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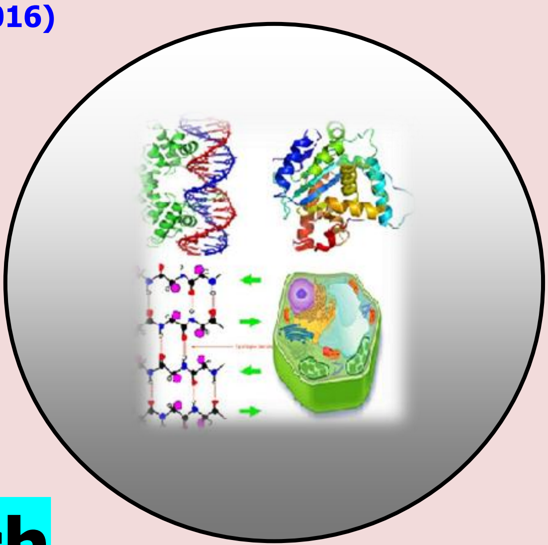

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RESEARCH PAPER

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Protective Effect of Propolis on Haematological and Biochemical Changes in Fresh Water Fish *Cirrhinus mrigala* Exposed to Pyrethroid Pesticide Fenvalerate

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ABSTRACT

Fenvalerate is a synthetic pyrethroid pesticide used in agriculture to protect a variety of crops and its exposure is associated with serious health consequences in several non target organisms, fish being one of the most prominent among these. Propolis has antioxidant properties. The main chemical classes found in propolis are flavonoids and phenolics. Bioflavonoids are antioxidant molecules that play important roles in scavenging free radicals, which are produced in neurodegenerative diseases and aging. This study was undertaken to determine the possible protective role of propolis in fresh water fish Cirrhinus mrigala, exposed with fenvalerate by assessing hamematological and biochemical analyses in the blood. Fish were divided into four groups: control, fenvalerate -treated, fenvalerate +propolis-treated and propolis-treated. MCV and MCH levels decreased slightly but significantly (p<0.05). RBC and Hb level decreased significantly (p<0.05). Maximum reduction was seen in the levels of WBC and MCHC (p<0.001). Significant reduction was seen in the activities of ACP (p<0.01), LDH (p<0.001) and AChE (p<0.001) in the blood of fishes exposed to fenvalerate while the activities of ALT (p<0.01), AST (p<0.001), and ALP (p<0.01), increased significantly. When propolis was exposed simultaneously along with fenvalerate and also in fishes exposed to propolis alone, all the enzyme activities were similar almost to levels of control fishes. The results demonstrated that the adverse effects, observed as a result of fenvalerate treatment, could be reversed by adding supplementary propolis which may improve some biochemical markers associated with physiological status of fish after exposure to fenvalerate.
Key words: Propolis, Fenvalerate, Cirrhinus mrigala, RBC, WBC, LDH and AChE.

INTRODUCTION

Improved industrialization, contemporary civilization and rapid green revolution ultimately lead to the environmental pollution. Several pollutants like metals and pesticides are being released into the environment and subsequently they affect the aquatic body. They ultimately interfere with the health of many aquatic organisms. Pesticides are known to contaminate a number of inland water bodies closer to areas of pesticide applications. The harmful effects on non-target organisms cannot be ignored though pesticides are needed for the management of pests.

In the present scenario the major environmental problem globally is the toxicity due to pesticides. The residues of organochlorines, organophosphates, carbamates and synthetic pyrethroids used widely in agricultural purposes, reach the water bodies finally (Das and Mukherjee, 2003). Synthetic pyrethroids have effectively replaced organochlorine and organophosphate pesticides because of their advantages like high toxicity to a wide range of insects, including resistant strains low toxicity to mammals and birds and rapid biodegradability. Fenvalerate is one of the pyrethroid insecticide and most widely used in agricultural crops such as cotton, paddy, jowar, maize, soya bean, tomato, lady's finger, cauliflower, tobacco and tea. But the use of this insecticide also tends to affect the biology of non-target species along with pests.

The quality of the aquatic environment can be assessed by fish which may be considered as a suitable bioindicator to monitor environmental pollution. Fishes are particularly sensitive to environmental contamination of water. Due to the unregulated use of pesticides, the pesticide residues are increasing in food materials and several other components of the environment ultimately having its toxic influence to fish, birds, domestic animals and human beings as well as several other non-target organisms (Anitha Smruthi et al., 2018). Pollutants like pesticides significantly damage certain physiological and biochemical processes when they enter into the organs of fishes (Banaee et al., 2011).

The growth and nutritional value of fish is affected when the concentration of pesticides in water goes beyond tolerance level. Due to the exposure of different pesticides the physiological functions of the fish is affected. The health of fishes is very important for human beings as they are important sources of proteins for humans and domestic animals. Several biochemical and physiological changes are evident in fishes exposed to variety of pesticides. The biochemical processes are very important aspect in assessing the toxicity of many toxicants and also finding proper protective mechanisms against the toxicants (Vasantharaja *et al.,* 2015 a). Blood parameters therefore considered as a useful tool in diagnosing the functional status of the body in response to various stressors (Houston and Carlile1997; Vasantharaja *et al.,* 2015 a and b). The toxicants are stressors which are accumulated in the fish through the food chain or absorption through the general body surface and severely affect the life supporting system at molecular and biochemical levels (Ogundiran and Fawole, 2018). Pollutants generally produce relatively quick changes in hematological characteristics of fish (Rizkalla *et al.,* 1999). Blood parameters are possibly more rapid and measurable variations under stress condition and are valuable in assessing the health state of human and animals (Hymavathi and Rao, 2000; Vasantharaja *et al*., 2015 a). Haematological and serum markers are commonly used to resolve systematic relationship and physiological adaptations including the evaluation of general health conditions in animals (Alkinson and Judd, 1978; Vasantharaja *et al.,* 2015 b). Hence, it has long been used as diagnostic tools to examine physiological, pathological and metabolic alterations in living systems (Bansal *et al.,* 1979; Hardikar and Gokhale, 2000). However, Fenvelerate toxicity study was poorly documented in different status but not elaborated in haematologic and serum levels.

Several researchers have attempted to prove the effects of natural therapeutics on pesticide induced damages in fish (Koru et al., 2007; Kanbur et al., 2009). Among the natural therapeutic antioxidants, propolis plays an important role (Kanbur et al., 2009). Recent attraction towards ameliorative point of view propolis is considered to be more effective. The therapeutic action has been attributed to several phenolic compounds with antioxidant properties, present in the propolis. The objective of the present study was to investigate the possible protective and therapeutic effects of bee propolis against fenvalerate induced toxicity on serum biochemical and haematological changes in fresh water fish *Cirrhinus mrigala*.

MATERIALS AND METHOD

Maintenance of Fishes

The present study was conducted in the Department of Zoology, Kanchi Mamunivar Centre for Post Graduate Studies, Puducherry, India. Fresh water fish *Cirrhinus mrigala* was selected for this study. The fishes were procured from Government fish pond, Puducherry. While collection, care was taken to avoid stress and injury to fishes, then they were carefully transported to the laboratory in oxygen pack. The active and healthy *Cirrhinus mrigala* of both sexes with weight of 95 ± 3 g were selected for acclimatization during which they were kept in glass aquaria for 10 days. During acclimatization the fishes were fed with commercial food pellets. The water was changed daily, the remaining food and faecal matters were removed and water quality is also monitored periodically. The healthy fishes were subsequently used for the present study. The fishes were examined carefully for any pathological symptoms and placed in water containing 0.1 mg/L of potassium permanganate solution to avoid the possibility of any dermal infection. Physico-chemical characteristics of the experimental medium such as temperature, pH, salinity, dissolved oxygen and total hardness were analyzed following standard procedure (APHA, 1998).

Experimental Design
Healthy and same sized *Cirrhinus mrigala* were chosen and sorted into 4 groups of 15 fishes each.
Group I: Control fishes
Group II : Fishes exposed to 1/10 of Lc50 value of fenvalerate (0.25µg/L) for 30 days
Group III : Fishes exposed to 1/10 of Lc50 value of fenvalerate (0.25µg/L) + 10 ppm propolis for 30 days
Group IV: Fishes exposed to 10 ppm propolis alone for 30 days.
Test solution was renewed daily, which facilitated the removal of nitrogenous waste excreted by the test fishes and for the removal of unconsumed food. The fishes were fed during the experiment at least twice (morning and evening) a day. Feeding was stopped 24 hours prior to sacrifice. The stock and test solution was prepared by dissolving the pesticide in acetone. Fishes kept in a pesticide free medium served as control. The same volume of acetone used in the dissolution of pesticide was maintained in the control.

Haematological investigation
The blood samples were collected into separate tubes containing ethylene diamine tetra acetic acid (EDTA) anti-coagulant for haematological studies. Haematological parameters including white blood cell count (WBC), erythrocytes (RBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were analyzed using Auto-hematology analyzer (MINDRAY-BC-2800, USA).

Serum biochemical analysis
Blood samples were collected by the method of caudal vein puncture. Collected blood samples were allowed to coagulate and the serum part was harvested immediately and utilized for biochemical analysis like, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) enzymes were assayed by the method of Reitman and Frankel, (1957), Acid phosphatase (ACP) was estimated by following the method of Andersch and Szezypinski, (1947) as modified by Tenniswood *et al*., (1976), Alkaline phosphatase (ALP) was assayed following the method of Bessey *et al*., (1946), lactate dehydrogenase (LDH) activity was assayed following the method of Srikanthan and krishnamurthy (1955) and acetylcholine esterase (AChE) activity was estimated according to the method of Ellman *et al.,* (1961).

Statistical analysis
The data were analyzed using Student's t-test and the data were expressed as mean ± standard error mean (SEM). The value of p<0.05 was considered as significant value against the control.

RESULTS AND DISCUSSION

The changes in haematological and biochemical parameters of the fish *Cirrhinus mrigala* exposed to fenvalerate and bee propolis are given in table 1 and 2. MCV and MCH levels decreased slightly but significantly (p<0.05). RBC and Hb level decreased significantly (p<0.05). Maximum reduction was seen in the levels of WBC and MCHC (p<0.001). In fishes exposed to propolis alone slight insignificant increase was observed only in WBC and no change in other haematological profiles. In fishes exposed to fenvalerate and propolis simultaneously, all the parameters studied were almost same as that of control fishes. Significant reduction was seen in the activities of ACP (p<0.01), LDH (p<0.001) and AChE (p<0.001) in the blood of fishes exposed to fenvalerate while the activities of ALT (p<0.01), AST (p<0.001), and ALP (p<0.01), increased significantly. When propolis was exposed simultaneously along with fenvalerate and also in fishes exposed to propolis alone, all the enzyme activities were similar almost to levels of control fishes.

Table 1. Changes in Haematological indices of the fenvalerate and propolis treated groups compared to control.

Parameters	Control	Fenvalerate	Fenvalerate + Propolis	Propolis
RBC (x10^{12}/L)	2.19 ± 0.05	1.783 ± 0.04**	2.09 ± 0.05	2.28 ± 0.06
WBC (10^{12}/L)	56.39 ± 1.69	42.36 ± 1.73***	55.86 ± 2.14	60.35 ± 2.27
HGB (g/dl)	9.53 ± 0.20	7.86 ± 0.189**	9.24 ± 0.21	10.43 ± 0.34
MCV (fl)	82.67 ± 1.98	75.63 ± 2.89	80.79 ± 3.12	83.47 ± 3.41
MCHC (g/dl)	37.29 ± 0.63	26.85 ± 0.72***	38.71 ± 0.69	39.64 ± 0.78
MCH (pg)	43.36 ± 0.95	36.23 ± 1.23*	42.96 ± 0.87	44.82 ± 0.85

The results are expressed as Mean ± SEM (n = 10) per treatment and respective control groups.
Levels of significance values are *p<0.05, **p<0.01, ***p<0.001 compared with control group. P <0.05 considered to be statistically significant.

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For assessing the impact of any toxicant in normal as well as in pathological conditions, the evaluation of haematological and biochemical profiles have been used as an effective indicators.

Haematological indices are valuable tool in the assessment of fish physiological status. In fish, exposure to chemical pollutants can induce either increase or decrease in haematological indices (Ogundiran, 2007; Adewoye, 2010; Yekeen and Fawole, 2011).

The decreased RBCs may be due to embarrassment of RBCs production and or due to the decreased synthesis of HGB. In the present study significant decrease in all the HCT parameters was observed when exposed to fenvalerate pesticide in fish *Cirrhinus mrigala* establishing an anaemic situation in the examined fish. Anaemia is associated with the diminution in the RBCs, HCT percentage and HGB contents which have also been reported by a number of researches after heavy metals and insecticides exposure (Adeyemo *et al*., 2003 and Ogundiran, *et al*., 2007; Jayaprakash and Shettu, 2013).

Decline in the WBCs count is typically pragmatic during toxic stress in organisms. Decrease of WBCs count shows the deteriorating of immune system. Substantial decrease of WBCs may be due to the sign of leucocytosis with heterophilia lymphopenia which are distinctiveness leucocytes responses in animals exhibiting stress (Thangam et al., 2014).

Uniformity decreases of MCV, MCH and MCHC value was apparent in this work and this is a hint that anaemia formed. MCHC is a good indicator of RBCs inflammation (Wepener *et al*., 1992 a & b). The MCHC is not in any way prejudiced by the blood volume or by the number of cells in the blood but can be interpreted mistakenly only when new cells with dissimilar HGB concentrations, are unconfined into blood circulation (Adewoye, 2010). The noteworthy decrease in the value of MCHC due to fenvalerate may possibly be accredited to RBCs inflammation. In another instance, a momentous decline in the values of MCH and MCHC have also been reported by Khahak and Hafeez (1996) in fish, *Cryprinion watsoni* exposed to broadly used agriculture pesticide malathion. A drop in the MCHC value was also reported by Qurestii *et al*., (1995) in fish, *Cryprinion watsoni* after exposure to various stresses like hypoxia, anaemia and hyperthermia.

A significant alteration in haematological parameters have also been documented by Sampath *et al*., (1993) and Omoregie *et al*., (1994) when fish are exposed to polluted environment. The alterations observed in the present study could be due to destruction of erythrocytes or the inhibition of erythrocyte production (Omoregie *et al*., 1994) and haemodilution (Sampath *et al*., 1993). Changes in the WBCs and RBCs in fish have been reported to be a strong indicator of stress due to the presence of toxicants in the aquatic environment. Both leucopenia and conversely leukocytosis have been observed in fish populations exposed to heavy metals (Ogundiran, *et al.,* 2007).

Generally blood parameters are one of the most sensitive indicators of metabolic disorderliness occurring in the body, therefore, blood test play a key role in the easy and rapid diagnosis of ailments occurring in the animal body. Enzymes of blood serum are considered as a relevant stress indicator (Firat *et al*., 2011). Therefore, activities of serum transferases, ACP, ALP, AChE and LDH activities have been commonly used in the diagnosis of fish diseases as well as in the detection of tissue damage caused by environmental pollution. Alteration of these enzyme activities in the extracellular fluid or serum is a sensitive indicator of even minor cellular damage (Palanivelu *et al*., 2005; Vasantharaja *et al.,* 2015 a).

Table 2. Changes in serum biochemical parameters of the fenvalerate and propolis treated groups compared to control.

Parameters	Control	Fenvalerate	Fenvalerate + Propolis	Propolis
AST (IU/l)	37.19 ± 0.84	48.24 ± 1.23***	36.32 ± 0.96	39.78 ± 0.78
ALT (IU/l)	48.63 ± 1.11	57.14 ± 1.89**	50.96 ± 2.17	52.14 ± 2.31
ACP (IU/l)	27.61 ± 0.74	21.84 ± 0.63**	26.37 ± 0.58	25.72 ± 0.67
ALP (IU/l)	52.12 ± 0.35	61.36 ± 2.42**	55.16 ± 2.31	50.75 ± 1.79
LDH (IU/l)	51.68 ± 1.22	43.65 ± 1.56***	49.36 ± 1.73	54.23 ± 2.14
AChE (IU/l)	7.31 ± 0.17	5.86 ± 0.14***	7.63 ± 0.18	8.11 ± 0.19

The results are expressed as Mean ± SEM (n = 10) per treatment and respective control groups.

Levels of significance values are *p<0.05, **p<0.01, ***p<0.001 compared with control group. P <0.05 considered to be statistically significant.

Increase in amino transferases (AST and ALT) and ALP may indicate degeneration changes and hypofunction or dysfunction of liver, as the toxicants effects on the hepatocytes and causes tissue damage in which the cellular enzymes are released from the cells into the blood serum. Therefore, increase in these enzyme activities observed in the present study of *Cirrhinus mrigala* is mainly due to the leakage of these enzymes from the liver cytosol into the blood stream as a result of liver damage by pesticide, which gives clear indication of the hepatotoxic effect of fenvalerate. Similar finding was reported by Sarkar *et al*., 1996. Significant increase in the activities of serum ALP was observed in *Rhamdia quelen* when exposed to cypermethrin by Borges *et al*., (2007). Cypermethrin exposure in *Labeo rohita* significantly increased the serum ALP activity (Das and Mukherjee, 2003). Deltamethrin exposure in Nile tilapia for 28 days resulted in enhanced serum ALP level (El-Sayed and Saad, 2008). It may be concluded that necrosis of liver and subsequent leakage of this enzyme into blood stream might be responsible for increase of this enzyme in blood.

AChE activity is very much sensitive for pesticides (Murthy et al., 2013). Alteration of AChE activity in fish may result in abnormal behavioral activities like, the swimming capability and ultimately reduced and disturbed which can attribute to more harmful consequences (Rao *et al*., 2007). Significant change in AChE activity has been observed in different tissue of different species after exposure to chlorpyrifos, carbosulfan in tilapia (Joseph and Raj, 2011). AChE activity in *Labeo rohita* was inhibited by cypermethrin significantly, brain being the most altered followed by muscles, gills and liver tissues (Marigoudar *et al*., 2010). Cypermethrin resulted in AChE inactivation which led to accumulation of acetylcholine within the cholinergic synapses, which ultimately led to hyperstimulation in *Labeo rohita* (Marigoudar *et al*., 2009; Marigoudar *et al*., 2010). Cypermethrin induced alterations in AChE, succinic dehydrogenase and LDH activities in nervous tissue of *Colisa fasciatus* (Singh *et al*., 2010). Glyposate induced AChE change in *Leporinus obtusidens* (Glusczak *et al*., 2006) and *Rhamdia quelen* (Glusczak *et al*., 2007) while karate decreased AChE activity in *Cyprinus carpio* (Bibi *et al*., 2014).

Now a day's lot of research has been conducted on the use of natural products as natural medicine or antioxidants because of their fewer side effects, easy and cheap availability. Studies on the neutralization of adverse effects caused by pesticides in fish are most significant point of view associated with human health but there are only few studies reported on the natural antioxidants against pesticide exposure in fishes (Singh and Srivastava, 2010). To eliminate and prevent the damages caused by pesticides, several antioxidants have been enrolled. Antioxidant therapy is one of the most important and protective method to prevent oxidative damage. Synthetic antioxidants are very effective but various side effects have been reported (Nabavi *et al*., 2012; Nabavi *et al*., 2013). Most of the herbal compound such as plant phenolics often exhibit antioxidant activities which may be helpful to improve the health status and stabilization living organisms (Sforcina and Bankovab, 2011).

To minimize damage and toxic effects caused by pesticides, cells should have improved immune systems consisting antioxidant molecules. When toxic agents overrun the normal level, external antioxidant and protective compounds should be taken. Consequently, the investigation of new antioxidants is a promising therapeutic agent in the field of toxicology (Orun *et al*., 2014). Bee propolis has an important place among these natural agents in particular (Kanbur *et al*., 2009; Gulhan *et al*., 2012).

The present study was undertaken to assess the effect of pyrethroid insecticide fenvalerate on a non-target organism, fresh water fish, *Cirrhinus mrigala*. The fish affected by the pesticides could pose a health problem to the consumers. Hence, it is worthwhile to investigate the effect of the pesticides on the fish like *Cirrhinus mrigala*. Of the several pesticides used, the fenvalerate toxicity and its effect on *Cirrhinus mrigala* and its amelioration by bee propolis is not so far investigated.

It is concluded from the present study that fenvalerate is capable of producing chronic toxicity to fish even at a low dose. The present results showed that fenvalerate caused haematological and biochemical alterations. The presence of propolis with fenvalerate showed protective effects against its toxicity and this may be due to the activity of propolis as antioxidant. Awareness of pesticide is the primary factor in preventing pesticide induced toxicity. Also, using propolis as supplement could be a beneficial way to overcome the toxicity of pesticides.

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