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# Acute toxicity of povidone-iodine (Betadine) in common carp (*Cyprinus carpio* L. 1758)

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**ABSTRACT:** Organisms in aquatic environments are exposed to a number of pollutants such as pharmaceutical residues. As such, the aim of the present study is to achieve the Lethal Concentration ($LC_{50}$) of Povidone-iodine (Betadine) for *Cyprinus carpio*. To do so, the study employs samples, weighing 4±1 [mean±SD] gr, and carries out an experiment in static condition. Based on OECD instructions, after a period of 4 days under controlled water, the physicochemical factors give the following results: pH= 8-8.3, BOD= 690 mg/l, total hardness= 210 mg, and CaCo3 and temperature= 17±0.1 °C. All fish are acclimatized for 10 days in an aquarium, 60×55×30 cm in size, which included the control group (no toxic concentration) as well as the treated aquariums, with Betadine concentration of 20, 40, 60, 80, 100, 120, 140, 160, 180, 200, 400, and 600 mg/l. $LC_{10}$, $LC_{20}$, $LC_{30}$, $LC_{40}$, $LC_{50}$, $LC_{60}$, $LC_{70}$, $LC_{80}$, $LC_{90}$, and $LC_{95}$ have been measured for 6, 12, 18, and 24 hours. $LC_{50}$ 24h Betadine for *C. carpio* has been 158.273 ml/l, showing no mortality after 24 hours (i.e. 48h, 72h, and 96h). Results of the present study suggest that Betadine is practically nontoxic and not irritant at low concentrations for this species and it has a short half-life in aquatic environments.

**Keywords:** acute toxicity, betadine, common carp, $LC_{50}$.



## INTRODUCTION

During the past decade, there has been growing concern for human and veterinary pharmaceutical compounds on ecosystem's health along with their potential toxicity on aquatic organisms. Also, water environments have been the last destination of several pollutants (Yalsuyi & Vajargah, 2017a). Pharmaceuticals are used in large quantities by human beings and livestock in industrialized countries and are frequently found in Sewage Treatment Plants (STPs) and surface waters (Huschek et al., 2004).

Povidone-iodine (Betadine) is a stable chemical complex of both polyvinylpyrrolidone (povidone, PVP) and elemental iodine. It is a broad spectrum of antiseptic for topical application in treatments and prevention of infection in wounds (Barabas & Brittain, 1998).

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Millions of kilograms of Povidone-iodine are annually used in the world. Huschek et al. (2004) reported that about 994 Ton Povidone-iodine was used in Hospital, Prescription, and Retail from 1999 to 2000 in Germany. With agitation, povidone-iodine is completely soluble in cold water at concentrations, exceeding 10%, while the aqueous solubility of elemental iodine is only 0.034% (Barabas & Brittain, 1998). Also, Betadine is usually used for disinfection of fish eggs (Eissa et al., 2007); therefore, it is essential to evaluate acute toxicity of Betadine in aquatic animals.

Common carp (*Cyprinus carpio* L. 1758) is a freshwater, benthopelagic fish of Cyprinidae family, Cypriniformes order, one of the oldest species for freshwater Aquaculture (Hedayati et al., 2014).

The contamination of aquatic ecosystems by chemicals, such as medicinal products or herbicides, has gained increased attention with several studies conducted on acute toxicity and the destructive effect of these chemicals on the fish (Vajargah et al., 2014; Petersen et al., 2014; Akbary & Jahanbakhshi, 2016b; Huschek et al., 2004; Amend, 1974). Pharmaceutical Residues (PhaR) reach aquatic environment through various pathways, such as direct disposal of domestic surplus drugs, expulsion from faeces/urine after therapeutic use, or effluents from the manufacturing industry (Li & Randak, 2009).

Currently different disinfectant substances such as ozone, malachite green, and ionic compounds are used to control pathogens; however, the toxicity of many of these compounds on fish has been proven. For example, Srivastava et al. (1995) stated $LC_{50}$ 96h of malachite green for Freshwater catfish (*Heteropneustes fossilis*) was 1 ml/l; also, Yalsuyi and Vajargah (2017b) stated that silver nanoparticles was highly toxic for Roach (*Rutilus rutilus*) and Goldfish (*Carassius auratus*). Results from previous studies have shown that these compounds can affect the survival and reproduction of fish (Srivastava et al., 2004; Eissa et al., 2007).

In spite of the wide application of Betadine in clinics and the possible eco-toxicological impact, associated to its use, there is scarce information concerning its effects on many inland waters' fish species, like common carp. With the development of industries, a progressive decline took place in water quality, resulting in poor ecological condition of the water bodies (Khabbazi et al., 2014).

Sensitivity of various fish species to toxic substances is different (Vajargah & Hedayati, 2014; Akbary et al., 2016a). The $LC_{50}$ 96h tests of pollutants are a way of finding different ingredients' toxicity; in other words, by means of these tests the strength and survival rate of different species, when facing different concentrations of pesticide and other risk factors can be found to some extent (Yalsuyi & Vajargah, 2017b; Vajargah et al., 2014; Hedayati et al., 2014; Chorehi et al., 2013).

## MATERIALS AND METHODS

According to previous studies (Hedayati et al., 2014; Chorehi et al., 2013) and laboratory facilities, a sum of 280 live specimens of common carp (weighing 4±1 gr) were obtained from fish ponds in Gorgan Province, Iran, and moved to laboratory (Laboratory of Department of Fisheries, Gorgan University of Agricultural Sciences and Natural Resources). The fish were acclimatized in 28 aquariums (60×55×30 cm in size) with natural and toxin-free environments for 10 days to determine natural mortality. Biological Oxygen Demand (BOD) was set on 690 mg/l, pH = 8 to 8.28, 17±1 °C of temperature, and hardness of 210 ppm (mg $CaCo_3$). The density was equal in all aquariums. Prior to the test, the fish were fed twice a day with Biomar feed at 2% body weight, but feeding was stopped 24 h before and throughout the test (Akbary et al., 2016a, 2016b).

Aquariums were siphoned off daily to

reduce ammonia content in the water and remove the fecal matter as well as other waste materials. All experiments were performed 16 hours of light and 8 hours of darkness. Static acute toxicity test was carried out, following the standard method guideline, offered by the Organization for Economic Co-operation and Development (OECD, 1994).

To measure $LC_{50}$ of Betadine, as many as 39 treated aquariums got involved (13 treatments and 3 replications), whose Betadine concentration ranged among 20, 40, 60, 80, 100, 120, 140, 160, 180, 200, 400, and 600 mg/l (Povidone-iodine 10% - Darudarman Co.) along with a control group (no toxic concentration), with 21 fish in each treatment. Mortality rates were recorded after 6, 12, 18, 24, 48, 72, and 96 hours and the dead fishes were quickly removed from the aquariums (Hedayati et al., 2014). Physicochemical condition of the water at the main test minus the concentration of Betadine was similar to the previous stage (in which BOD was set on 690 mg/l, pH = 8-8.28, temperature of 17±1 °C, and hardness of 210 ppm).

Spearman test (2-tail) was used to find the correlation of different Betadine concentrations on mortality. Acute toxicity test was carried out, in accordance with Hotos and Vlahos. The nominal concentration of Betadine, assumed to result in fish mortality ($LC_{10}$, $LC_{20}$, $LC_{30}$, $LC_{40}$, $LC_{50}$, $LC_{60}$, $LC_{70}$, $LC_{80}$, $LC_{90}$, and $LC_{95}$) within, 12, 18, 24, 48, 72, and 96 hours was estimated, using SPSS Software program (IBM SPSS Statistic 20), via probit analysis, with confidence level of 95% (Vajargah et al., 2013; Hedayati et al., 2014).

## RESULTS AND DISCUSSION

There was no mortality during the acclimation stage. The results of the main test showed that there was a significant correlation between the Betadine concentration and mortality during the first 24 hours of the test ($P<0.01$). After 24h (i.e. at 48h, 72h, and 96h) mortality was not observed in the treatments (Table 1).

Betadine concentrations of 200, 400, and 600 ml/l killed all the fish in the first 24 hours. However in lower concentrations (0, 20, 40, 60, 80, 100, 120, 140, 160, and 180 ml/l), there was no mortality after the first 24h. According to these results, it seems that the toxicity of Betadine plummeted after 24 hours; however, to ensure this, still further and more complete researches are required. Due to ethical considerations and laboratory facilities, acute toxicity test of Betadine was not performed for higher concentrations.

**Table 1. Mortality rate of common carp (*Cyprinus carpio* L. 1758), facing different concentrations of Betadine (n= 21 each treatment). After 24 (48h, 72h and 96h) hours no mortality was observed in any of the treatments.**

| Concentration (mg/l) | Number | No. of mortality | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 6 h | 12h | 18h | 24h | 48h | 72h | 96h |
| 20 | 21 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 40 | 21 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 60 | 21 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 80 | 21 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 100 | 21 | 0 | 3 | 3 | 3 | 3 | 3 | 3 |
| 120 | 21 | 1 | 2 | 3 | 3 | 3 | 3 | 3 |
| 140 | 21 | 2 | 5 | 6 | 7 | 7 | 7 | 7 |
| 160 | 21 | 3 | 4 | 6 | 8 | 8 | 8 | 8 |
| 180 | 21 | 5 | 11 | 13 | 13 | 13 | 13 | 13 |
| 200 | 21 | 14 | 18 | 20 | 21 | 21 | 21 | 21 |
| 400 | 21 | 21 | 21 | 21 | 21 | 21 | 21 | 21 |
| 600 | 21 | 21 | 21 | 21 | 21 | 21 | 21 | 21 |
| Control | 21 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

**Table 2. Lethal concentration of Betadine for common carp (*Cyprinus carpio* L. 1758). All concentration are shown as Mean ± SD**

| Point | Concentration (mg/l) | | | |
|---|---|---|---|---|
| | 6 h | 12 h | 18 h | 24 h |
| $LC_{10}$ | 144.90 ± 11.6 | 117.67± 23 | 112.85± 14.1 | 110.96± 9.05 |
| $LC_{20}$ | 162.99± 11.6 | 136.74± 23 | 130.02± 14.1 | 127.20± 9.05 |
| $LC_{30}$ | 174.48± 11.6 | 150.49± 23 | 142.40± 14.1 | 138.91± 9.05 |
| $LC_{40}$ | 184.30± 11.6 | 162.24± 23 | 152.98± 14.1 | 148.92± 9.05 |
| **$LC_{50}$** | **193.48± 11.6** | **173.23± 23** | **162.87± 14.1** | **158.27± 9.05** |
| $LC_{60}$ | 202.66± 11.6 | 184.21± 23 | 162.87± 14.1 | 167.62± 9.05 |
| $LC_{70}$ | 212.49± 11.6 | 195.96± 23 | 172.76± 14.1 | 177.63± 9.05 |
| $LC_{80}$ | 223.98± 11.6 | 209.71± 23 | 195.72± 14.1 | 189.34± 9.05 |
| $LC_{90}$ | 239.92± 11.6 | 228.78± 23 | 212.89± 14.1 | 205.58± 9.05 |
| $LC_{95}$ | 253.09± 11.6 | 244.53± 23 | 227.07± 14.1 | 218.99± 9.05 |

$LC_{50}$ 24h of Betadine for common carp was 158.273 ml/l (Table 2). Also Table 2 shows the nominal concentration of Betadine, causing mortality ($LC_{10}$, $LC_{20}$, $LC_{30}$, $LC_{40}$, $LC_{50}$, LC60, $LC_{70}$, $LC_{80}$, $LC_{90}$, and $LC_{95}$) within 6, 12, 18, and 24 hours for each concentration ($P$<0.05).

The fish, exposed to acute concentration of Betadine, showed clinical symptoms, such as anxiety, fast swimming, loss of balance, darkening of the color, increased mucus secretion, enhancement of operculum movement, and death with open mouths.

Amend (1974) reported that acute concentration of Betadine was toxic for fish. Results of his study showed that exposure time and Betadine concentration can be effective on its toxicity. Results from the present study were similar to his study, even though Betadine was not toxic to the fish at lower concentrations (less than 100 ml/l).

Eissa et al. (2007) stated that the use of a Betadine bath at concentration level of 60 ml/l for 30 min and 70 ml/l for 10 min, before collecting oocyte and milt, can reduce the transmitted colony of broodstock to lay eggs in the reproductive cycle. They stated these concentrations of Betadine had no fatal effect on treatment fish. Results of the present study was similar to their studies, too. What is more, in the present study there was no mortality at concentrations, lower than 100 ml/l.

Betadine (Povidone-iodine) Toxicity can vary, depending on the species, size, temperature, and pH, e.g. Moreover, previous studies have shown that Betadine toxicity ascends as the temperature is decreased (Amend, 1974).

## CONCLUSION

Many studies in this regard have shown that iodized antiseptic compounds (such as Povidone-iodine) can be considered the best surface disinfectant that could effectively be used to control fish pathogens (Eissa et al., 2007). Results from the current study showed Betadine was not lethal to fish at low concentrations and after 24 hours practically loses its toxicity.

The present study was to investigate the lethal concentration of Betadine (Povidone-iodine) for common carp (*Cyprinus carpio*). Further researches can conduct a histo-pathological study of the effects of sub-lethal and lethal Betadine concentration on fish organs, and compare its impacts with other antiseptics, such as malachite green and formalin.

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