Application of rosemary Rosmarinus officinalis extract as an anthelmintic agent against Monogenean parasite in common carp Cyprinus carpio

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Cyprinids are one of the most farmed fish in fresh water ecosystems. At present, one of the biggest problems in intensive cyprinid species production is ectoparasitic diseases. They affect the health and productivity of cyprinids. Parasites can cause skin erosion, mechanical damage, immunosuppression, secondary infectionsa and chronic stress. In addition, they cause economic losses, which result from mortality in fish stocks and high treatment costs.

Natural products such as medicinal plants have alternative approaches for parasitic treatments. They have an antibacterial, antiparasitic, antipathogenic, antitumour, immunostimulant activites. In addition, they were reported to have low toxicity to fish and have fewer adverse environmental impacts. The aim of this study is to determined to find new effective candidate herb against fish parasites. In addition, effective doses are determined to treat infected fish by herb extract in bath and oral treatment methods. In the second part of this study, toxicology and pharmacokinetic studies were investigated during the herb extract treatment.

First part, I examined the ffects of some herbs, such as rosemary, clary sage and thyme leaf extracts against *Ichthyophthirius multifiliis* (protozoa), *Trichodina* spp. (protozoa), *Dactylogyrus minutus* (monogenea), *D. extensus* (monogenea) and *Argulus* spp. (arthropoda) *in vitro*. Rosemary extract showed strong antiparasitic activity against protozoa and monogenea parasites. Based on the result of screening, I focused on the effects of rosemary extract against monogenean parasites and conducted various experiments for applying herb to control parasitic diseases. In addition, I evaluated the efficacy of rosemary extract by immersion and oral method to prevent and control monogenean infestation. In last part, I examined the safety of rosemary extract against fish. Also, I investigated 1,8-Cineole component (active component of rosemary extract) in fish blood and skin mucus.

In vitro parasite survival experiments revealed that both of ethanol and aqueous rosemary extracts reduce parasite survival time in dose dependent manner and ethanol extracts of rosemary had higher anti-parasitic activity against the *Ichthyophthirius multifiliis*, *Trichodina* spp., *D. minutus* and *D. extrensus* than that of aqueous extracts. The pure component of rosemary extract obtained commercially used in *in vitro* experiments showed that 1,8-Cineole was the most toxic component against *D. minutus*. The parasite intensity and prevalence in fish exposed to 50 and 100 g aqueous rosemary solution/L water for 30 min were significantly lower than those in controls ($p < 0.05$). In oral treatment experiments, diets of *C. carpio* were supplemented with eight different concentrations of aqueous rosemary extract. The intensity of *D. minutus* was significantly less in fish fed for 30 days with feed containing 60, 80 and 100 ml aqueous extract/100 g feed than in control ($p < 0.05$). No abnormal behavior was observed in any experimental fish group in the 30 day feeding period. Together these results indicate that rosemary is a promising candidate for prevention and control of monogenean infection. Aqueous rosemary extract demonstrated lower toxicity against host, and although its anthelmintic activity was slightly inferior to that of the ethanolic extract, the aqueous extract is a relatively safe and beneficial method for practical control of monogenean parasites.

In order to examine the safety of rosemary aqueous extract, toxicological study were conducted in fish were fed experimental diets supplemented with 10, 20, 40, 80, 100 ml extract/100 g feed for 20 days. According to the histopathological examination, the notable histological changes were detected in liver and kidney. Atrophy and nuclear pyknosis of hepatocytes were caused in the groups of ≥ 20 ml aqueous extract/100 g feed administration. In kidney, granular degeneration lead to necrosis and vascular irregularity were observed in groups of ≥ 40 ml aqueous extract/100 g feed. It was estimated that the LOAEL of rosemary extract against fish was 20 ml aqueous extract/100 g feed by the hepatotoxic effect. These

findings indicate that high doses of rosemary extract in diet for 20 days can be damage to liver and kidney.

In plasma chemistry analysis, fish were fed three experimental diets: control (without extract), 10 ml and 80 ml rosemary extract/100 g of feed for 20 days. Serum samples were taken at the end of 10 and 20 days feeding. AST (aspartate aminotransferase) activity of rosemary diet groups was tended to higher than of control group although the difference was not significant. The number of specimens that showed higher AST activity was increased with dose dependent manner. The AST is indicator of hepatocytes damage in vertebrates. It is suggested that the results of AST activity reflected hepatotoxicity of rosemary extract obtained by histopathological examination. In other clinical biochemical parameters, there were no significant differences among the experimental groups. These results suggest that the liver function and kidney damage were not serious.

1,8-Cineole was detected in blood 80 ml aqueous extract/100 g feed for 1 day. Following oral administration, 1,8-Cineole level in blood was reached to peak (117.89 ± 3.47 ng/ml) at 60 min after administration. After that, the amount of 1,8-Cineole in blood decreased exponentially from 2 to 72 h. The elimination half-lives ($T_{1/2}$) of 1,8-Cineole was calculated as 248 min in blood. 1,8-Cineole were detected 15.08 ± 7.6 ng, 49.8 ± 41.1 ng and 60.9 ± 37.7 ng in 10 mg crude dried mucus 5, 10 and 20 days administration, respectively. These results indicate that components of rosemary extract, such as 1,8-Cineole, absorbed by oral administration and travel through the blood, and can be secreted to mucus.

In summary, rosemary extract and its active compound of 1,8-Cineole showed anthelmintic activity against monogenean parasite. Both of oral administration and immersion of rosemary extract were effective to cure monogenean parasitic infection. In addition, component of rosemary extract was absorbed by oral administration and travel through the blood and

secreted into mucus. Based on these results, I concluded that rosemary extract would be candidate anthelmintic agent that apply to control of parasitic infection of farmed fish. On the other hand, it was revealed that rosemary extract had hepatotoxity and nephrotoxity in histopathological study and its LOAEL might be lower than that of effective dose. Further studies are recommended to establish suitable treatment regime for practical use of rosemary extract to cure parasitic infection in farmed fish.