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Effects of Rut in on Helminth Infected Fish, *Channa punctata* – Hematological, Biochemical and Immunostimulatory Study

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Abstract—*Fisheries being one of the promising sectors of agriculture has been recognized as a employment generator, source of nutritious food and a foreign exchange earner. Fishes harbors numerous parasites causing huge economic loss, hence it is necessary that the fish should be free from all types of pathogens like viruses, bacteria, algae, protozoans, helminths, annelids, arthropods and mollusks. Immunostimulants obtained from natural sources are presently gaining importance in present times instead of using chemically synthesized drugs and antibiotics which enhances the non-specific immune responses (innate immunity). Secondary metabolites present in plants like flavonoids are known for their various functions like antioxidant, antibacterial, antiviral, immunomodulatory and anticancer. In the present study, Rutin a flavonol glycoside comprising of the flavonol quercetin and the disaccharide rutinose was observed for their role in helminth infected fishes and immunomodulatory function. Hematological, biochemical and nonspecific immune parameters were studied for rutin injected fishes and their effect on helminth infested fishes.*

In the present study results shows that the serum parameters, hemoglobin, RBC count, WBC count, nitric oxide, in Channa punctata, had significantly changed over a period of 7 days after injection of rutin. Haemoglobin, RBC count, nitric oxide were low in infected fishes which increased significantly in treated fishes. WBC levels also showed significant increase in helminth infected fishes than those of injected and control fishes. Thus in can be concluded that dietary supplementation of rutin, extracted from various plants has shown to increasethe O_2 levels, and thereby enhancing immunity of Channa punctata and its resistance against various parasites.

Keywords*: biochemical, Channa punctata, helminth, hematological, immunostimulatory, rutin.*

1. INTRODUCTION

Aquaculture is a fast growing food production industry that contributes nearly 50% of the annual fisheries production(1). Indiscriminate use of antibiotic has lead to the emergence or development of antibiotic-resistant pathogens, thus safer and more effective alternatives should be used (2) . Studies on the use of immunostimulants derived from natural bioactive products are presently gaining importance as an option instead of using chemotherapeutants and antibiotics. Immunostimulants enhance the non-specific immune responses (innate immunity) as well as the specific immune response mechanisms (adaptive or acquired immunity) of a certain organism (3).In coastal, marine and fresh water environments, most fish harbours very diverse parasite fauna (4,5, 6) and these infections with some parasites may result in a modulation of physiological or immune responses (4) which are considered as indicators of biological effects of parasitic infestation.

Among various biochemical, cellular and physiological systems, certain innate immune responses are considered as suitable biomarkers for monitoring biological effects of parasitic infection. The non-specific immune system of fishes constitutes their first line of defence against pathogens (7) hence cellular and humoral response of the innate immune system are of great importance. They are unspecific although effective against pathogens which try to invade the host. Activity of phagocytic cells, such as endocytosis or respiratory burst, and plasma lysozyme levels are part of innate immune mechanisms, which respond immediately to pathogen challenge.

Flavonoids are also known as nature's tender drugs as they possess various pharmacological activities and the name has been derived from the Latin word *flavus* meaning yellow The flavonoids in general possess antioxidant (8, 9) anti-inflammatory (10, 11), anticancer (12,13, 14) antimicrobial (15,16), antiviral (17,18), immunomodulatory (19,20) and antiulcergenic (21, 22) activities.

Rutin ($C_{27}H_{30}O_{16}$) a polyphenolic bioflavonoid (2-(3,4-dihydroxyphenyl)-4,5-dihydroxy-3-[3,4,5-trihydroxy-6-[(3,4,5-trihydroxy-6 methyl-oxan-2-yl) oxymethyl]oxan-2-yl]oxy-chromen-7-one) (Figure 1) also known as quercetin-3-

rutinoside or sophorin .It is a flavonol glycoside comprised of the flavonol quercetin and the disaccharide rutinose (23) which is found in fruits, vegetables and plant-derived beverages such as tea and wine. It has been detected in various plants showing anthelminthic activity like *Calotrophis gigantea, Phyllanthus amarus* (24), *Nicotiana tabacum* (25), *Tecomella undulata* (26), *Rauwolfia serpentine* (27).Rutin has significant scavenging properties on oxidizing species such as OH radical, superoxide radical, and peroxyl radical. Rutin has significant scavenging properties on oxidizing species such as OH radical, superoxide radical, and peroxyl radical (28).Therefore, it shows several pharmacological activities including antiallergic (29), anti inflammatory and vasoactive (30), antitumor (31), antibacterial, antiviral, and antiprotozoal properties (32).Moreover, it has also been reported that rutin has other therapeutic effects such as hypolipidaemic (33), anticarcinogenic (34) and antidiabetic effect (35).

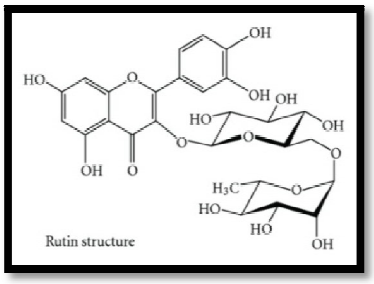

Figure 1

This study was therefore undertaken to investigate the protective role of rutin on helminth parasite infected damage in fishes by measuring some hematological, biochemical and nonspecific immune parameters activity as indicator of oxidative stress.

2. MATERIALS AND METHODS

2.1 Collection of host fishes

Live host specimen randomly sampled and were collected from fish farms of Krishnagar, Nadia West Bengal.They were then brought alive at the Parasitology Laboratory of the Department of Zoology, Krishnagar Government College for examination. The collection of hosts have been done during the study period of January 2018– April 2018. Specimens were kept for one week under observation for acclimatization in glass aquaria (40 × 60 × 100 cm). Water was changed after every 24 hr and commercial fish food was supplied to fish during acclimatization period.

2.2 Collection and identification of helminth parasites

Adult fish specimens of nearly similar weight and length were dissected in physiological saline (0.75% NaCl solution)for collecting helminth parasites. Standard procedure was used for collection of helminth parasites were then preserved in coupling jars containing 70% ethanol and 5% glycerine (36, 37). The relative parameters were measured and identification was performed using selected identification keys (36,37).The approval of Institutional Animal Ethics Committee, University of Kalyani was not taken since the experiment were made on commonly available edible fishes.

2.3 Experimental Design

Effect of rutin on the intensity of helminth infection to *Channa punctata* was evaluated.Fishes individually were injected into the ventral side of the body with 1 mg /ml rutin solution to achieve doses of 10 mg/ g body weight on day 1.Experimental and control fishes, 10 in each treatment, were maintained in 60 L glass aquaria containing 40 L of water. Water was renewed daily, and the experiment lasted for 7-10 days.

2.4 Haematological parameters

2.4.1 Sample preparation

Blood samples collected by caudal puncture method were immediately transferred into EDTA containing assay tubes at an approximate concentration of 5 mg ml^{-1} of blood (38). Blood with EDTA was used for determination of total count of RBC, WBC and hemoglobin.

2.4.2 Haemoglobin (Hb)

Haemoglobin estimation was done by Sahli's method with the help of a haemometer.

2.4.3 Total count of Red blood cell (RBC) count and White blood cell (WBC) count

Total count of RBC and WBC was done by diluting blood samples with RBC and WBC diluting fluids and the counts were determined using an improved Neubauer hemocytometer and then calculated (38, 39).

2.5 Estimation of serum biochemical parameters

2.5.1 Collection of blood

The blood samples collected by caudal puncture method in a clean, dry sample container (without anticoagulant) were centrifuged at 2000 rpm for 5-6 minutes at 4°C. The supernatant containing the serum was collected and stored in sterile eppendorff tube at -20°C till further use.

2.5.2 Quantitative estimation of total protein (TP)

Total protein concentration in serum was analyzed by Biuret method (40).

2.6 Immunological parameters

2.6.1 Respiratory burst activity estimation by Nitroblue tetrazolium assay method

The nitroblue tetrazolium assay (41) was followed with modification (42).

2.6.2 Nitric Oxide estimation by Griess reagent

Determination of NO was done by Griess reagent (43).

2.7 Statistical analysis

The experiments were conducted in triplicates. All the values are given as the mean ± standard error of the mean (S.E.M). The values of the hematological and biochemical data between the control and infected groups of fish blood were compared statistically by using student's t test (2-tailed). The mean values were compared at the 1% level of significance (P<0.01).

3. RESULTS AND DISCUSSION

3.1 Effect of rutin on the susceptibility of *Channa punctata* to helminth infection

All control fishes survive. In contrast, death began to occur after 24 h in challenged fishes. After 48–72 h of challenge, survival rates of fishes that received rutin at 10 mg g^{-1} were significantly higher than those of fishes that received saline and control fishes (P < 0.05).Survival rates were 92 % for fishes that had been injected with rutin at 10 mg g^{-1} after 72 h.

3.2 Physiological parameters of *Channa punctata* injected with rutin

Among the hematological parameters, hemoglobin levels were significantly higher in treated fishes than those of infected and control fishes levels after 7 days (P <0.05). (Figure 2).

However, slight changes in RBC cell count levels were observed among the treated fishes in contrast with the infected fishes although marked changes in control and treated fishes were not observed after 7 days (P <0.05). (Figure 2).The WBC count levels were elevated in infected fishes which decreased in treated fishes. Control and infected fishes did not show much difference in levels after 7 days (P <0.05)(Figure 3).Serum protein levels of fishes that received rutin at 10 mg g^{-1} were higher than those of fishes that those of infected fishes after 7 days (P <0.05), although control fishes recorded higher levels than treated ones (Figure 4).

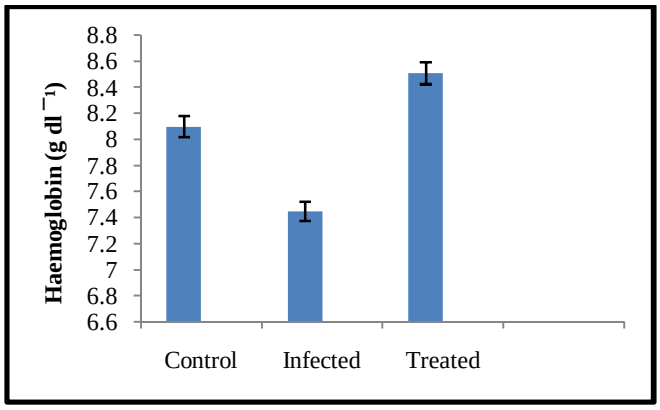

Figure 2: Test for estimation of haemoglobin

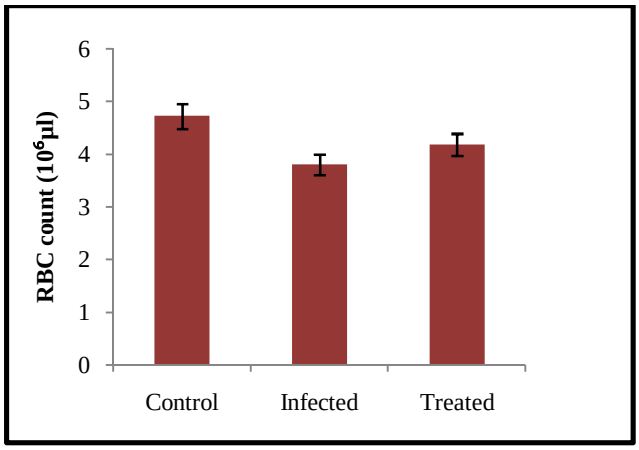

Figure 3: Test for estimation of RBC count

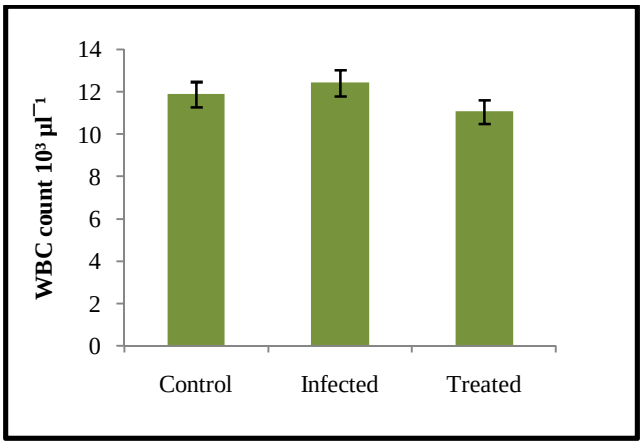

Figure 4:Test for estimation of WBC count

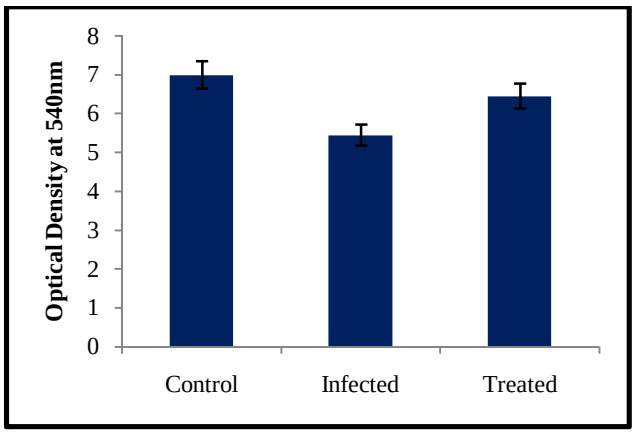

Figure 5: Test for estimation of Total Protein

3.3 Immunological Parameters

3.3.1 Nitric Oxide Estimation

The nitric oxide value in treated fishes was found to be higher than control fishes at 10 mg g^{-1} after 7 days of treatment (Figure 7). Standard curve of sodium nitrite was prepared for the estimation of nitric oxide (Figure 6).

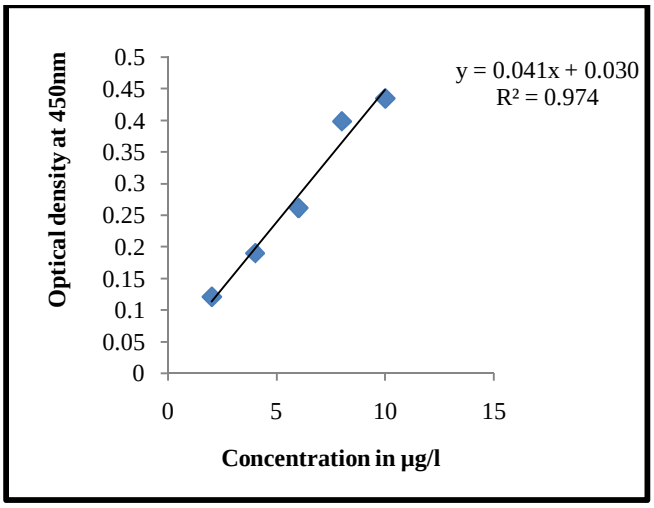

Figure 6: Standard curve of sodium nitrite

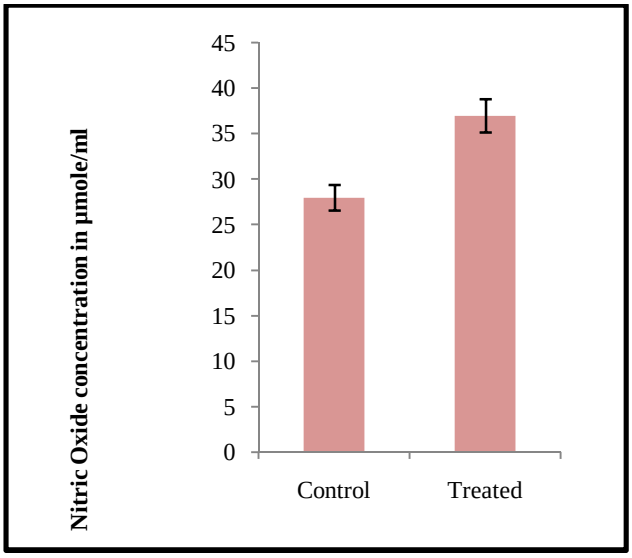

Figure 7: Test for estimation of Nitric Oxide

Studies on neutrophil activity presented in Figure 8 clearly showed the enhancing effect of rutin on neutrophil respiratory burst activity, which is evident from the increased NBT reduction. The neutrophil activity was enhanced in test groups but the highest significant NBT reduction was achieved 7th day.

The NBT assay is a quick inexpensive test focusing on the ability of phagocytes to reduce the dye by the production of oxygen radicals. In animals, the oxygen radicals are focused at the destruction of bacterial invaders. The ability of macrophages to kill pathogenic parasites like trematodes is probably one of the most mechanisms of protection against disease among fishes. The higher optical density in the NBT assay was observed in all the treatments. Similar results were obtained by other researchers (44) Some authors have also demonstrated that respiratory burst (NBT) activity in levamisole fed group was found significantly higher as compared to the CYP-treated control group(45).

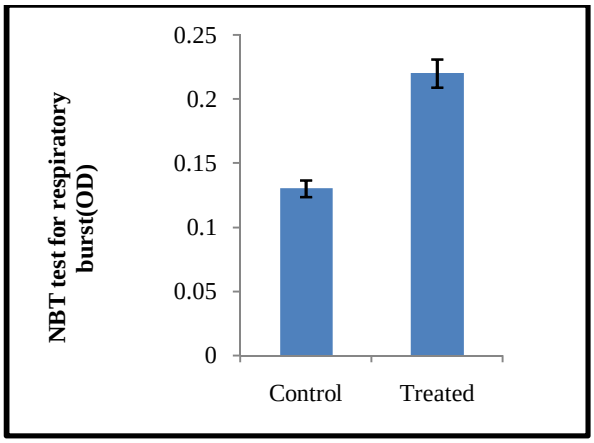

Figure 8: Test for estimation of respiratory burst in fishes by NBT method

4. DISCUSSION

Plants are the storehouses and rich sources of huge number of natural compounds. These natural plant products have been reported to have various activities like antistress, growth promotion, appetite enhancement, tonic, immunostimulant, and antimicrobial (46). In this study, we demonstrated the rutin, showed positive effects on improved fish immunity against helminth infections. Many natural compounds from various sources (bacterial components, chemical agents, animal or plant extracts etc.) have been investigated as prospective immunostimulants against pathogen infection in aquaculture. The immunostimulatory activities of algal extracts in fish and shrimp have been reported previously (47, 48).

Results in this study showed that the serum parameters of heamoglobin, RBC count, WBC count, Nitric Oxide, total protein in *Channa punctata*, were significantly changed over a period of 7 days after injection of Rutin. WBC levels were significantly increased in helminth infected fishes than those of injected as well as control fishes. Haemoglobin, RBC, nitric oxide were low in infected fishes which increased treated fishes.Thus dietary supplementation of rutin, extracted from various plants has shown to increase the O_2 levels, and thereby the *Channa punctata* immunity is enhanced and its resistance against various parasites were improved.

Conflict of Interest: None

5. ACKNOWLEDGMENT

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Ethical Approval : The fishes were collected in fresh condition from different fish farms of West Bengal. Animal ethical care guidelines were followed as the fish were used in the study. It has been informed that as per CPCSEA instruction's protocol for experimentation on fishes, does not require approval.

REFERENCES

[1] Food and Agriculture Organization of the United Nations (FAO) (2012) Main Cultured Species. http://www.fao.org/fishery/topic/13531/en

[2] Pachanawan A, Phumkhachorn P, Rattanachaikunsopon P (2008) Potential of Psidium guajava supplemented fish diet in controlling Aeromonas hydrophila infection in tilapia (Oreochromis niloticus).Biosci Bioeng 106(5):419-424

[3]Anderson DP (1992) Immunostimulants, adjuvants, and vaccine carriers in fish: applications to aquaculture. Annu Rev Fish Dis 1:281-307

[3] Anderson D P and Siwicki AK (1995) Basic hematology and serology for fish health programs. In: Diseases in Asian Aquaculture II. (M. Shariff, J.R. Auther & R.P. Subasinghe ed.), pp. 185-202, Fish Health Section, Asian Fisheries Society, Manila.

[4] Overstreet RM (1997) Parasitological data as monitors of environmental health. Parassitologia 39:169–175

[5] Broeg K, Zander S, Diamant A, K_rting W, Krner G, Paperna I, Westernhagen H von (1999) The use of fish metabolic, pathological and parasitological indices in pollution monitoring. I. North Sea. Helgol Mar Res 53:171–194

[6] Schmidt V, Zander S, K_rting W, Steinhagen D (2003) Parasites of flounder (Platichthys flesus) from the German Bight, North Sea, and their potential use in biological effects monitoring. A. Infection characteristics of potential indicator species. Helgol Mar Res 57: 262–271. doi 10.1007/s10152-003-0159-x

[7] Ingram GA (1980) Substances involved in the natural resistance of fish to disease: a review. J Fish Biol 16:23–60

[8]Williams RJ, Spencer JPE, Rice-Evans C (2004) Flavonoids: antioxidants or signaling molecules? Free Radic Biol Med 36: 838-849

[9] Petkov E, Nickdor N, Uzunov P (1981) Inhibitory effects of flavonoids on xanthine oxidase. Planta Med 43:183-187

[10] Gonza´lez-Gallego J, Sa´nchez-Campos S, Tun˜o´n MJ (2007) Anti-inflammatory properties of dietary flavonoids. Nutr Hosp 22:287-293

[11] Garcia-Mediavilla V, Crespo I, Collado PS, et al (2007) The anti-inflammatory flavones quercetin and kaempferol cause inhibition of inducible nitric oxide synthase, cyclooxygenase-2 and reactive C-protein, and down-regulation of the nuclear factor kappa B pathway in Chang Liver cells. Eur J Pharmacol 557: 221-229

[12] Hertog MGL, Hollman PCH, Katan MB (1993) Intake of potentially anticarcinogenic flavonoids and their determinants in adults in the Netherlands. Nutr Cancer 20:21-29

[13] Hirano T, Oka K, Akiba M (1989) Antiproliferative effects of synthetic and naturally occurring flavonoids on turner cells of the human breast carcinoma cell line, 2R- 75-1. Res Commun Chem Pahol Pharmacol 64: 69-78

[14] Catherine C, Malc S, Esther HL, et al (1996) Lack of tumour-Promoting effects of flavonoids: studies on rat liver preneostatic foci and on in vivo and in vitro gap junctional inter cellular communication. Nutr Cancer 26:251-263

[15] Gabor M, Eperjessy E (1966) Antimicrobial activity of flavonoids from leaves of chinese herb. Nature 212:1273-1279

[16] Tereschuk ML,Riera MVQ, Casteo GR (1997) Antimicrobial activity of flavonoids from leaves of Tagetes minuta. J Ethnopharmacol 56:227-236

[17] Mahmood N, Pizza C, Aquino R (1993) Inhibition of HIV infection by flavonoids. Antiviral Res 22:189-197

[18]Tripathi VD, Rastogi RP (1981) In vitro anti-HIV activity of flavonoids isolated from Garcinia multifolia. J Sci Indian Res 40: 116-121

[19] Kais N, Rahman MM, Rashid MA (1996) Effect of flavonoid compounds on the immune system. Fitoterapia 67: 554-561

[20] Middleton E Jr (1998) Effect of plant flavonoids on immune and inflammatory cell function. Adv Exp Med Bio 439:175-182

[21] Hirasawa T, Shoji T, Hoshino E (1982) Clinical study of effect of SU-88 on gastric ulcer combination of treatment. Japan Pharmacol Ther 10:15

[22] La Casa C, Villegas I, Alarcon De La Lastra C, Motilva V, Martin Calero M.J(2000). Evidence for protective and antioxidant properties of rutin, a natural flavone, against ethanol induced gastric Lesions. J Ethnopharmacol. 71: 45-53.

[23] Calabro ML, Tommasini S, Donato P, et al (2005) The rutin/beta-cyclodextrin interactions in fully aqueous solution: spectroscopic studies and biological assays. J Pharm Biomed Anal 36:1019-1027

[24] Shukla P, Gopalkrishna B and Shukla P (2012) Isolation of rutin from *Phyllanthus amarus*. IJPSR 3(4): 1198-1201

[25] Dubey S, Ganeshpurkar A, Bansal D, Dubey N(2013) Experimental studies on bioactive potential of rutin. Chron Young Sci 4:153-157

[26] Saraf A, Sankhala S(2014) Simultaneous Determination of Rutin and Quercetin in Different Parts of *Tecomella undulata* (Seem): An Endangered Medicinal Plant. IJPPR 6(3); 434-439

[27] Gupta J, Gupta A (2015) Isolation and identification of flavonoid rutin from *Rauwolfia serpentina* International Journal of Chemical Studies 3(2): 113-115

[28]Mauludin R, Mu¨ller RH, Keck CM (2009) Development of an oral rutin nanocrystal formulation. Int J Pharm 370:202-209

[29] Yoo H, Ku SK, Baek YD, Bae JS. Anti-inflammatory effects of rutin on HMGB1-induced inflammatory responses in vitro and in vivo. Inflamm Res. 2014;63:197–206

[30] Ihme N, Kiesewetter H, Jung F, et al (1996) Leg oedema protection from a buckwheat herb tea in patients with chronic venous insufficiency: a single-centre, randomised, double-blind, placebo-controlled clinical trial. Eur J Clin Pharmacol 50:443-447

[31] Deschner EE, Ruperto J, Wong G, et al. (1991) Quercetin and rutin as inhibitors ofazoxymethanol-induced colonic neoplasia. Carcinogenesis 12: 1193-1196

[32] Panasiak W, Wleklik M, Oraczewska A, et al(1989) Influence of flavonoids on combined experimental infections with EMC virus and Staphylococcus aureus in mice. Acta Microbiol Pol 38:185-188

[33] Park SY, Bok SH, Jeon SM, et al (2002) Effect of rutin and tannic acid supplements on cholesterol metabolism in rats. Nutr Res 22:283-295

[34] Webster RP, Gawde MD, Bhattacharya RK(1996) Protective effect of rutin, a flavonol glycoside, on the carcinogen-induced DNA damage and repair enzymes in rats. Cancer Lett 109:185-191

[35] Ahmed OM, Moneim AA, Yazid A, Mahmoud AM (2010). Antihyperglycemic, antihyperlipidemic, antioxidant effects and

the probable mechanisms of action of Ruta graveolens infusion and rutin in nicotinamide- Streptozotocin-induced diabetic rats. Diabetol Croat 39:15-35
[36] Anderson R C (2000) Nematode Parasites of Vertebrates their Development and Transmission. 2nd Edition. CABI Publising
[37] Yamaguti S (1961) The Nematodes of Vertebrates, in Systema Helminthum. Volume III. New York: John Wiley and Sons
[38] Blaxhall PC, Daisley KW(1973)Routine haematological methods for use with fish blood. J Fish Biol: 771-81
[39] Hesser EF (1960). Methods for routine on fish haematology. The Progressive Fish Culturist 22: 164-171
[40] Doumas BT(1975)Standards for total serum protein assays-a collaborative study. Clin Chim; 21: 1159-66
[41]Secombes CJ (1990) Isolation of salmonid macrophage and analysis of their killing activity. In: T.C. Fletcher, D.P. Anderson, B.S. Roberson and W.B. Van Muiswinkel (Eds.), Techniques of fish immunology, SOS Publications, Fair Haven, NJ: 137-154
[42] Stasiak AS and Baumann, CP (1996) Neutrophil activity as a potential bioindicator for contaminant analysis. Fish and Shellfish Immnunology 6 : 37-39
[43]Grisham MB, Johnson GG, Lancaster JR Jr (1996) Quantitation of nitrate and nitrite in extracellular fluids. Methods Enzymol 268:237–246
[44] Gopalakannan A and Venkatesan A (2006) Immunomodulatory effect of dietary intake of Chitin, Chitosan and Levamisole on the immune system of Cyprinus carpio and control of *Aeromonas hydrophila* infection in ponds. Aquaculture 255: 179-187
[45] Kumari J, Swain T, Sahoo PK (2003) Dietary bovine lactoferrin induces changes in immunity level and disease resistance in Asian catfish *Clarias batrachus*. Vet Immunol Immunopathol 94:1–9
[46] Citarasu T, Sekar RR, Babu MM, Marian MP (2002) Developing artemia enriched herbal diet for producing quality larvae in Penaeus monodon. Asian Fish Sci 15:21–32
[47] Fujiki K, Matsuyama H, Yano T (1993) Effect of hot-water extracts from marine algae on resistance of carp and yellowtail against bacterial infections. Sci Bull Fac Agr Kyushu Univ 47:137–141
[48] Yeh ST, Lee CS, Chen JC (2006) Administration of hot-water extract of brown seaweed *Sargassum duplicatum* via immersion and injection enhances the immune resistance of white shrimp *Litopenaeus vannamei*. Fish Shellfish Immunol 20:332–345