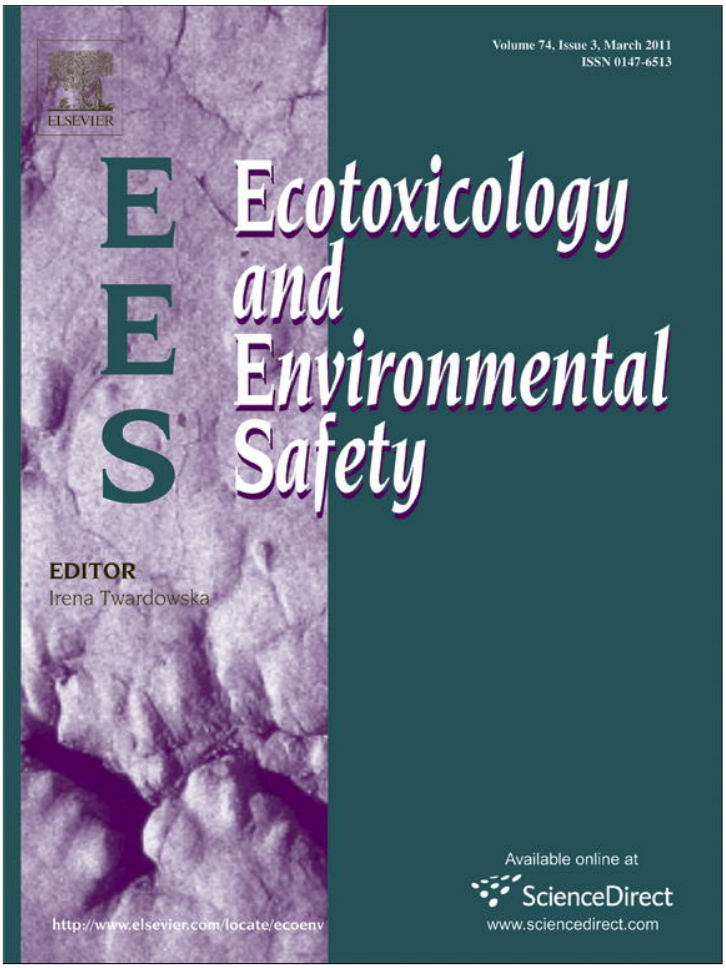

Ecotoxicology and Environmental Safety 74 (2011) 315–318

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Long-term effects of propolis on serum biochemical parameters of rainbow trout (*Oncorhynchus mykiss*)

Omid Beyraghdar Kashkooli [a,*], Eisa Ebrahimi Dorcheh [a], Nasrollah Mahboobi-Soofiani [a], Abdolhossein Samie [b]

[a] Department of Natural Resources, Isfahan University of Technology, Isfahan 84156-83111, Iran
[b] Department of Agriculture, Isfahan University of Technology, Isfahan 84156-83111, Iran

ARTICLE INFO

Article history:
Received 26 February 2010
Received in revised form
22 September 2010
Accepted 3 October 2010
Available online 27 October 2010

Keywords:
Propolis
Rainbow trout
Serum biochemistry
Toxicity
Long-term effects

ABSTRACT

Long-term effects of propolis administration on serum biochemical parameters of rainbow trout (*Oncorhynchus mykiss*) were investigated. To determine the possible toxicity and side effects of propolis, fish were fed on diets containing 0, 0.5, 1.5, 4.5 and 9 g propolis/kg diet for 8 weeks. At the end of the experiment, various seric biochemical parameters were determined. Our results showed that all dosages induced no significant alterations in growth parameters and the seric levels of total protein, albumin, globulin, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol and triglycerides and activities of glutamic pyruvic transaminase, glutamic oxaloacetic transaminase, alkaline phosphatase and lactate dehydrogenase, when compared to the control group. On the basis of our findings, propolis is a non-toxic substance for rainbow trout and its long-term administration might not have any side effects.

1. Introduction

Propolis (bee glue) is a complex resinous sticky substance that honeybees collect from buds and exudates of various plants, mix it with their own salivary secretions and waxes, and thought to be used as a protective barrier and sterilant in beehives. Due to its numerous pharmacological properties, it has been used in folk medicine since ancient times. The precise composition of raw propolis varies with the source. In general, it is composed of 50% resin and vegetable balsam, 30% wax, 10% essential and aromatic oils, 5% pollen and 5% various other substances, including organic debris (Burdock, 1998). More than 300 constituents have been identified in different propolis samples (Khalil, 2006). Propolis 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 (Bankova et al., 2000).

Due to propolis versatile biological and pharmacological activities, it has wide applications in medicine, cosmetics and food industry (Bankova et al., 1998). Propolis and its derivatives possess several biological properties such as antibacterial (Grange and Davey, 1990; Sforcin et al., 2000; Mohammadzadeh et al., 2007a), antioxidant (Kumazawa et al., 2004; Mohammadzadeh et al., 2007b), antiviral (Amoros et al., 1992), antifungal (Dobrowolski et al., 1991; Mohammadzadeh et al., 2007a) anti-inflammatory (Dobrowolski et al., 1991), antitumoral (Banskota et al., 2002; Bazo et al., 2002) and immunomodulatory (Sforcin, 2007).

Substances obtained from natural sources being biodegradable and biocompatible have received attention with regard to fish (Christybapita et al., 2007). Propolis is one of these substances, due to its proven immunostimulatory and anti-inflammatory effects in mammals and also antioxidant properties, most recently propolis and has attracted aquaculture researchers' interest (Cuesta et al., 2005; Chu, 2006; Abd-El-Rahman, 2009; Zhang et al., 2009; Talas and Gulhan, 2009); furthermore, propolis constituents may enter aquatic environments from agricultural and rural fields by rainwater and have long-term effects in fish (Talas and Gulhan, 2009). Possible toxicological effects of propolis have been widely studied and reviewed by Burdock (1998) and Sforcin (2007), but to our knowledge there is no report about the possible long-term side effects of propolis administration on aquatic animals.

Biochemical and hematological parameters of fish are determined as an index of their health status as well. As a sign of stress, the use of hematological and biochemical methods provides valuable knowledge about physiological reactions occurring against changing conditions, especially understanding the physiological, biochemical and hematological changes occurring at various concentrations of compounds, to predict the possible level of threat to life (Talas and Gulhan, 2009).

Talas and Gulhan (2009) tested the effects of propolis on rainbow trout (*Oncorhynchus mykiss*), for the first time. These

* Corresponding author. Fax: +98 311 3912840.
E-mail address: omid_beyraghdar63@yahoo.com (O. Beyraghdar Kashkooli).

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doi:10.1016/j.ecoenv.2010.10.014

authors suggested that, there is still a need for further dose-dependent studies regarding the use of propolis extracts or its constituents and environmental distribution as natural antioxidant, possible food supplement and natural protective agent.

In view the lack of publication about possible long-term effects of propolis administration on aquatic animals, this study was designed for the first time to assess the effects of orally administered propolis on rainbow trout (*O. mykiss*), which is one of the most important fish in Iran and human diet through analyzing several biochemical parameters of blood serum. Furthere, the aim was to evaluate the safety of this natural product (recently has been attracting researchers' attention for use in aquatic animals; also its possible entrance and distribution in aquatic environments) for fish health and hence for the consumers health.

2. Materials and methods

2.1. Extract preparation

Ethanolic extract of Iranian propolis was prepared by adding 90 g of absolute ethanol to 10 g of minced propolis. Bottles were sealed and shaken in darkness for 14 days at room temperature. Extract was then filtered twice and stored in sealed bottles at 4 °C until use (Krell, 1996). This extract was supplemented in commercial diet using the method, described by Cuesta et al., (2005) and Abd-El-Rahman (2009). Commercial basal diet was crushed and mixed with water and sufficient amount of extract to obtain supplemented diet with 0 (control), 0.5, 1.5, 4.5 and 9 g propolis/Kg diet; in all cases, the diet contained the same volume of ethanol. The diets were reformed into pellets, allowed to dry; and coated with fish oil. The diets were stored at 4 °C until use.

2.2. Experimental design

Juvenile rainbow trout (*O. mykiss*) were purchased from a private farm near Isfahan (Iran), acclimated and fed with commercial diet for 20 days prior to the experiment. After acclimation, fish (about 14 g/individual) were pooled and randomly distributed into 20 tanks (groups of 12 fish) with each dietary treatment being given to four tanks (four replicates for each treatment). Fish were hand-fed 3% of body weight twice daily at 10:00 and 17:00. At each feeding stage, all food were consumed and no leftover food was observed. Experiment lasted 8 weeks and rations were adjusted after day 21 and day 38 following weighing and measuring. The feeding trail was conducted in 100 L plastic tanks in an indoor air-conditioned recirculation culture system. Tanks were covered with plastic mesh to minimize disturbance and prevent escape, and supplied with continuously aerated fresh water (11 L min $\pm$ 1) at a temperature of 16 $\pm$ 1 °C.

Temperature, dissolved oxygen, total ammonia, nitrite and pH were measured three times weekly and did not exceed values recommended for rainbow trout (Tarazona and Munoz, 1995; Farhangi and Carter, 2001).

This study was performed under ethics code of Iran's Veterinary Organization.

2.3. Analytical procedures

After the intake of propolis-containing diets, growth parameters were determined. At the end of the trial, fishes were starved for 24 h and anesthetized with MS-222 at a concentration of 100 mg/L. Blood samples were obtained from the caudal vein of four randomly chosen fish from each tank. Serum was collected after centrifugation (3000 rpm for 10 min), stored in a freezer at 20 °C for biochemical analysis. Glutamic pyruvic transaminase (GPT), glutamic oxaloacetic transaminase (GOT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH) activities, total protein (TP), albumin, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol and triglycerides (TG) contents, were determined by an automated analyzer system for serum chemistry (Roche, COBAS-MIRA Plus CC) based on the manual.

2.4. Statistical analysis

The data are reported as mean $\pm$ standard error of mean (S.E.M) of four replicate groups (each tank was considered as one experimental unit). The data were subjected to one-way analyzes of variance (ANOVA) and if significant differences between means were found, Duncan's multiple range test (whose significance level was defined as $p < 0.05$) was used (SPSS 15.0).

3. Results

At the end of the experiment, the survival rates (%), weight gain (%), specific growth rate (SGR) and liver index (LI) of juvenile rainbow trout fed on the experimental diets for 8 weeks were determined and presented in Table 1. No deaths were observed in any of the groups over the entire period of the experiment. Weight gain, SGR and LI of the fish were not significantly ($P > 0.05$) influenced by the dietary supplementation of propolis.

The effects of different concentrations of orally administered propolis on rainbow trout (*O. mykiss*), observed through serum biochemical parameters, are summarized in Table 2. It can be seen that changes in enzymes activities including, GOT, GPT, ALP and LDH with different concentrations of propolis (0.5, 1.5, 4.5 and 9.0 g propolis/kg diet) are not statistically significant as compared with the control group and also, ethanolic extract of propolis did not exert any influence on the seric contents of TP, albumin, globulin, LDL and HDL cholesterol and TG in different experimental groups ($P > 0.05$).

4. Discussion

In this study, we have tested for the first time the possible effects of long-term administration and toxicity level of propolis on the rainbow trout (*O. mykiss*), one of the most popular and economical fish in Iran. In recent years, there has been a great deal of studies carried out on propolis metabolism but to our knowledge to date, there is only one research about propolis and its effects on biochemical parameters of aquatic animals for determining biological activities of this substance (Talas and Gulhan, 2009).

Natural products have been used lately as an alternative for different proposals. However, there must be a control of the indiscriminate use of such products. There are no works dealing with high propolis consumption, and with the long-term

Table 1
Survival rates (%), weight gain (%), specific growth rate (SGR) and liver index (LI) of juvenile rainbow trout fed the experimental diets containing various amounts of propolis for 8 weeks.

Propolis concentration (g/kg diet)	Initial weight (g per fish)	Final weight (g per fish)	Survival rate (%)	Weight gain of fish (%)	SGR[a]	LI (%)[b]
Control	13.9 $\pm$ 0.2	60.9 $\pm$ 0.9	100	339.11 $\pm$ 11.60	2.64 $\pm$ 0.05	1.38 $\pm$ 0.08
0.5	14.0 $\pm$ 0.2	57.5 $\pm$ 0.8	100	311.83 $\pm$ 4.36	2.53 $\pm$ 0.02	1.35 $\pm$ 0.05
1.5	13.9 $\pm$ 0.1	60.2 $\pm$ 2.0	100	332.83 $\pm$ 18.33	2.61 $\pm$ 0.07	1.34 $\pm$ 0.06
4.5	13.8 $\pm$ 0.3	55.6 $\pm$ 1.1	100	304.41 $\pm$ 13.72	2.49 $\pm$ 0.06	1.30 $\pm$ 0.05
9.0	13.7 $\pm$ 0.2	59.7 $\pm$ 1.5	100	335.68 $\pm$ 9.83	2.63 $\pm$ 0.04	1.33 $\pm$ 0.02

Values (means of four replicates $\pm$ SEs), no significant differences were seen ($P > 0.05$).

[a] SGR = (Ln final weight of fish − Ln initial weight of fish) × 100/days of feeding trial.

[b] LI = (Weight of liver/body weight) × 100.

Table 2
Changes in the biochemical parameters in rainbow trout serum with different concentrations of propolis.

Biochemical parameters	Control	Concentrations of propolis			
		0.5 (g/kg diet)	1.5 (g/kg diet)	4.5 (g/kg diet)	9.0 (g/kg diet)
GOT (U/l)	309.50 ± 7.97	322.38 ± 7.50	325.25 ± 6.12	313.00 ± 8.87	306.75 ± 7.52
GPT (U/l)	11.88 ± 1.24	10.50 ± 1.31	11.50 ± 1.08	11.62 ± 0.97	9.86 ± 0.55
ALP (U/l)	1005.00 ± 126.73	1312.13 ± 34.83	1047.25 ± 115.29	1132.38 ± 63.36	1129.38 ± 94.27
LDH (U/l)	1803.00 ± 105.59	1644.13 ± 109.89	1930.88 ± 66.90	1688.75 ± 54.46	1870.75 ± 115.41
TP (g/dl)	6.00 ± 0.21	6.18 ± 0.21	5.78 ± 0.18	6.16 ± 0.09	6.21 ± 0.28
Albumin(g/dl)	1.86 ± 0.83	2.14 ± 0.10	2.01 ± 0.06	2.03 ± 0.08	2.01 ± 0.12
Globulin(g/dl)[a]	4.13 ± 0.13	4.04 ± 0.12	3.76 ± 0.21	4.13 ± 0.03	4.20 ± 0.17
TG(mg/dl)	450.00 ± 12.87	479.63 ± 30.48	428.75 ± 39.41	445.88 ± 21.95	408.63 ± 21.94
HDL(mg/dl)	210.50 ± 9.74	222.50 ± 7.67	210.13 ± 8.07	216.75 ± 5.85	219.50 ± 4.20
LDL(mg/dl)	83.00 ± 7.64	99.38 ± 8.32	94.25 ± 7.80	83.38 ± 4.63	91.63 ± 7.13

Values (means of four replicates ± SEs), no significant differences were seen ($P > 0.05$).

[a] Globulin content was determined by subtracting albumin from the total protein.

consumption of this honeybee product. Thus, studies revealing the effects of intake of propolis are very important (Mani et al., 2006). The large number of chemical components in propolis may justify its many biological activities. However, it is possible hypothesize that its complex composition may lead to damage in the organism (Sforcin et al., 2002). Acute and chronic toxicity studies on propolis are still inconclusive (Jasprica et al., 2007). Dose-dependent effects of propolis on blood of fish can be favorable, opening new perspectives of investigation on their biological properties and utilization (Talas and Gulhan, 2009). Possible physiological effects of dietary propolis were determined in some animals like rats and mice (Sforcin et al., 1995; Sforcin et al., 2002; Mani., et al., 2006; Mohammadzadeh et al., 2007a; Ponte et al., 2007) for using in human studies, and in poultry like broilers (Biavatti et al., 2003), quails (Denli et al., 2005) and laying hens (Galal et al., 2008) but to our knowledge there have been no investigations about this area of subject in fish. Therefore, the present work attempted to address lack of information by describing the non-toxic level of use and general action of propolis ethanolic extract (after long-term administration) on overall health of fish through determining some of serum biochemical parameters of rainbow trout.

Diets containing propolis showed no adverse effects on growth parameters of rainbow trout (*O. mykiss*) and our results are in agreement with the results observed for sea bream (*Sparus aurata* L.) after dietary intake of propolis (Cuesta et al., 2005).

Long-term administration of propolis at different concentrations in diet resulted in no significant alterations in the levels of serum biochemical parameters such as, content of TP, albumin, globulin, LDL, HDL, TG, and activity of enzymes including, GPT, GOT, ALP and LDH. Liver index (LI) and various serum parameters are often used for the evaluations of liver condition and possible tissue damages (indicators of fish health). Talas and Gulhan (2009) reported significant changes in some of the seric variables after short-term administration of propolis extract (exposed to different concentrations of propolis in water for 96 h) in rainbow trout (*O. mykiss*). According to these authors report, preservation role of propolis on hematological and biochemical parameters of fish was observed at a concentration of 0.01 g/L, whereas 0.02 and 0.03 g/L concentrations appeared to be unfavorable for blood tissue of rainbow trout.

Propolis and its constituents might harm organisms when they are exposed to amounts higher than the normal level (Burdock, 1998; Jingtao, 1999). Our results about serum biochemical parameters demonstrated that administration of propolis ethanolic extract (to concentration 9 g/kg diet for 8 weeks) is non-toxic for juvenile rainbow trout, and might not harm the organism (possible side effects were not observed).

Due to the presence of some of the effective compounds such as flavonoids (flavones and flavanones), phenolic acids and their esters in propolis and propolis extracts, if the positive physiological properties and the non-toxicity of the propolis sample are proven it could be used as a mild antioxidant and preservative (Mohammadzadeh et al., 2007a). Due to antioxidant and preservative properties of propolis, it may not only prolong the physiological functions of some aquatic living organisms, but also contribute to the health benefit of consumers who consume aquatic animals (Talas and Gulhan, 2009).

5. Conclusions

It can be concluded that long-term administration of ethanolic extract of propolis in the diet of juvenile rainbow trout induced no adverse physiological and side effects on fish, and it can be tested safely as an immunostimulant and antioxidant in rainbow trout diet to the highest mentioned concentration. Moreover, additional studies must be carried out to determine the exact effects of propolis on aquatic animals and aquatic environments, using different doses, different indicators and various aquatic spices.

Acknowledgment

This study was supported by Isfahan University of echnology (IUT).

References

Abd-El-Rahman, A.M.M., 2009. Antagonism of *Aeromonas hydrophila* by propolis and its effect on the performance of Nile tilapia, *Oreochromis niloticus*. Fish. Shellfish Immunol. 27 (3), 454–459.

Amoros, M., Simoes, C.M.O., Girre, L., Sauvager, F., Cormier, M., 1992. Synergistic effect of flavones and flavonols against Herpes simplex virus type I in cell culture. Comparison with the antiviral activity of propolis. J. Nat. Prod. 55, 1732–1740.

Bankova, V.S., Christov, R.S., Delgado Tejera, A., 1998. Lignans and other constituents of propolis from the Canary Islands. Phytochemistry 49 (5), 1411–1415.

Bankova, V.S., De Castro, S.L., Marcucci, M.C., 2000. Propolis: recent advances in chemistry and plant origin. Apidologie 31, 3–15.

Banskota, A.H., Nagaoka, T., Sumioka, L.Y., Tezuka, Y., Awale, S., Midorikawa, K., Matsushige, K., Kadota, S., 2002. Antiproliferative activity of the Netherlands propolis and its active principles in cancer cells lines. J. Ethnopharmacol. 80, 67–73.

Bazo, A.P., Rodrigues, M.A.M., Sforcin, J.M., Camargo, J.L.V., Ribeiro, L.R., Salvadori, D.M.F., 2002. Protective action of propolis on the rat colon carcinogenesis. Teratog. Carcinog. Mutagen. 22, 183–194.

Biavatti, M.W., Bellaver, M.H., Volpato, L., Costa, C., Bellaver, C., 2003. Preliminary studies of alternative feed additives for broilers: *Alternanthera brasiliana* extract, propolis extract and linseed oil. Braz. J. Poult. Sci. 5 (2), 147–151.

Burdock, G.A., 1998. Review of the biological properties and toxicity of bee propolis (propolis). Food Chem. Toxicol. 36 (4), 347–363.

Chu, W.H., 2006. Adjuvant effect of propolis on immunization by inactivated *Aeromonas hydrophila* in carp (*Carassius auratus gibelio*). Fish. Shellfish Immunol. 21 (1), 113–117.

Cuesta, A., Rodríguez, A., Esteban, M.A., Meseguer, J., 2005. *In vivo* effects of propolis, a honeybee product, on gilthead seabream innate immune responses. Fish. Shellfish Immunol. 18 (1), 71–80.

Christybapita, D., Divyagnaneswari, M., Michael, R.D., 2007. Oral administration of *Eclipta alba* leaf aqueous extract enhances the non-specific immune responses and disease resistance of *Oreochromis mossambicus*. Fish. Shellfish Immunol. 23 (4), 840–852.

Denli, M., Çankaya, S., Silici, S., Okan, F., Uluocak, A.N., 2005. Effect of dietary addition of Turkish propolis on the growth performance, carcass characteristics and serum variables of quail (*Coturnix coturnix japonica*). Asian. Australas. J. Anim. Sci. 18 (6), 848–854.

Dobrowolski, J.W., Vohoraq, S.B., Sharma, K., Shah, S.A., Naqvi, S.A.H., Dandiya, P.C., 1991. Antibacterial, antifungal, antiamoebic, antiinflammatory, and antipyretic studies on propolis bee products. J. Ethnopharmacol. 35 (1), 77–82.

Farhangi, M., Carter, C.G., 2001. Growth, physiological and immunological responses of rainbow trout (*Oncorhynchus mykiss*) to different dietary inclusion levels of dehulled lupin (*Lupinus angustifolius*). Aquacult. Res. 32 (Suppl. 1), 329–340.

Galal, A., Abd El-Motaal, A.M., Ahmed, A.M.H., Zaki, T.G., 2008. Productive performance and immune response of laying hens as affected by dietary propolis supplementation. Int. J. Poult. Sci. 7 (3), 272–278.

Grange, J.M., Davey, R.W., 1990. Antibacterial properties of propolis (bee glue). J. R. Soc. Med. 83, 159–160.

Jasprica, I., Mornar, A., Debeljak, Z., Smolcic-Bubalo, A., Medic-Saric, M., Mayer, L., Romic, Z., Bucan, K., Balog, T., Sobocanec, S., Sverko, V., 2007. *In vivo* study of propolis supplementation effects on antioxidative status and red blood cells. J. Ethnopharmacol. 110 (3), 548–554.

Jingtao, Z., 1999. A refined propolis which is lowly-toxic and non-allergens. World Phytomed. 14 (1), 37.

Khalil, M.L., 2006. Biological activity of bee propolis in health and disease. Asian Pac. J. Cancer Prev. 7, 22–31.

Krell, R., 1996. Value-added products from beekeeping. Chapter 5: propolis. FAO Agricultural Services Bulliten. 124.

Kumazawa, S., Hamasaka, T., Nakayama, T., 2004. Antioxidant activity of propolis of various geographic origins. Food Chem. 84 (3), 329–339.

Mani, F., Damasceno, H.C.R., Novelli, E.L.B., Martins, E.A.M., Sforcin, J.M., 2006. Propolis: effect of different concentrations, extracts and intake period on seric biochemical variables. J. Ethnopharmacol. 105, 95–98.

Mohammadzadeh, S., Shariatpanahi, M., Hamedi, M., Ahmadkhaniha, M., Samadi, R., Ostad, S.N., 2007a. Chemical composition, oral toxicity and antimicrobial activity of Iranian propolis. Food Chem. 103 (4), 1097–1103.

Mohammadzadeh, S., Sharriatpanahi, M., Hamedi, M., Amanzadeh, Y., Sadat Ebrahimi, S.E., Ostad, S.N., 2007b. Antioxidant power of Iranian propolis extract. Food Chem. 103 (3), 729–733.

Ponte, F.L.R., Silva, A.A.R., Maia, M.B.S., 2007. Preclinical toxicity study of the phytomedicine—bee honey, propolis and *Mikana glomerata* extract in rodents. Phcog. Mag. 12 (3), 204–212.

Sforcin, J.M., 2007. Propolis and the immune system. J. Ethnopharmacol. 113 (1), 1–14.

Sforcin, J.M., Fernandes Jr, A., Lopes, C.A.M., Bankova, V., Funari, S.R.C., 2000. Seasonal effect on Brazilian propolis antibacterial activity. J. Ethnopharmacol. 73, 243–249.

Sforcin, J.M., Funari, S.R.C., Novelli, E.L.B., 1995. Serum biochemical determinations of propolis-treated rats. J. Venom. Anim. Toxins 1, 31–37.

Sforcin, J.M., Novelli, E.L.B., Funari, S.R.C., 2002. Seasonal effect of Brazilian propolis on seric biochemical variables. J. Venom. Anim. Toxins 8, 244–254.

Talas, Z.S., Gulhan, M.F., 2009. Effects of various propolis concentrations on biochemical and hematological parameters of rainbowtrout (*Oncorhynchus mykiss*). Ecotoxicol. Environ. Saf. 72 (7), 1994–1998.

Tarazona, J.V., Munoz, M.J., 1995. Water quality in salmonid culture. Res. Fish. Sci. 3, 109–139.

Zhang, G., Gong, S., Yu, D., Yuan, H., 2009. Propolis and Herba Epimedii extracts enhance the non-specific immune response and disease resistance of Chinese sucker, *Myxocyprinus asiaticus*. Fish. Shellfish Immunol. 26 (3), 467–472.