration to infected fish non-treated group. Administration of propolis with *Aeromonas hydrophila* showed a significant improvement in biochemical parameters when compared with infected non-treated group. These improvements in the liver function might be due to bactericidal effect of the drug combination administered in feed for 10 consecutive days (M.S.E) (n=5) effects of *A. hydrophila* in the liver as well as the regenerative process which takes place in the liver cells (26). Mahran et al. (28) added that the positive effect of propolis on biochemical parameters could be attributed to non-antifungal activity (colony), anti-toxicity and hepatoprotective effect. Hegazi et al. (29) added that the activity of ALT, AST and serum lipids returned to the control level after administration of propolis in rats infected with *S. aureus* and *E. coli*. Furthermore, Deng et al. (30) indicated that the potential use of propolis as a growth promoter, hepatoprotective agent, and immunostimulant for rainbow trout. About the improvement of kidney function could be due to the anti-inflammatory effect of propolis in which inhibit the release of prostaglandins and leukotrienes (31).

It could be concluded that administration of propolis have a mitigated effect when used in combination administered in feed for 10 consecutive days on infected fish non-treated treatment (gm/dl) (M.S.E) (n=5) with *Aeromonas hydrophila*. Also the present study displayed that propolis had a synergistic effect when used with florfenicol to increased its efficacy. So the combination between propolis and florfenicol was the most effective treatment in the present study (control) (G1)
 Infected non treated (G2) 1.93 ± 0.09 c
 Infected & treated with propolis (G3) 3.43 ± 0.41 a
 Infected & treated with Florfenicol (G4) 1.91 ± 0.06 c
 Infected & treated with propolis and florfenicol (G5) 3.10 ± 0.06 ab

Different letters at the same column means that there was a significant changes at p<0.05

florfenicol, and their combination for treatment of *Aeromonas hydrophila* infected fish displayed a significant increase at (p<0.05) in albumin and total protein compared with infected non-treated group as shown in Table (7 & 8) respectively.

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الملخص العربي

تأثير البروبليز(صمغ النحل) والفلورفينيكول و كلاهما معا في علاج أسماك القرموط النيلي المصابه معمليا بميكروب الايرومونات هيدروفيل

سوسن محمد على الشيخ، حسنى عبد الفضيل إبراهيم، رفعت خضري محمد، داليا إبراهيم محمد إبراهيم
قسم الفارماكولوجيا - كلية الطب البيطرى - جامعة الزقازيق
قسم الفارماكولوجيا - معهد بحوث صحة الحيوان فرع الزقازيق

استهدفت هذه الدراسة مقارنة تأثير كلا من البروبليز(صمغ النحل) والفلورفينيكول و كلاهما معا في علاج أسماك القرموط النيلي المصابه بميكروب الايرومونات هيدروفيل معمليا وذلك من خلال دراستهم تأثيرهم على صورة الدم ووظائف الكبد والكلى . تم تقسيم مائة وخمسة وعشرين من أسماك القرموط النيلي إلى خمس مجموعات متساوية ٢٥ سمكة فى كل مجموعة. المجموعة الأولى تم تغذيتها على العليقة الطبيعية كمجموعة ضابطة بينما المجموعة الثانية فتم حقنها بريتونيا بميكروب الايرومونات هيدروفيل كمجموعة معده و غير معالجه . الأسماك فى المجموعة الثالثة فقد تم اصابتها تجريبيا بالمثل كما فى المجموعة الثانية ولكن تم اعطائها دواء البروبليز فى العليقة بجرعة علاجيه قدرها ١٠ جم / كيلو جرام عليقه/ يوم لمدة ١٠ أيام متتالية، بينما المجموعة الرابعة فقد تم استبدال البروبليز بدواء الفلورفينيكول فى العليقة أيضا بجرعة علاجية قدرها ١٠ مجم / كيلو جرام وزن حى لمدة ١٠ أيام متتالية أما المجموعة الخامسة فقد تم اعطاء البروبليز والفلورفينيكول معا ايضا فى العليقة بجرعتهما لمدة ١٠ أيام متتالية. أظهرت نتائج هذه الدراسة أن اعطاء البروبليز(صمغ النحل) والفلورفينيكول معا أدى الى تحسن صورة الدم ووظائف الكبد والكلى فى الأسماك المعده بميكروب الايرومونات هيدروفيل معمليا مقارنة باعطائهما كلا منهما منفردا.