Research Article

Impact of Oberon (insecticide) and remedial impact of Tulsi (*Ocimum sanctum*) on hematological parameters of air-breathing fish *Channa punctatus*

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ABSTRACT

Aim: Indiscriminate use of insecticides has increased many folds in the recent times. Farmers for the better yield of crops, are widely utilising the pesticides. These pesticides through agricultural run offs have reached the surface water like ponds and has contaminated the flora and fauna of it. Fish is the animal which are extensively cultivated in these ponds. And humans are consuming these fishes as food as they have high nutritious value. These contaminated if consumed by the human has caused various health hazards to humans. Thepresent research work is therefore focused to know the bioremedial effect of Tulsi on Oberon induced toxicity in fish Channapunctatus. **Materials and Methods:** Oberon insecticide(Oberon SC 240) was administered directly in water contained in aquarium at the dose of 1.5ppm and observed 24hrs, 48 hrs, 72hrs and 96hrs after the dose calculation through LC50. Thereafter, aqueous extract of Tulsi leaves was administered orally, daily by gastric intubation method at the dose of 100 mg/Kg body weight per day for 15 days. **Results:** The study reveals that after the exposure of Oberon, there was significant damage at the haematological and hematopoietic Stem cells in fish Channapunctatus. But, after theadministration of Tulsi leaves, there was significant normalisationin the haematological and hematopoietic Stem cells of fish. **Conclusion:** This denotes that Tulsinot only possesses ameliorating and rejuvenating property but also maintains the normal functioning of the body of the fish. Thus, it proves to be one of the best antidote against Oberon induced toxicity.

Keywords: Hematology, hematopoietic stem cells, Oberon, Tulsi leaves

Introduction

The wide use of agrochemicals under conventional agriculture has caused severe health hazards for human beings. It also has caused numerous other side effects on the environment including destruction of the biodiversity. The pesticide enters aquatic ecosystems through various routes and poses a risk to many non-target aquatic organisms, particularly those inhabiting water bodies adjacent to agricultural fields. Although synthetic pyrethroids have been claimed as safe and environmentally friendly because of their selective toxicity to insects, low persistence and low toxicity to mammals and birds, they are highly toxic to a number of other non-target organisms including fish, lobster, shrimp, mayfly nymphs, and many species of zooplankton. Oberon SC is compatible with many other pesticides, plant growth regulators, and micronutrients.[1-3] It has a new mode of action inhibiting lipid biosynthesis. The biological activity of tetronic acids correlates with inhibition of lipogenesis especially triglycerides and free fatty acids. Spiromesifen is a systemic product belonging to the chemical group of spirocyclic tetronic/tetramic acid derivatives widely used against certain pests such as mites and whiteflies.[4]This insecticide/acaricide has become an important element in the resistance management program[5] due to its mode of action and low toxicity against no target organisms. It acts on lipid synthesis by inhibiting acetyl CoA carboxylase.[6]

In the recent years, research on medicinal plants has attracted a lot of attention globally. Large body of evidence has accumulated to demonstrate the promising potential of medicinal plants used in various traditional, complementary, and alternate systems of treatment of human diseases.

Tulsi is a branched, fragrant and erect herb having hair all over. These are aromatic because of the presence of a kind of scented oil in them.

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Due to its medicinal virtues, Tulsi is used in ayurvedic preparations for treating various ailments.

The objective of the present investigation is to ascertain the toxic impact of Oberon a most commonly used insecticide on hematological parameters and hemopoietic tissue of an air-breathing fish *Channa punctatus* (Garai) and remedial property of a medicinal plant, Tulsi (*Ocimum sanctum* L.) to generate data for medicinal use of Tulsi as detoxifying and antioxidant agent.

Materials and Methods

Animals

Live specimens of *C. punctatus* (Garai) were procured from Dumka fish market and were acclimatized in the laboratory before experimentation. The fishes were kept in big aquaria (50-gallon capacity). The animals were fed with chopped goat liver and earthworms. Care was taken to keep the animals healthy and free from parasites. The experiment was established in the Department of Zoology, SKM University, Dumka, Jharkhand. The ethical approval was obtained from the Postgraduate Research Council of SKM University, Dumka.

Test chemical

Oberon SC 240 (Bayer CropScience Ltd., India) was administered directly in water contained in an aquarium at the dose of 1.5 ppm and observed 24 h, 48 h, 72 h, and 96 h after the dose calculation through LC_{50}.

Preparation of Tulsi leaves dose

In the present study, Tulsi leaves were plucked from the local garden, and aqueous extract of Tulsi leaves was made to the dose of 100 mg/Kg body weight per day after the LD_{50} estimation. The aqueous extract of Tulsi was administered orally, daily by gastric intubation method to the fish.

Study groups and sampling

The control group of fish received no treatment. The "treatment" groups received Oberon at the dose of 1.5 ppm and observed for 24 h, 48 h, 72 h, and 96 h in the aquariums. The third aquarium fish were first treated with Oberon insecticide at the dose of 1.5 ppm and observed 24 h, 48 h, 72 h, and 96 h then after were administered Tulsi at the dose of 100 mg/kg body weight per day orally, daily by gastric intubation method to the fish for 15 days.

Hematological evaluation

The hematological parameters red blood cell count (RBC's), hemoglobin percentage (HGB), mean cell hemoglobin (MCH), MCH concentration (MCHC), clotting time (CT), and white blood cell (WBC's) count and differential counts - neutrophils, eosinophil, basophils, lymphocytes, and monocytes were done manually.

Hematopoietic stem cells evaluation

After opening the abdominal cavity, head kidney was collected. The impression smears were prepared on a clean micro slide from the cut surface of the freshly dissected head kidney as described by Agrawal and Mahajan 1980.[7] The imprints were air dried for 24 h, fixed in methanol for 30 s and stained using Wright-Giemsa solution. Apart from this, RBC diameter was measured in control, Oberon treated, and Tulsi treated fish group to establish a correlation.

Statistical analysis

Results are presented as mean ± SD, and total variation present in a set of data was analyzed through one-way analysis of variance. Difference among mean values has been analyzed by applying Dunnett's test. Calculations were performed with the GraphPad Prism Program (GraphPad software, Inc., San Diego, U.S.A.). The criterion for statistical significance was set at $P < 0.05$.

Results

Hematological evaluation

In the present study, in the hematological study, there was a significant decrease in the RBC counts, total lymphocyte count (TLC) counts, HGB, MCH, and MCHC levels after Oberon exposure while after Tulsi treatment there was a significant decrease in their levels. There was a huge decrease in the Basophil counts and Monocyte counts after Oberon exposure but in Tulsi treated group there was a significant decrease in their level. The Neutrophil counts, Eosinophil counts, Lymphocyte counts, and CT showed a significant decrease in their levels after Oberon exposure, but after Tulsi treatment, there was a significant increase in their levels [Tables 1 and 2].

Hematopoietic stem cells evaluation

In the present study, hematopoietic stem cells of the head kidney of fish were analyzed which showed a high degree of degeneration in Oberon treated group, but in Tulsi treated group, there was significant normalization in the architecture of the hematopoietic stem cells denotes that Tulsi plays the key role to normalize the function of stem cells. The results of hematopoietic tissue analysis revealed a gradual increase in the percentage of the earliest blast stages. The percentage of neutrophil lineage decreased and did not recover until the end of the experiment. This was also the case with basophil and thrombocyte lineages. No significant differences occurred in the percentage of lymphocyte, monocyte, and of eosinophil cell lineages. The increase in the frequency of early blast cells in the head kidney showed a general activation of a hematopoietic function, and the increase in the frequency of RBC precursors among hematopoietic cells indicates that the recovery of blood cells was proportional to their loss [Tables 3 and 4].

Discussion

Environmental problems have increased exponentially in recent decades mainly because of rapid growth in human population and increased demand for several household materials. On the one hand, technological development has improved the quality of life while on the other hand, it has created a number of health hazards. The toxic chemicals discharged into the air, water, and soil get into food chain

Table 1: Mean hematological parameters of *Channa punctatus* exposed to sublethal concentration (1.5 ppm) of Oberon in 24 h, 48 h, 72 h, and 96 h

Parameter	Concentration	Control	Oberon treated			
			24 h	48 h	72 h	96 h
RBC ($\times 10^6$ mm^{-3})	1.5 ppm	3.125±0.047	3.001±0.031	2.748±0.019	2.504±0.08	2.145±0.012
TLC ($\times 10^3$m^{-3})	1.5 ppm	17.78±0.015	19.56±0.032	16.73±0.032	15.57±0.039	14.16±0.030
Neutrophil (%)	1.5 ppm	21.70±0.260	23.50±0.166	24.60±0.163	25.1±0.100	25.68±0.029
Eosinophils (%)	1.5 ppm	4.09±0.027	4.55±0.016	4.92±0.014	5.55±0.016	6.02±0.032
Basophils (%)	1.5 ppm	2.44±0.016	1.85±0.016	1.53±0.021	1.04±0.010	0.68±0.020
Lymphocytes (%)	1.5 ppm	62.30±0.152	63.50±.166	64.60±0.266	66.50±0.223	67.30±0.152
Monocytes (%)	1.5 ppm	10.74±0.039	7.72±.024	6.44±0.042	6.18±0.032	5.19±0.499
Hb (g/100 ml)	1.5 ppm	10.83±0.036	8.77±0.059	6.45±0.031	5.85±0.028	5.10±0.023
MCH (10^5 pg)	1.5 ppm	3.75±0.023	3.43±0.023	2.66±0.016	2.46±0.016	2.28±0.039
MCHC (g %)	1.5 ppm	34.74±0.235	32.79±0.348	27.14±0.150	24.92±0.176	22.64±0.164
CT (s)	1.5 ppm	25.2±0.041	28.16±0.003	30.91±0.3555	32.87±0.738	35.37±0.138

RBC: Red blood cell count, TLC: Total lymphocyte count, MCH: Mean cell hemoglobin, MCHC: Mean cell hemoglobin concentration, CT: Clotting time

Table 2: Mean hematological parameters of *Channa punctatus* exposed to sublethal concentration (1.5 ppm) of Oberon followed by Tulsi treatment for 5 days, 10 days, and 15 days

Parameter	Concentration	Control	Oberon treated 1.5 ppm for 96 h followed by *Ocimum sanctum* treated - 100 mg/kg b.w.			
			96 h Oberon treated	5 days Tulsi treated	10 days Tulsi treated	15 days Tulsi treated
RBC ($\times 10^6$ mm–^{3})	1.5 ppm	3.125±0.047	2.145±0.012	2.583±0.017	2.842±0.012	2.938±0.010
TLC ($\times 10^3$mm–^{3})	1.5 ppm	17.78±0.015	14.16±0.030	15.41±0.031	15.91±0.018	16.66±0.037
Neutrophil (%)	1.5 ppm	21.70±0.260	25.68±0.029	23.60±0.163	23.30±0.152	22.57±0.030
Eosinophils (%)	1.5 ppm	4.09±0.027	6.02±0.032	5.48±0.035	5.14±0.016	4.86±0.020
Basophils (%)	1.5 ppm	2.44±0.016	0.68±0.020	0.89±0.007	1.24±0.016	1.64±0.016
Lymphocytes (%)	1.5 ppm	62.30±0.152	67.30±0.152	65.20±0.133	63.60±0.221	63.10±0.179
Monocytes (%)	1.5 ppm	10.74±0.039	5.19±0.499	6.86±0.016	7.24±0.037	8.81±0.027
Hb (g/100 ml)	1.5 ppm	10.83±0.036	5.10±0.023	5.55±0.055	6.49±0.034	8.06±0.011
MCH (10^5 pg)	1.5 ppm	3.75±0.023	2.28±0.039	2.69±0.033	2.85±0.026	3.08±0.021
MCHC (g %)	1.5 ppm	34.74±0.235	22.64±0.164	24.30±0.196	26.33±0.158	28.60±0.116
CT (s)	1.5 ppm	25.21±0.041	35.37±0.138	31.73±0.611	30.03±0.120	27.83±0.318

RBC: Red blood cell count, TLC: Total lymphocyte count, MCH: Mean cell hemoglobin, MCHC: Mean cell hemoglobin concentration, CT: Clotting time

Table 3: Changes in mean size and surface area of erythrocytes (µm) of *Channa punctatus* exposed to sublethal concentration (1.5 ppm) of Oberon in 24 h, 48 h, 72 h, and 96 h

Parameter	Concentration	Control	Oberon treated			
			24 h	48 h	72 h	96 h
Length (µm)	1.5 ppm	7.875±0.172	7.461±0.142	7.035±0.121	6.884±0.102	6.145±0.082
Breadth (µm)	1.5 ppm	5.264±0.205	5.00±0.235	4.786±0.200	4.573±0.142	4.216±0.121
Area (µm)	1.5 ppm	41.3224±1.665	37.305±1.806	33.669±1.168	31.480±1.152	25.907±1.026

Table 4: Changes in mean size and surface area of erythrocytes (µm) of *Channa punctatus* exposed to sublethal concentration (1.5 ppm) of Oberon followed by Tulsi treatment for 05 days, 10 days, and 15 days

Parameter	Concentration	Control	Oberon treated 1.5 ppm for 96 h followed by *Ocimum sanctum* treated - 100 mg/kg b.w.			
			96 h Oberon treated	5 days Tulsi treated	10 days Tulsi treated	15 days Tulsi treated
Length (µm)	1.5 ppm	7.875±0.172	6.145±0.082	6.456±0.128	6.789±0.146	7.018±0.1675
Breadth (µm)	1.5 ppm	5.264±0.205	4.216±0.121	4.525±0.131	4.691±0.147	4.986±0.187
Area (µm)	1.5 ppm	41.3224±1.665	25.907±1.026	29.213±1.089	31.847±1.092	34.991±2.030

from the environment. By entering into the biological system, they disturb the biochemical processes leading to health abnormalities, in some cases to fetal consequences.[8] The degradation of an aquatic system is a worldwide phenomenon originated from the intense

population and the corresponding increase in agriculture practices as well as industrial and domestic activities. Pesticides are a major cause of concern for aquatic environment because of their toxicity, persistency, and tendency to accumulate in the organisms. The impact of pesticides on aquatic organisms is due to the movement of pesticides from various diffuse or point sources. These pesticides are posing a great threat to aquatic fauna especially to fishes, which constitute one of the major sources of protein-rich food for mankind.[9] The fish serves as bioindicator of water quality, and the impact of the pesticide can be well understood by analyzing either blood or serum of the fish because blood is a pathophysiological reflector of whole body.[10,11]

Hematological study is important in toxicological research because a hematological alteration is a good method for rapid evaluation of the chronic toxicities of a compound. A thin epithelial membrane separates fish blood from the water and an unfavorable change in the water body is reflected in the blood. In the present study, in the hematological study, there was a significant decrease in the RBC counts, TLC counts, HGB, MCH, and MCHC levels after Oberon exposure while after Tulsi treatment there was a significant decrease in their levels. There was huge decrease in the Basophil counts and Monocyte counts after Oberon exposure, but in Tulsi treated group, there was a significant decrease in their level. The Neutrophil counts, Eosinophil counts, Lymphocyte counts, and CT showed significant decrease in their levels after Oberon exposure, but after Tulsi treatment, there was significant increase in their levels.

This effect of Oberon on *C. punctatus* might have been through failure or suppression of normal mechanisms promoting erythropoiesis or deficiency of some factors required for the maturation of the red cell. The causes of leukopenia observed in the present study are supposed to be according to the degeneration, depression, depletion, and destruction of the blood-forming materials by these compounds. The observed depletion in the hemoglobin percentage in the fish could also be attributed to the lysis of erythrocytes. Thus, the significant reduction in these parameters is an indication of severe anemia. In the values obtained in the hematological indices, slight fluctuations were recorded in the mean corpuscular volume and MCHC, but there was a significant change in the MCH, due to the reduction in cellular blood iron, resulting in reduced oxygen carrying capacity of blood and eventually stimulating erythropoiesis.

The WBCs in fish respond to various stressors including infections and chemical irritants. Thus, increasing or decreasing numbers of WBCs are a normal reaction on the exposure of toxicants. In the present investigation, the gradual decrease in WBC (leukocytosis) may have resulted from the detoriated defense mechanism of the fish to counter the effect of the toxicant. A sharp increase was observed in percentage of neutrophils, while slight significant increase of lymphocyte was recorded in this investigation.

In the present study, the clotting was significantly increased in Oberon treated fish but was observed in normal condition in Tulsi treated group. The clot is formed under normal conditions undergoes contraction, when serum is expressed from the clot, and finally, the clot becomes denser. The high percentage of prothrombin, the blood clotting substance is responsible for blood clotting and a substance called thromboplastin, released by the platelet also responsible for blood clotting reaction.[12,13] Due to the increased concentration of toxicant, the production of these substances is decreased, so, the blood takes more time to clot.[14]

In the present study, the RBC diameter was observed in the blood smear of the fish. In the Oberon treated group, there was a decrease in the size lengthwise while there was a gradual decrease in the breadth size and the surface area of RBC. However, after the treatment of Tulsi their size was normalized to the normal architecture denotes that Tulsi plays the major role to control the cellular architecture as well as its integrity. This is a novel study done on Oberon and Tulsi in the field of toxicology. Similar, studies on other fishes have been well documented by Metelev[15] and Jay Prakash and Shett.[14] They have also proposed the mode of action as the surface area reduction of erythrocytes causes hypoxic effect prevailing over the body tissues of fishes due to the damaging effect on the gill tissue. The erythrocytes in the exposed fish were shrinked, with cell wall distortion showing crenated margin and the nuclei were hypertrophied.

In the present study, hematopoietic stem cells of head kidney of fish were analyzed which showed a high degree of degeneration in Oberon treated group but in Tulsi treated group there was significant normalization in the architecture of the hematopoietic stem cells denotes that Tulsi plays the key role to normalize the function of stem cells. The results of hematopoietic tissue analysis revealed a gradual increase in the percentage of the earliest blast stages. The percentage of neutrophil lineage decreased and did not recover until the end of the experiment. This was also the case with basophil and thrombocyte lineages. No significant differences occurred in the percentage of lymphocyte, monocyte, and of eosinophil cell lineages. The increase in the frequency of early blast cells in the head kidney showed a general activation of hematopoietic function, and the increase in frequency of RBC precursors among hematopoietic cells indicates that the recovery of blood cells was proportional to their loss. The obtained results showed the very high potential of common carp head kidney hematopoietic tissue. These results indicate that under good environmental and nutritional conditions fish are able to compensate for anemia very quickly. This is the novel study ever done in this field on this pesticide.

Conclusions

Oberon causes severe damage to the fish by the means of hematological level, hematopoietic stem cells level. However, Tulsi plays the key role in combating the deleterious effects of Oberon at each level. Thus, Tulsi is the best antidote against the Oberon induced toxicity.

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