Effect of long term exposure to sublethal concentration of imidacloprid on some biochemical and haematological parameters of Grass carp and Goldfish

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Abstracts: During the present research, *C. idella* and *C. auratus* fish were exposed to 2 ppm concentration of imidacloprid for 28 and 24 days, respectively, and the effect on biochemical and haematological parameters was investigated. During the study of biochemical parameters, there occurred significant increase (P<0.05) in the serum levels of ALT and creatinine of imidacloprid exposed groups of both species of fish. The level of serum albumin of imidacloprid exposed groups of both fish species was significantly lower as compared to control group (P<0.05). Serum globulin level in imidacloprid exposed group of *C. idella* was insignificantly lower as compared to control group, however the serum globulin level of *C. auratus* was significantly lower than the control group (P < 0.05). The level of total proteins in serum of imidacloprid exposed groups of both fish species was insignificantly lower as compared to control groups (P> 0.05). During the study of haematological parameters, TLC of *C. idella* was insignificantly (P>0.05) higher than control group but the TLC of *C. auratus* was significantly (P<0.05) higher than control. There was also observed increasing trend in the percentage of neutrophils and lymphocytes of imidacloprid exposed group of each fish species. The platelets count of imidacloprid exposed group of each fish species was significantly (P<0.05) lower than control group. The haemoglobin concentration of imidacloprid exposed group of *C. idella* was significantly lower than control group (P<0.05). In case of *C. auratus*, the haemoglobin level of imidacloprid exposed group was insignificantly lower than control group (P>0.05). From the finding of the present research it was concluded that 28 days exposure of *C. idella* and 24 days exposure of C. *auratus* to 2 ppm concentration of imidacloprid does not cause mortality however the exposure causes alteration in the normal level of biochemical and haematological parameters.

Keywords: ALT, creatinine, leucocytes, platelets, haemoglobin.

INTRODUCTION

Neonicotinoids are agricultural systemic insecticides which are modeled on naturally occurring nicotine (Gullan and Cranston, 2005). They act as agonists on nicotinic acetylcholine receptor (nAChRs) opening cation channel (Casida and Durkin 2013). Imidacloprid is one of the neonicotinoids that is used for the control of insect pests in agricultural fields and also in settlements throughout the world (Jeschke *et al*. 2011). Imidacloprid is highly toxic for aquatic invertebrates (US EPA, 2008). Its solubility is 580 ppm, aerobic soil half-life is 520 days, and aerobic aquatic half-life is 1040 days, therefore imidacloprid has the potential to reach and contaminate the surface water (Starner and Goh, 2012). Aquatic organisms may be adversely affected by contamination of aquatic habitat with imidacloprid due to spray drift and or runoff (Armbrust and Peeler, 2002; Hilz and Vermeer, 2012). Fish which constitute the important source of human food may be exposed to pesticide pressure in the aquatic habitat due to current increasing trend of pesticides application in agricultural fields. Pesticides adversely affect the health and survival of fish and other aquatic organisms (El-Sayeed *et al*., 2007). Recently, Tyor and Harkrishan (2016) reported the adverse effects of imidacloprid on the embryos and larvae of common carp, *Cyprinus carpio* in the laboratory. Qadir and Iqbal (2016) exposed *Labeo rohita* to 120 ppm of imidacloprid for short (2 to 8 days) and long term (16 to 64 days). They observed adverse effect of imidacloprid on the heart, liver and kidney of *L. rohita*. Sumon *et al*. (2018) studied the fate and effects of imidacloprid on structural and functional endpoints of freshwater, sub-tropical ecosystems. They concluded that sub-tropical aquatic ecosystems is more sensitive to imidacloprid than temperate ecosystems. There are few reports about the presence of imidacloprid in surface water of agricultural areas (Kreuger *et al*., 2010). Starner and Goh (2012), detected imidacloprid in 67 water samples out of 75 samples collected in agricultural areas in California. It was concluded that imidacloprid contaminate surface water that could harm aquatic organisms following its use in agricultural fields. It has been reported that the concentration of imidacloprid in water of a small pond was high (ranged from 1.8 ppm to 7.3ppm) due to accidental spill of imidacloprid in the pond (SERA, 2005). The present research aimed to study the effect of long term exposure of two fish species, grass carp (*Ctenopharyngodon idella*) and goldfish (*Carassius*

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auratus) to a sublethal concentration of imidacloprid on some biochemical and haematological parameters.

MATERIALS AND METHODS

Collection of fish

Experiment was conducted on two fish species i.e., grass carp (*Ctenopharyngodon idella*) and goldfish (*Carassius auratus*). Both the species of fish belong to the family Cyprinidae and order Cypriniformes. Healthy *C. idella* fish of 13.55±1.4cm length were obtained from a fish hatchery at Thana Malakand agency, Khyber Pakhtunkhwa, Pakistan. Fish were brought to laboratory within 30 minutes of capture in 7L plastic jars with water of pond from which fish were collected. No fish died during transport. Similarly, healthy *C. auratus* fish of 14 ±1.4cm length were collected with the help of fishing net from River Swat near the campus of University of Malakand, Khyber Pakhtunkhwa Pakistan. Fish were brought to the Entomology and Ecotoxicology laboratory at the Department of Zoology, University of Malakand within 30 minutes of capture in 7 L plastic jars with water of collection site. The species of the fish was authenticated by an expert in fish taxonomy at fish hatchery, Thana Malakand agency, Khyber Pakhtunkhwa, Pakistan. In the laboratory the fish were maintained in small fish aquaria containing non-chlorinated tap water which were aerated by using air pumps. Air pumps were used to keep the water fully aerated. Water was changed every twenty hours. Fish were kept for seven days in the laboratory for acclimation. All the experiments were conducted in the Entomology and Ecotoxicology laboratory at the Department of Zoology, University of Malakand. For conduction of experiments on fish, permission was taken from the University of Malakand Animal Ethics Committee.

Experimental design

Two fish aquaria were arranged and labelled accordingly for two groups of *C. idella*, control group and imidacloprid exposed group. Each fish aquarium was 45 cm × 40cm × 40cm. Aquarium for control group was filled with 40L non-chlorinated tap water. For imidacloprid exposed group of grass carp fish, initially 30 L non-chlorinated tap water was added to the aquarium. Then 80 mg of imidacloprid (20% w/w powder of Jiangsu Fengshan Group Co. Ltd, China) was dissolved in 30 ml non-chlorinated tap water in a glass petri dish and then poured into the aquarium which was already containing 30 L water and labelled for insecticide treatment group of fish. Then further non-chlorinated tap water was added so that a volume of 40L was reached. Thus, 40L imidacloprid solution of 2ppm in fish aquarium was prepared. Then 12 fish of same age and same size were added to each aquarium. The period of exposure was 28 days. The fish were normally fed once a day by providing fixed quantity of fish food to each aquarium. The same arrangement of aquaria and the same procedures were performed for exposing the fish of *C. auratus* species to 2 ppm of imidacloprid. The period of exposure was 24 days. The behavior and mortality of fish was checked every day.

Estimation of biochemical and haematological parameters

On the day 29th of exposure of *C. idella* and day 25th of exposure of *C. auratus*, each fish was transversely cut just posterior to the gills with the help of a sharp blade. Immediately after cutting, the outflowing blood from bulbus arteriosus of each fish was collected into gel and EDTA tubes. All the tubes that contained blood were immediately carried to the laboratory. The blood of gel tube was analyzed for estimating the biochemical parameters i.e., alanine transaminase (ALT), creatinine, albumen, globulin and total proteins by using COBAS chemistry automation machine and Roche Diagnostic kits. The blood of EDTA tube was analyzed for estimating the haematological parameters i.e., total leucocyte count (TLC), percentage of neutrophils and lymphocytes, platelet count and hemoglobin concentration. TLC, percentage of neutrophils and lymphocytes and platelet counts were determined by automated blood cell analyzer (Sysmex Co, Japan). The hemoglobin (Hb) concentration was measured by the cyanmethemoglobin method. The data were presented as mean and standard deviation. For comparing the biochemical and hematological parameters of fish of imidacloprid exposed group with the control group, the data was subjected to un-paired sample T-test in SPSS, 16 software.

RESULTS

Mortality and behavior

There occurred no mortality in the control or imidacloprid exposed groups of fish during experiment. No abnormal changes were observed in the behavioural and swimming patterns of the control or imidacloprid exposed groups of fish.

Effect on biochemical parameters

Table 1 shows some biochemical parameters of control and imidacloprid exposed (exposed for 28 days) groups of *C. idella*. The mean serum ALT levels of control and imidacloprid exposed groups of grass carp fish were 35.6 ±15.6 and 181.2±4 U/L, respectively. The difference was significant ($P<0.05$). Similarly, the serum creatinine level of imidacloprid exposed group of *C. idella* was 0.6±.189 mg/dl which was significantly higher than the serum creatinine level of control group (0.4±0.05 mg/dl) of *C. idella* ($P<0.05$). The serum albumin level of imidacloprid exposed group was 0.3±0.05 mg/dl which was significantly lower than the serum albumin level of control group (0.5±.1mg/dl) ($P<0.05$). The serum globulin level of imidacloprid exposed and control groups of *C.*

Table 1: Effect of long term exposure to low concentration of imidacloprid on some biochemical parameters of *C. idella*

Groups	ALT (U/L)	Creatinine (mg/dl)	Albumin mg/dl)	Globulin (mg/dl)	Total protein (mg/dl)
Control	35.62±15.6	0.4±0.1	0.5±.1	2.3± 1.2	3.9±0 .6
Imidacloprid	181.2±41.4	0.6±.2	0.3±.05	2.1±1.4	2.7±1.51
Statistics	T=-2.2, P<0.05	T=-2.1 P<0.05	T=4.8 P<0.05	T=0.8 P>0.05	T= 1.6 P>0.05

Table 2: Effect of long term exposure to low concentration of imidacloprid on some biochemical parameters of *C. auratus*

Groups	ALT (U/L)	Creatinine (mg/dl)	Albumin (mg/dl)	Globulin (mg/dl)	Total protein mg/dl)
Control	120±21.7	0.5±0.1	0.5±0.1	2.6±0.5	3.02±0.67
Imidacloprid	311±12.2	0.9±0.4	0.3±0.3	1.9±0.1	2.38±0.1
Statistics	T=4.2 P<0.05	T=-2.1 P<0.05	T=7.73 P<0.05	T=2.5 P<0.05	T=1.9 P>0.05

Table 3: Effect of long term exposure to low concentration of imidacloprid on some haematological parameters of *C. idella*

Groups	TLC / cm^2	Neutrophils %	Lymphocytes %	Platelets /cm^2	Hb g/dl
Control	20500±3410	2.50±0.4	97.5±0.4	10000±1632.9	4.6±0.5
imidacloprid	27250±4140	3.5±0.4	97.3±0.5	76660±618.2	3.2±0.6
Statistics	T=-1.8, P>0.05	T=3.4, P<0.05	T=0.6, P>0.05	T=2.7, P<0.05	T=3.6, P<0.05

Table 4: Effect of long term exposure to low concentration of imidacloprid on some haematological parameters of *C. auratus*

Groups	TLC / cm^2	Neutrophils %	Lymphocytes %	Platelets /cm^2	Hb g/dl
Control	110500±408.2	2.0±0.1	94.1±1.5	110000±12247	2.6±0.2
Imidacloprid	175000±8164.9	2.7±0.47	98±1.3	70000±7500	1.9±0.2
Statistics	T=-15.8, P<0.05	T=-2.3, P>0.05	T=2.3, P<0.05	T=-6.5, P<0.05	T=2.5, P=0.05

idella were 2.1±1.4mg/dl and 2.3±1.2mg/dl, respectively. The difference in the serum globulin level between imidacloprid exposed and control groups of *C. idella* was not significant (P>0.05). Similarly, the serum level of total proteins of imidacloprid exposed and control groups of *C. idella* were 2.7±1.51 mg/dl and 3.9±0 .6 mg/dl, respectively. The difference in the serum total proteins level between imidacloprid exposed and control groups of *C. idella* was not significant (P>0.05). Table 2 shows the serum levels of some biochemical parameters of control and imidacloprid exposed groups of *C. auratus*. The serum levels of ALT of control and imidacloprid groups of *C. auratus* fish were 311±12.2 U/L and 120± 21.7 U/L, respectively. The serum ALT level of imidacloprid group was significantly higher than control group (P<0.05). The serum creatinine level of imidacloprid exposed group (0.9±0.4 mg/dl) was significantly higher than the serum creatinine level of control group (0.5±0.1 mg/dl) of *C. auratus* fish (P<0.05). The level of serum albumin of imidacloprid exposed group (0.3±0.24 mg/dl) was significantly lower than the serum albumin level of control group (0.5±0.1 mg/dl) (P<0.05). Similarly, the level of serum globulin of imidacloprid exposed group (1.9±0.1 mg/ dl) was significantly lower than the serum globulin level of control group (2.6±0.54 mg/ dl) (P<0.05) of *C. auratus* fish. The level of total proteins in serum of imidacloprid exposed was 2.38±0.1 mg/dl which was insignificantly lower than the serum total proteins of control group (3.02±0.67 mg/dl) (P>0.05).

Effect on haematological parameters

Table 3 shows some haematological parameters i.e., TLC, percentage of neutrophils and lymphocytes, platelets count and haemoglobin concentration of control and imidacloprid exposed (exposed for 28 days) groups of grass carp fish (*C. idella*). TLC of imidacloprid exposed and control groups of *C. idella* were 27250 ± 4140 cells /cm^2 and 20500 ± 3410 /cm^2, respectively. The difference was insignificant (P>0.05). The neutrophils of imidacloprid exposed group of *C.* idella was 3.5 ± 0.4 % which was significantly higher than the neutrophils of control group (2.5 ± 0.4 %) (P<0.05). The percentage of lymphocytes of imidacloprid exposed group (97.3 ± 0.3%) and control group (97.5 ± 0.2 %) of *C. idella* was nearly similar. The platelets count of imidacloprid exposed group was 7666± 618.2 cells/cm^2 which was significantly lower than the platelets count of control group (10000 ± 1632.9 cells/cm^2) (P<0.05). Similarly,

the haemoglobin concentration of imidacloprid exposed group (3.2± 0.6 g/dl) was significantly lower than the haemoglobin concentration of control group (4.6 ± 0.5) (P<0.05). The effect of 24 days exposure to 2 ppm of imidacloprid on some haematological parameters of *C. auratus* is also shown in table 4. TLC of imidacloprid exposed group (175000±8164.9 cells/cm^2) was significantly higher (P<0.05) as compared to control group (110500±408.2 cells/cm^2). The neutrophils of imidacloprid exposed group (2.7±0.47%) and control group (2.0%) was similar statistically (P>0.05). The percentage of lymphocytes of imidacloprid exposed group was 98±1.3% which was significantly higher (P<0.05) than the percentage of lymphocytes of control group (94.1±1.5%). The platelets count of imidacloprid exposed group was 70000±7500 cells/cm^2 which was significantly lower than the platelets count of control group (110000±12247 cells/cm^2) (P<0.05). Similarly, the haemoglobin concentration of imidacloprid exposed group was 1.9± 0.2 g/dl which was significantly lower than the haemoglobin concentration of control group (2.6±0.2 g/dl) (P<0.05).

DISCUSSION

During the study of biochemical parameters, the serum ALT level was significantly elevated in imidacloprid exposed groups as compared to the control groups (P<0.05). Alanine aminotransferase (ALT) plays an important role in synthesis and deamination of amino acids during condition of stress-imposed energy demand for the organism and occurs in the liver (Srivastava *et al*, 2004), heart and skeletal muscle (Petrovic *et al*., 1996) and some other organs or tissues (Bhattacharya *et al*., 2008). Elevated level of ALT in response to pesticide stress has been reported in different fish species (Qadir *et al*., 2014). The serum creatinine level of imidacloprid exposed groups of both species of fish was significantly higher as compared to control group (P<0.05). Creatinine is a byproduct product of metabolism which is normally filtered from the blood and excreted in urine. Creatinine level is usually estimated for detection of renal damage and kidney dysfunction (Toffaletti and McDonnell, 2008). Elevated blood creatinine level in fish exposed to pesticides has been reported (Mirghaed *et al*., 2018). The level of serum albumin of imidacloprid exposed groups of both fish species was significantly lower as compared to control group (P<0.05). Serum globulin level in imidacloprid exposed group of *C. idella* fish was insignificantly lower as compared to control group (P> 0.05), however, the serum globulin level of *C. auratus* fish was significantly lower than the control group (P< 0.05). The level of total proteins in serum of imidacloprid exposed groups of both fish species was insignificantly lower as compared to control group (P>0.05). Albumin and globulin constitute the important part of total protein in plasma part of the blood. Tests of albumin, globulin and total protein are used for diagnosis of liver dysfunction, immune diseases and impaired kidney function (Banaee *et al*., 2008). The reduction in the level of total protein may be due to chronic liver diseases, malnutrition or starvation (Martin *et al*., 2010). Decrease in albumin, globulin and total protein of fish exposed to pesticides has been reported (Velisek *et al*., 2008).

During the study of haematological parameters, increase was observed in TLC and percentage of neutrophils and lymphocytes of imidacloprid exposed groups of both species of fish. Leucocytes in fish body respond to various environmental stressors such as infectious agents and toxic chemical agents (Christensen. *et al* 1978). The increase in fish leucocyte count in response to toxicant exposure, is due to immunological reaction for production of more antibodies against the toxicant (Shanthi. *et al*., 2009). Neutrophils and lymphocytes are the important types of leucocytes. Increase in neutrophils and lymphocytes of imidacloprid exposed fish groups was observed when compared to control groups. The increase in neutrophils was significant (P<0.05) in case of *C. idella* and insignificant (P>0.05) in case of *C. auratus*. The increase in lymphocytes was insignificant (P>0.05) in case of *C. idella* and significant (P<0.05) in case of *C. auratus*. The increased percentage of circulating neutrophils of imidacloprid exposed fish group may be due to mobilization of neutrophils from the bone marrow reserves into the blood stream in response to presence of toxicant (Banaee *et al*., 2008). Increase in fish circulating neutrophils in response to presence of toxicant has been reported (Banaee *et al*., 2008). The increase in percentage of circulating lymphocytes during the present study could be the result of stimulated lymphopoiesis and increased release of lymphocytes from lymphomycloid tissues in response to the toxicant (imidacloprid). Elevation in lymphocyte count may be the compensatory response of lymphoid tissues to the destruction of circulating lymphocytes (Shah and Altindag, 2005). Increase in fish lymphocyte in response to the presence of toxicant in water has been reported (Parkash, 2016). During the present study, the concentration of Hb of *C. idella* was insignificantly lower than control group (P>0.05), however the haemoglobin level of imidacloprid exposed group of *C. auratus* was significantly lower when compared to control group (P<0.05). This reduction may be due to the blood haemorrhage as a result of unequal osmotic pressure inside and outside blood cells (Heath, 1987), due to haemodilution of blood as a result of damage and bleeding of fish organs (Movotny and Beeman, 1990). Increase in fish haemoglobin concentration in response to pesticide has been reported (Sweilum, 2006). Platelets are smallest among the blood cells and are very important in blood clotting. They swell and clump together to form a sticky mass for preventing blood flow (Nassar *et al*., 2016). During the present study, the platelets counts of imidacloprid exposed groups of

both species of fish were significantly lower when compared to control groups ($P<0.05$). Decrease in platelets count due to pesticide toxicity has been reported (Qadir *et al*., 2014; Nassar *et al*., 2016). From the findings of the present research it was concluded that 28 days exposure of *C. idella* and 24 days exposure of C. *auratus* to 2 ppm concentration of imidacloprid does not cause mortality however the exposure causes alteration in the normal level of biochemical and haematological parameters. The information obtained during this research provides a basis for laboratory, semifield and field works on the effects of sublethal concentration of imidacloprid on fresh water fishes.

CONCLUSION

The findings of this study suggest the minimum and safe application of imidacloprid in the agricultural areas which are adjacent to the water bodies.

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