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ORIGINAL ARTICLE

Acute Toxicity of Captan on Blood Factors Total Immunoglobulin, Liver, and Gill Tissues of Fingerling Grass Carps: *Ctenopharyngodon idella*

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KEYWORDS

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ABSTRACT: The majority of fungicides, including captan, used in gardens and farms are washed into the aquatic environments. These compounds can lead to extensive side effects on the inhabitants living in that exposed area. In this research, toxicity of captan on fingerling grass carps (3-5 g) was studied. Based on the results, captan was categorized as an acute toxin with LC_{50}96h and MAC values of 0.9 and 0.09 mg/ml, respectively. This toxin damaged severely the gills and liver of fishes and the toxic severity increased in higher doses of the toxin. Furthermore, hematochemical studies showed significant drops in all red blood cell criteria, except for MCH (mean corpuscular haemoglobin) and MCV (mean corpuscular volume). Besides, captan decreased significantly the total immunoglobulin and white blood cell counts, except for monocytes and neutrophils. As a conclusion, captan causes detrimental effects on several tissues of *Ctenopharyngodon idella*, and therefore limited utilisation of this fungicide is recommended strongly.

INTRODUCTION

Water pollution with different types of chemicals is a global problem confronting all living beings, humankind included. The growth in population and their demands for food, energy and many other comforts have led to increases in the processes of industrialization and urbanization worldwide. These industries introduce a variety of harmful chemicals into the environment and therefore cause serious problems for habitants in that area. Agricultural industries are considered as one of main sources of chemical contamination due to employment of several fertilizers, herbicides, fungicides and pesticides [1].

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These compounds are finally released into water sources, which can affect the ecological balance in these environments.

Since most agriculturally important plants are susceptible to a variety of fungal diseases, they are protected using several types of fungicides [2, 3]. These toxins can access to water sources through direct applications of pesticide in aquatic systems and/or their indirect discharges of agricultural wastewater [4]. Fungicides are normally toxic for cells and any exposure of aquatic animals to these compounds after their wash into water sources can cause adverse effects on different tissues of these animals, leading to increase in their mortality rate and/or reduction in their growth rate [5, 6]. These adverse effects sometimes are incredibly massive, involving thousands of fishes, frogs, turtles, mussels and waterbirds [7]. Fishes are considered as one of the most susceptible animals to pesticide pollution due to their Anatomy and Physiology. In addition to drops in the fishery industries, exposure of aquatic animals to fungicides can lead to accumulation of these toxins through the food chains in their hunters, human being include [8].

Captan (N-trichloromethylthio-4-cyclohexen-1/2-dicarboximide), as a very common curative and protectant fungicide, is a water insoluble chemical belonging to dicarboximide family. This chemical kills fungi via blocking thiol-containing compounds such as oxidases, hydrogenases and Coenzyme A [9].

In this study, toxic effects of captan on several tissues of fingerling grass carp (*Ctenopharyngodon idella*) were investigated.

MATERIALS AND METHODS

Totally, 18 aquariums were filled with 20 L of well water and were aerated for 24 h before beginning the tests (water temperature 22 ± 1, dissolved oxygen below 7 mg L^{-1}, water hardness 230 mg L^{-1} and pH 7.5-8). After physical examination of the fingerling grass carps for their health condition, 10 healthy fishes with 3-5 gr weight were transferred to the aquariums for adaptation to the environment and further tests.

Determination of Captan toxicity on Grass carps

The fishes were exposed to different titre of captan (between 0.5 and 2.5 mg/L) under standard conditions and continuous aeration. Toxic effects of captan on the subjects were investigated according to the OECD 2001 (Organization Economic Cooperation Development) guidelines for the acute toxicity testing in fishes [10, 11]. Based on the guidelines, the fishes were exposed to different concentrations (between 0.5 to 2.5 mg/L) of captan for 96 h (24, 48, 72 and 96 h) to determine the LC_{50}. The fishes were not fed through this period [12]. This test was repeated three times with additional three aquariums without treatment as a control. Finally, three different concentrations of the captan (25%, 50% and 75% of the calculated LC_{50}96h) were chosen for further pathological studies.

Blood and serum preparation

The blood samples of fishes, taken from dorsal aorta of the fishes, were heparinised and were assayed for different serological and haematological tests, including total immunoglobulin (using indirect ELISA assay of a serial dilution of the samples), total RBC (red blood cells; using hemocytometer), Hb (haemoglobin; using Cobas method), PVC (packed cell volume; haematocrit; based on glass capillary system), MCV (mean corpuscular volume;

$$\frac{Hematocrit(\%)\times 10}{\text{RBC count }(\frac{\text{millions}}{\text{mm3}}\,blood)}$$

MCHC (mean corpuscular haemoglobin concentration;

$$\frac{Hemoglobin\ (\frac{g}{100ml})\times 100}{hematocrit\ (\%)}$$

, total white blood cells (WBC; by Hemacytometer chamber) and percentages of neutrophils, lymphocytes, monocytes, eosinophils (using flow cytometer).

Tissue preparation

After sampling from the tissues (gill and liver) and a staining process using Eosine and Hematoxiline (E&H) procedure [13], the samples were observed microscopically (×40) .

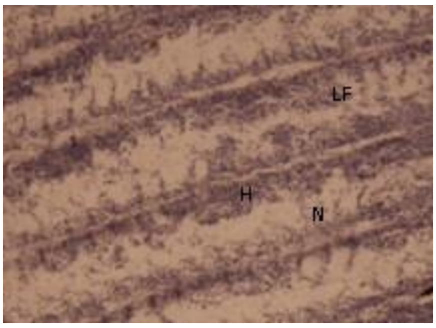
STATISTICAL ANALYSIS

In order to determine LC_{50}96h, the data were analysed by Probit program (USEPA, 1985). Other statistical studies were performed with SPSS software version 20 (Chicago, IL, USA).

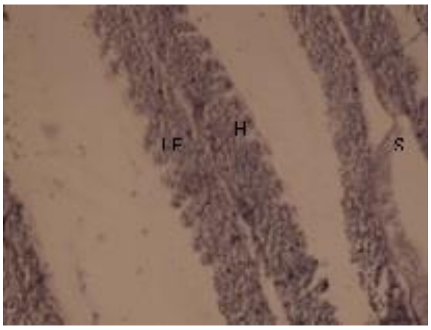
RESULTS

The LC_{50}96h (lethal concentration of a toxin on 50% of the population in 96 h) and MAC value (Maximum Allowable Concentration Value) of captan were calculated 0.9 mg/L and 0.09 mg/L, respectively.

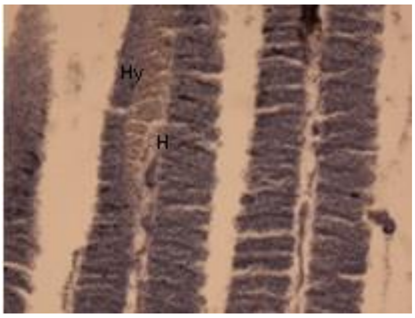
The tissue studies on the gill samples showed severe gill damages, including hyperemia, haemorrhage, and hyperplasia and gill cohesion and cell necrosis in this tissue (Figure 1).



H: Hyperplasia, Lf: Secondary blade attachment, N: Necrosis, T 25% - T1



H: Hyperplasia, Lf: Secondary blade attachment, S: Swelling, T 50% - T2

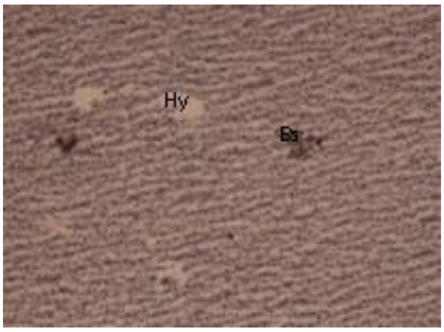


H: Hyperplasia, HY: Congestion, T 75% - T3

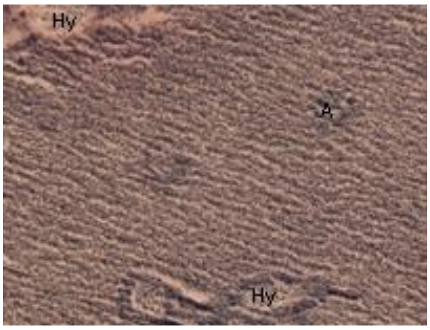
Figure 1. Pathological changes in gill.

In addition, the tissue studies on the liver samples showed severe liver damages, including hyperplasia and cohesion and

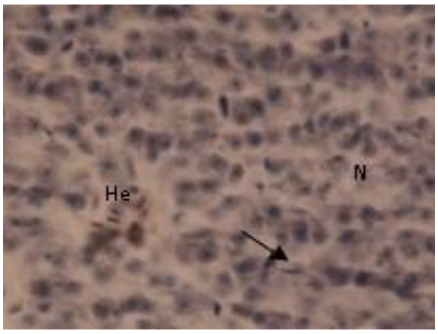
Secondary blade attachment in this tissue (Figure 2).



Bs: Bile stagnation, HY: Congestion, T 25% - T1



HY: Congestion, A: Atrophy, T 50% - T2



N: Necrosis, He: Coopfer, T 75% - T3

Figure 2. Pathological Changes in the Liver

Our observations showed more injuries in higher concentrations of captan. However, the different degrees of injuries were observed in liver varied based on the concentration of the toxin. In other word, although treatment with 25% toxin was associated with only hyperemia and reduced bile secretion, the signs extended to cell atrophy and a rise in the Kupffer cells when the fishes were treated with higher concentrations of the toxin. The control samples showed normal conditions in their liver tissues except for slight cell atrophy. No severe damages were seen in both gill and liver samples of the controls.

Furthermore, based on hematologic studies, meaningful changes were observed in blood indexes of the treated samples (P<0.05). Treatment with captan decreased significantly the levels of RBC count, Hb, MCHC and PVC of the blood samples, and the reduction trend was sharper in high concentrations. However, the levels of MCV (except for treatment with 25%) and MCH (except for treatment with 25%) increased meaningfully, and the increase was in parallel to the toxin concentration (Table 1).

Although treatment with captan could dramatically decrease total immunoglobulin, total WBC count and the number of lymphocytes, the quantity of neutrophil and monocyte showed gradual but meaningful increases from lower concentrations of the toxin to higher concentrations, reached to its peak at 75% (P<0.05). Furthermore, the level of eosinophil in the treated samples starts to increase at 50% of Lc_{50}96h, but the amount showed a sudden decrease at higher concentration (75%) (Table 1).

Table 1. The mean levels of changes (±SD) in hematological parameters of fingerlings carp (*C. idella*) exposed to 25%, 50% and 75% of captan (P<0.05)

Blood Indexes	Concentrations of captan			
	Control	25%	50%	75%
Erythrocyte (10^6)	1.18±0.7	1.12±0.9	0.88±0.04	0.7±0.09
Hb (g/100ml)	7.8±0.47	6.9±0.3	4.6±0.56	6.3±1.26
Hematocrit	37±2.1	33.33±1.37	28.5±1.05	30.45±5.68
MCV	314.35±2.65	297.34±16.20	325.27±5.1	329.55±9.22
MCH	66.11±0.67	61.55±3.57	66.17±0.13	67.0±1.17
MCHC	21.03±0.30	20.70±0.7	20.35±0.30	20.30±0.44
WBC (10^4)	0.843±0.03	0.722±0.03	0.378±0.015	0287±0.04
Neutrophil	39.8±2.86	48±3.16	70.66±3.93	55.37±12.73
Lymphocyte	58.33±2.58	48.83±2.8	32±2.2	26.83±6.34
Monocyte	1.33±0.51	2.66±0.51	3.66±0.51	4.33±0.51
Eosinophil	0.5±0.54	0.5±0.54	1.33±0.81	0.66±0.51
Total Immunoglobulin	0.97±0.01	0.84±0.08	0.72±0.08	0.49±0.11

DISCUSSION

The destructive effects of industrial chemicals on aquatic organisms and/or accumulation of these compounds in their body are the main concern of environmentalists [14, 15]. Indeed, it is very essential to address these environmental hazards since the toxicity information of these chemicals on different aquatic organisms will shed light on the bioavailability and behaviour of these chemicals in the aquatic system. Since a majority portion of the pesticides is washed into aquatic environments and also since the half-life of many of these compounds is quite long, they are potentially able to cause irreversible or long lasting damages in the habitants of these environments [16-18]. For some chemicals, this obstacle is magnified after 96 h exposure time due to decreases in their LC_{50} and LC_{90} values, probably as a result of immunological defects and general weakness in the animals [19, 20].

Since *C. idella* is one of main protein sources for human beings [8, 21], release of toxins, captan in this study, in the aquatic environments may lead to decrease in the production of this food source and/or accumulation of the toxin in the fish tissues, which in turn threatens the health of the consumers. Captan, a chlorated derivative molecule classified into phthalimide category, is one of the popular fungicides used in agricultural industries. Captan is a lethal poison for animals, leading to a variety of clinical manifestations, including skin irritation [22], diarrhoea, anorexia, weight loss [22], hypothermia [23] and depression [23]. Based on our results, 0.9 mg L^{-1} (LC_{50}96h= 0.9mg/L) of this compound for 96 hours is highly toxic for grass carp fingerlings. This value varies between different fishes as has been listed in Table 2. In addition, while an average MAC value of most toxins is 0.1 mg L^{-1}, the tests showed that the MAC value of captan on the fingerlings is 0.09 mg L^{-1}. Overall, these findings show that *C. idella* is highly susceptible to the toxin in comparison with many other fishes.

Table 2. LC_{50}96h value in different aquatic animals

Fish species	LC_{50}96h	Ref.
Anguilla japonica	0.072 mg L^{-1}	[28]
Carassius auratus	1.34 mg L^{-1}	[29]
***Cyprinus carp*io**	0.037 mg L^{-1}	[30]
Ictalurus punctatus	0.078mg/L	[30]
Lepomis marochirus	0.140 mg/L	[30]
Cyprinodon variegatus	56 µg L^{-1}	[31]
Americamysis bahia	8.4 mg L^{-1}	[31]
Cyprinodon variegates	1.9 mg L^{-1}	[31]
Lepomis macrochirus	72 µg L^{-1}	[31]
Oncorhynchus kisutch	56 µg L^{-1}	[31]
Oncorhynchus tshawytscha	56 µg L^{-1}	[31]

Based on microscopic observations, exposure to this toxin could cause different pathological and clinical manifestations in gill and liver of this fish. The levels of damages varied based on the tissue of interest and toxin concentration. The gill samples of the fishes were highly abnormal and showed several pathological manifestations, including hyperaemia, haemorrhage, hyperplasia, cohesion of gill layers and cell necrosis. Furthermore, the liver tissues were severely damaged and several inflammatory signs, including hyperaemia, cell atrophy, decreases in bill secretion and increases in the Kupffer cells were observed in these samples. Based on this experiment, the pathological manifestations in both gill and liver samples worsen because of increase in the concentration of captan.

Furthermore, hematologic studies showed that the levels of blood factors in the fingerlings were dramatically changed because of exposure to captan. Because of a decrease in the levels of RBC count, HCV, Hb and MCHC, the fishes had to increase erythrocyte volume (MCV) and the level of haemoglobin in each cell (MCH). Furthermore, it was shown that captan could reduce the functions of the immune system of these fishes by decreasing the levels of total WBC, monocytes, eosinophils, neutrophils and total immunoglobulin. Similarly, other researchers who worked on toxicity of different toxins on the blood factors of various fishes showed the same changes in blood factors, with some exceptions such as *Pseudoleuronectes americanus* and *Channa punctatus* [24, 25]. However, the pathological effects of different toxins on specific and general immunological responses are highly dependent on the fish species and type of toxins. For instance, hexachlorocyclohexane could not effectively destroy humeral immunological responses in *Cyprinus carpio* [26], but heavy metals could diminish the immunological activity of different fishes [27].

CONCLUSIONS

Captan is an acute toxic compound for *C. idella* and causes severe damages in its tissues and supresses its immune system. Such high-scale damage on the fingerlings is a serious environmental and economic disaster.

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Conflict of Interests

There is no conflict of interest.

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