

Effects of Streptococcinum, Hepar Sulfur, *Rosmarinus officinalis* and erythromycin on cultured rainbow trout (*Oncorhynchus mykiss*) with experimental streptococcosis

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Homeopathy or "similar therapy" is a phylum of "alternative medicines" is belonging to 7000 years ago. Hippocrates (350-460 BC), had used homeopathic remedies to cure his patients and he believed, we should cure similar with similar (Salehi, 2011). Galenus (130-200AC) was a famous anatomist, physiologist and homeopath. His books were used to cure the patients during about 1000 years.

Samuel Hahnemann was a German physician and chemist; he proved some of the homeopathic remedies on him and established the symptoms in the books: "organon of medicines" is about the philosophy of homeopathy and "rules of homeopathy" (1805), "chronic diseases" and "materia medica pura".

Homeopathic remedies can be used to treat acute and chronic diseases of humans, animals and herbs, without any side effects. About 90 percent of homeopathic remedies are made from herbs (from roots, bark, skin of barks,

flowers, seeds, fruits,...) and 10 percent of them are made from the minerals (aurum, silver, iron, mercury,...) and animals (discharges of animals such as the venom of snake, butterfly,...) (Salehi, 2010).

Diluting and shaking are two fundamental procedures in homeopathic remedies production. For example, more diluted remedies are more effective in treatment.

Sometimes there is an aggravation about 24-48 hours after the prescription; it is a good sign, because after this aggravation, every symptoms of the patient will be cured forever (Salehi, 2012).

This disease was found in *Oncorhynchus mykiss* (Eldar and Ghittino, 1999). Stressors such as: vibration of water temperature, manipulation of fish, fish ultra density, sudden vibration of physical and chemical factors of water, anomalous food,...increase sensitiveness of fish to

get any diseases such as streptococcosis (Roy, 2009).

In this study, there were 4 treatments and 2 controls, in 300 liter tanks, each tank contained 40 juvenile rainbow trout fish with 25 ± 5 g arranged weight. Pure culture of *S.iniae* in sheep blood agar from the Fish Health Department of the Shiraz University". 500 rainbow trout juvenile fish from the "Ghezelmah Rearing Center" in Uremia, were arrived in the "Iranian Artemia Research Center". Extruded food in FFT₁ size from the Faradaneh Company. 3 times feeding in a day, with 2 percent of the fish weight. The juveniles were adapted in 3 days and in forth day, 10^6 of *S.iniae* were injected in the peritoneum of all juveniles.

After 24 hour, some of streptococcosis symptoms occurred, and the remedies were prescribed: (every morning at 8 am, the circulation of water was stop for half hour and one globe or drop of the remedy were put in the water).

Treatment 1: erythromycin 20%, 0.1 weight of the body with food every morning.

Treatment 2: one globe of Streptococcinum C30, in the water, every morning.

Treatment 3: one drop of *R. officinalis* Q, in the water, every morning.

Treatment 4: one globe of Hepar Sulfur C30 in the water, every morning.

Control 1: without any injection and any therapy.

Control 2: with injection of the *S.iniae* but without any therapy.

Daily estimation of the water temperature, oxygen, pH, salinity and some chemical factors were measured. Statistical analyses were performed using SPSS (version 18). One way ANOVA was used to compare treatments and controls ($p < 0.05$) and survival and mortality compared by Tukey test ($p \leq 0.05$) (Table 1).

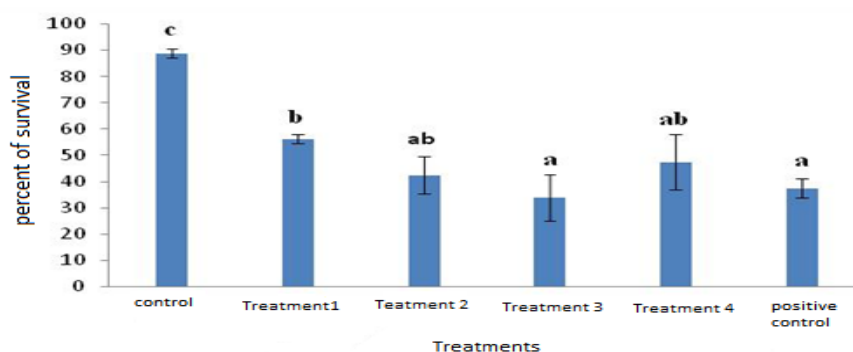
According to the survival results there was significant difference between the control 2 and the other treatments. Also there was significant difference between treatment 3, treatment 4 and treatment 2 ($p < 0.05$), this difference is due to the high dose (Q) of *R.officinalis*, while the two other homeopathic remedies were in a moderated dose (C30), 30×100 diluted dose (Table 2, Fig. 1).

Table 1: Estimation of physical and chemical factors of water.

Days	1	2	3	4	5	6	7	8	9	10
Factors										
water temp ° c	16.5	16.5	16.5	16.5	16.5	17	17	17	16	16
DO	6.8	7	7	7	7	7	7	7	7	7
N-NO (ppm)	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
NO ₂ pm	0.05	0.07	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04
NO ₃ mg/l	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
NH ₃ mg/l	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
NH ₄ mg/l	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Alkalinity mg/l	282	290	302	302	302	302	300	300	300	300
HCO ₃ mg/l	262	258	286	286	285	286	284	280	286	285
CO ₃ mg/l	20	32	16	16	16	16	16	16	16	16
pH	6.8	6.8	6.8	7	7	6.9	7	7	7	7
CO ₂ mg/l	1	0	1	2	1	1	1	2	2	3

Table 2: Number of mortality and survival of the *Oncorhynchus mykiss*

Drugs	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Control 1	Control 2
Dates						
30.1.2013	14	15	17	16	4	15
31.1.2013	5	5	8	10	3	7
1.2.2013	6	7	6	6	2	8
2.2.2013	5	7	6	4	0	7
3.2.2013	5	5	5	4	0	4
4.2.2013	4	3	6	3	0	5
5.2.2013	3	5	5	4	1	6
6.2.2013	3	4	5	4	2	5
7.2.2013	1	4	4	2	1	3
8.2.2013	2	3	3	2	0	5
9.2.2013	1	2	3	2	0	4
10.2.2013	1	3	4	1	2	5
11.2.2013	0	1	3	1	0	5
12.2.2013	2	1	3	1	0	4
13.2.2013	0	2	2	1	1	6
14.2.2013	0	2	1	0	0	5
Total	52	69	81	61	16	95
Mortality	43.3	57.5	67.5	50.8	13.3	79
Survival	56.7	42.5	32.5	49.2	86.7	21

**Figure 1: Percent of survival, in different treatments.**

Values marked with the same superscripts are not significantly different ($p < 0.05$).

Clinical and pathological surveys showed: Bleeding in brain, around and under the eyes, under the fins, abdomen, anus, branches, one or two side exophthalmia, septicemia, hyperplasia in branches (Fig. 2), melanosis in branch, heart (Fig. 3), a few of *S. iniae* in the blood (Fig. 4), necrosis of kidney and liver (Fig. 5), bleeding with congestion in connective tissues and internal organs.

Darkening of skin, branches were full of blood and opercula was open, congestion of liver, gall bladder and spleen, petechial bleeding in swim bladder and heart.

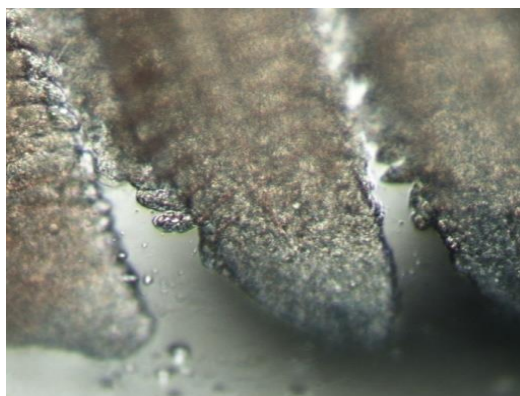


Figure 2: Brantial necrosis and hyperplasia



Figure 3: Melanosis in the heart

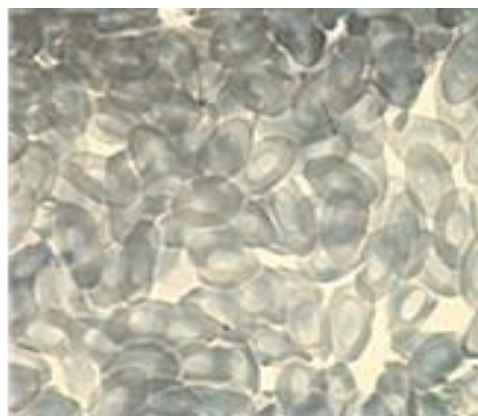


Figure 4: *S. iniae* in the blood

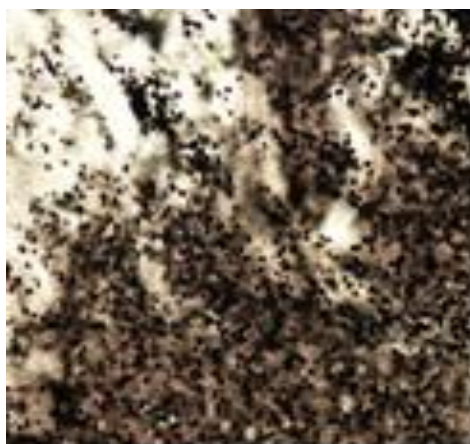


Figure 5: Melanosis in kidney

Homeopathic remedies are used to cure any diseases of herbs, animals and human. Streptococcinum is a "Nosod" (discharges of a patient who has streptococcosis).

In homeopathy we can use any product of microorganisms such as the pus, expectorations. The name of these materials is "Nosodes" that is very effective to cure related diseases.

Abutbul, *et al.*, (2004) compared sixteen accessions of *R. officinalis*, were extracted in several solvents and tested against *S. iniae* by disk diffusion assay in vitro.

All extracts were effective for inhibiting bacterial growth. The most effective was accession no.1 when

extracted in ethyl acetate, which inhibited bacterial growth by 37.5 mm/mg extract.

During the first 36h of incubation, after which bacterial growth recovered and reached the rate of the control, probably due to the decomposition or degradation of the inhibitory compounds by the bacteria.

Soltani (2008) studied the pathological effects of *S. iniae* in *O. mykiss*. For this purpose, he separated and purified *S. iniae*, 1.5×10^3 up to 1.5×10^8 then injected in peritoneum of fish and the following symptoms occurred:

Exophthalmia, hernia with bleeding, swelling of abdomen, decrease appetite, bleeding, a swelling in eyes, spleen and hearth, septicemia of internal organs and swelling of brain, increasing of melanosis in some organs, hyperplasia in lungs.

Gupta and Srivastava (2002) studied the effects of doses Q, C30, C200, 1M, 10M, 50M of *Thuja occidentalis* on the fungus *Aspergillus niger* (the factor of infection of internal ear or Otomycosis) and *A. flavus* (hyperpigmentation with severe itching). The result was shown *T. occidentalis* C 30 and C 200 had good effects on prevent the growth of *A. flavus*, while *T. occidentalis* 50M had good effects on the *A. Niger*.

Nash (2013) introduced Hepar Sulphuris Calcareum to cure the symptoms such as: pains, weakness with pain, cough and bronchitis, pause forming in little injuries, itching with

cough and emphysema in esophagus, pause in kidney and mucus membrane. In this study, bleeding from brain, around the eyes, under the fins, under abdomen, under anus, in gills and in other organs of diseased fish, were observed.

Septicemia in internal organs, hyperplasia in gills, melanosis in gills, kidney, liver and intestine was observed. Kidney was black.

Many *S. iniae* were observed in the blood. Liver, gallbladder and spleen were swollen. There were bleeding under the skin, in swim bladder and hearth.

The effect of Hepar Sulfur C30 was relatively similar to erythromycin. Erythromycin is a chemical drug, while Hepar sulfur is a natural drug and has not any side effect on the fish and any other creatures.

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