Can Povidone-Iodine (Betadine) Act On The Survival Rate and Gill Tissue Structure of Oranda Goldfish (*Carassius Auratus*)?

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Research Article

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Abstract

Industrial chemical solutions are widely used as a method to disinfection of aquaculture water and environments. The aim of the present study was to evaluate the toxicity effect of povidone-iodine (betadine) as a disinfectant solution on the survival and gill tissue of Oranda goldfish (*Carassius auratus*). For these purposes, 225 fingerling Oranda goldfish with an average weight 5 ± 0.67 were divided into 15 groups with 3 replication. Fish were exposed to series of concentrations (0, 10, 20, 40, 60, 80, 90, 100, 120, 140, 160, 180, 200, 220 and 240 ppm) of Betadine for 96h. The mortality of fish and the samples of gill were observed at 6, 12, 18, 24, 48, 72 and 96h after exposure. The results of the present study showed that the half-life Betadine was less than 24h and mortality were not record after 24h. The highest mortality rate were at 240 ppm, and LC_{50} 24h of Betadine was 158.800 ppm. Histopathological results showed that lethal concentrations of Betadine lead to hyperemia, hypertrophy, hyperplasia and adhesion of secondary lamellar of the gill. Moreover, fish that were exposed to these concentrations displayed clinical signs such as anxiety, darkening of the skin. Overall results showed that betadine had short half-life in the aquatic environment and are toxic to fish at very high concentrations therefore it can be considered as practically non-toxic and be useful for disinfection of the aquatic environment.

Introduction

Aquaculture as a growing industry faces with many limitations that can lead to reduced production (Yalsuyi and Vajargah, 2017a, b). Water pollutants, dehydration and climate change are some of these limitations (Sattari et al., 2020; Bueno et al., 2015). However, diseases can be marked as the most important cause of aquaculture growth limiter and its economic losses (Fred and Meyer, 1991). FAO (2014) reported that only acute hepatopancreatic necrosis disease in Penaeid shrimps (AHPND) results with serious economic losses in shrimp farms of China, Vietnam, Thailand and Malaysia in 2010. Also, Kubitza et al. (2013) reported that about 15% of the mortality of farm-fish is directly related to diseases. Consequences of the disease are not limited only to mortality, they can lead to economic loss trough different ways, such as increasing feed conversion rate, reducing productivity and growth (Monir et al., 2015). According to previous studies, the globally estimated average economic loss of aquaculture due to diseases was about 5 billion of US dollars per year (Shinn et al., 2015).

Managers of aquaculture farms are constantly trying to control diseases by different methods: use of food additives (vitamins, enzymes and antibiotics) in diet to strengthen the immune system (Overton et al., 2010; Vajargah et al., 2018; Hoseinifar et al., 2020; Hamed et al., 2021; Morshedi et al., 2021; Rashidian et al., 2021); the use of chemical disinfectants. Disinfectants are a relatively common method of prevention and management of pathogens in aquaculture (Katharios et al., 2007; Harikrishnan et al., 2021; Forouhar Vajargah, 2021). Disinfectant compounds can very vary and are used in several different shapes (Bregnballe, 2015). For example, sodium chloride (NaCl) or salt is a common disinfectant compound that it is used as a disinfectant compound in hatcheries and freshwater fish farms (Boyd and Mcnevin, 2015). Formalin solution is also a famous disinfectant in aquaculture (Hodkovicova et al., 2019). It is used to eliminate pathogen agents (Guimaraes et al., 2012). However, the use of these chemical compounds have some limitations. Formalin is highly toxic and has a stable structure remaining in the environment for a long time (Buchmann al., 2004). The results of Buchmann et al. (2004) study showed lengthy exposure (24 h) to formalin at lower concentrations caused a decrease in mucous cell density of rainbow fish (*Oncorhynchus mykiss*). Leal et al. (2018) stated half-life of formalin was more than 30 h during which formalin led to several damages on fish and alteration mucosal cells. The common methods didn't showed efficient on formalin removal from water environments, and the advanced oxidation processes can be a good alternative. However, these methods are expensive and sometimes haven't economic justification (Leal et al., 2018).

The development of aquaculture and the need of farms for more effective chemical disinfectants, along with restrictions on the use of chemical compounds (e.g. formalin and green malachite) led to the introduction of metallic nanoparticles (MNPs) as more effective disinfectants for use in aquaculture (Shah and Mraz, 2020; Sinha et al., 2021a, b). Metallic nanoparticles such as silver nanoparticles (Ag-NPs), cadmium nanoparticles (Cd-NPs) and copper nanoparticles (Cu-NPs) are more effective and they have a more stable structure than previous compounds and protect the environment against pathogens for a longer period of time (Yalsuyi and Vajargah, 2017; Mohsenpour et al., 2020). Thus, their applications in aquaculture are expanding rapidly (Yalsuyi et al., 2017). However, the previous studies showed that these compounds were high toxicity for fish and other aquatic organisms, Their sub-lethal concentration induced l tissue lesions such as hypertrophy and hyperplasia of gills, necrosis of liver, alteration epithelial and mucosal cells and reduce reproduction and growth of fish (Vajargah et al., 2018; Forouhar Vajargah et al., 2020). Ag-NPs can remain in natural water for several months and their half-life is more than 28 days (Zhang et al., 2019). Ag-NPs were "highly toxic" and "toxic" for rainbow trout (*Oncorhynchus mykiss*) eleuthero-embryo-larva and juvenile (Johari et al., 2013) showed Silver nanoparticles increased cortisol and cholinesterase in fish blood, reduce growth rate and survival rate, damaged tissue and increase stress of fish. All this suggest that direct applications in aquatic environment or aquaculture should be banned. Therefore, there are concerns about emissions of these substances and their wide distribution in nature and in a more comprehensive approach, the use of efficient and environmentally friendly compounds is an inevitable necessity (Dreher, 2004; Nel et al., 2006).

Povidone-iodine (Betadine) is a water-soluble compound. It is used as a common disinfectant around the world. Betadine is usually produced as a 10% solution of povidone-iodine, and it is used millions of kilograms each year in the world (Huschek et al., 2004; Vajargah et al., 2017). The connection of iodine to S-H and N-H groups in amino acids (i.e. cysteine, lysine) can lead to denaturation of structural and enzymatic proteins in cells. Also, iodine can react to fatty acids and nucleotides (Zawada et al., 2014). Finally, these changes in the cytoplasm and membranes of cells can lead to their mortality. Iodine has a short half-life in aquatic environments (Vajargah et al., 2017). The results of Lin et al. (2018) study showed that the half-life of iodine was about 5 h. iodine also is a strong biocide that even has good performance at 0 ●C. It doesn't damage metal and/or plastic tanks. Iodine has better performance as compared with chlorine compounds. Vajargah et al. (2017) reported that povidone-iodine (Betadine) was practically non-toxic for Common carp (*Cyprinus carpio*) and didn't irritate skin and mucous membranes at sub-lethal concentrations (below 80 $mgl^{-1}$of povidone-iodine). However, there are few reports to mention the use of povidone-iodine in aquaculture (Chen et al., 2018). The biocide efficacy of iodine is dependent on environmental factors (Amend, 1974). The organic matter colloids can significantly reduce the antibacterial effect of povidone iodine (Rodriguez Ferri et al. 2010). The present study aims to evaluate the toxicity effect of povidone-iodine as a disinfectant solution, trough the survival chances and histology of gill tissue of Oranda goldfish (*Carassius auratus*), famous commercial freshwater species.

Materials And Methods

In order to the test, 250 fingerling Oranda goldfish (*Carassius auratus*) with an average weight 5 ± 0.67 were bought from the freshwater ornamental fish farm in Langeroud city (Guilan providence, Iran) and transferred to the laboratory environment. Fish were adapting to laboratory conditions into 5 tanks (400 l) for 2 weeks. Water physicochemical parameters were checked every day and were: DO 7.5–8.5 ppm, NH_3 < 0.05, temperature 21 ± 2 C° and $CaCo_3$ 230 ppm. The fish were fed by Biomar feed equivalent 2% of biomass weight twice a day during the adaption period (Vajargah et al., 2021).

Static acute toxicity test was performed following guidelines the Organization for Economic Co-operation and Development OECD standard method (1994), references (Rodriguez Ferri et al., 2010; Vajargah et al., 2017) and laboratory facility. After the adaption period, 225 individuals were transferred in the test tanks (55×50×30 cm aquarium) and divided in 15 groups (treatments) with 3 replication 24 before began of the acute toxicity test (LC_{50} 96h). The treatments were 0, 10, 20, 40, 60, 80, 90, 100, 120, 140, 160, 180, 200, 220 and 240 ppm of the nominal concentration of Betadine (povidone-iodine solution 10% - Darudarman Salafchegan Co., Tehran, Iran). Each group was 15 fishes and they weren't fed during the test. The mortality of fishes was recorded at 6, 12, 18, 24, 48, 72 and 24 h after povidone-iodine exposure. Water physicochemical parameters were the same as during that adaption period (DO 7.5–8.5 ppm, NH_3 < 0.05, temperature 21 ± 2 C° and $CaCo_3$ 230 ppm).

According to Mohsenpour et al. (2020) and Forouhar Vajargah et al. (2018) studies the gills samples were collected at 6, 12, 18, 24, 48, 72, and 96 h after povidone-iodine exposure and they were fixed by formalin solution (Formaldehyde 10%, Sigma-Aldrich®, Missouri, United States). The formalin of the sample was replaced 24 h after sampling. An automatic tissue processor (TP1020, Leica Microsystems Incorporation, Buffalo Grove, IL) was used the prepare tissue slides which were analyzed, light microscopy (Model RH-85 UXL, UNILAB®).

The lethal concentration of povidone-iodine (for 50% of the population) in intervals of 24, 48, 72 and 96 hours (24 h LC_{50}), 48 h, 72 h and 96 h were estimated through probit test with a 95% confidence (Forouhar Vajargah et al., 2018). According to the Hedayati et al. (2017) study, the correlation between different concentrations of povidone-iodine on mortality was checked by Spearman test (2-tail) in SPSS software (IBM SPSS Statistics 20). The histopathological lesions were determined and bolded according to Mohsenpour et al. (2020) study.

Results

No mortality was observed during the adaptation period. A significant correlation was observed between fish mortality rate and Betadine concentration. (Fig. 1). The highest mortality was reported at concentration 240 ppm. The mortality of fish were not followed after 24 h of Betadine exposure. The highest the mortality rate of fish was recorded after 6 h Betadine exposure (Table 1). The LC_{50} 24h Betadine (nominal lethal concentration after 24h) was 158.800 ppm, while the LC_{50} 6, 12 and 18 hours were bolded in Table 2.

Table 1
The Mortality rate of fingerling Oranda goldfish, *Carassius auratus* after different concentrations of Betadine exposure (povidone-iodine solution 10% - Darudarman Salafchegan Co., Tehran, Iran)

Concentration (ppm)	**Number 0f fish**	**No. of mortality**						
		6 h	**12h**	**18h**	**24h**	**48h**	**72h**	**96h**
0	15	0	0	0	0	0	0	0
10	15	0	0	0	0	0	0	0
20	15	0	0	0	0	0	0	0
40	15	0	0	0	0	0	0	0
60	15	0	0	0	0	0	0	0
80	15	0	0	0	0	0	0	0
90	15	0	0	0	0	0	0	0
100	15	0	0	1	1	1	1	1
120	15	0	3	3	3	3	3	3
140	15	1	3	3	4	4	4	4
160	15	3	5	7	9	9	9	9
180	15	3	4	7	11	11	11	11
200	15	7	9	12	13	13	13	13
220	15	8	10	11	14	14	14	14
240	15	10	11	13	15	15	15	15

Note: all Concentrations of Betadine are nominal

Table 2
lethal concentration of Betadine (povidone-iodine solution 10% - Darudarman Salafchegan Co., Tehran, Iran) for Oranda Goldfish, *Carassius auratus*.

Point	Concentration (ppm)			
	6 h	**12 h**	**18 h**	**24 h**
LC_{10}	153.366	127.178	114.310	114.251
LC_{20}	173.966	151..066	135.765	129.544
LC_{30}	188.820	168.291	151.236	140.571
LC_{40}	201.512	183.009	164.455	149.993
LC_{50}	**213.375**	**196.766**	**176.811**	**158.800**
LC_{60}	225.238	210.522	189.166	167.606
LC_{70}	237.930	225.240	202.385	177.029
LC_{80}	252.784	242.465	217.856	188.056
LC_{90}	273.384	266.354	239.311	203.348
LC_{95}	290.396	286.080	257.30	215.977

Note: all Concentrations of Betadine are nominal

The fish exposed to nominal lethal concentrations of Betadine showed some clinical signs such as fast swimming, anxiety, a tendency to swim near the surface, and finally death with an open mouth.

The lethal concentration of Betadine lead to hypertrophy, hyperplasia, hyperemia and secondary lamellar adhesion, hemorrhage and necrosis of gills (Fig. 2). Also, there was a significant correlation between Betadine concentration and gill lesion (Table 3).

Table 3
The correlation between Betadine concentration (povidone-iodine solution 10% - Darudarman Salafchegan Co., Tehran, Iran) the damages of the gills of Oranda Goldfish (*Carassius auratus*)

Tissue damages	**Concentration (ppm)**							
	0	**60**	**100**	**140**	**160**	**180**	**220**	**240**
Hyperemia	-	++	++	++	+++	+++	++++	++++
Hyperplasia	-	-	++	++	+++	++++	++++	++++
Hypertrophy	-	-	-	++	++	+++	++++	++++
Secondary lamellar adhesion	-	-	-	-	++	+++	++++	++++
Hemorrhage	-	-	-	-	++	+++	++++	++++
Necrosis	-	-	-	-			++++	++++

Note: all Concentrations of Betadine are nominal. Also, None (-), Mild (++), Moderate (+++) and Severe (++++) of tissue damages.

Discussion

The povidone-iodine complex itself has no antimicrobial activity. Iodine gets free from povidone-iodine solution in an aqueous medium and its concentration reaches an equilibrium (Ripa et al., 2002). The germicidal process is an iodine-consuming process and along with that iodine is retrieve by the povidone-iodine complex until its concentration reaches equilibrium again (Selvaggi et al., 2003). Free iodine like chlorine (Cl) can destroy or oxidizes pathogen vital structures (such as enzymes, DNA and RNA). These destructions can result with inhibition of production and release of microorganism enzymes such as lipase, β-glucuronidase (Fig. 3) and finish with death (König et al., 2017).

Even though the mechanism of action of povidone-iodine (free iodine) is like chlorine, it is much less toxic than chlorine. Zhein et al. (2010) studied the survival ratio of *Carassius auratus* juveniles subjected to free chlorine. They reported LC_{50} 96h of free chlorine was 0.3 ppm. The results of the present study showed a concentration of 90 ppm of povidone-iodine was approximately non-toxic. Also, chlorine has a long half-life in an aqueous medium. The results of the present study showed all of the deaths occurred within the first 24 hours and weren't recorded mortality after that time. These results were the same as the results of the Forouhar Vajargah et al. (2017) study.

Tissue lesions of an aquatic organism are one of the common consequences of the use of chemical compounds (Forouhar Vajargah et al., 2018; Vaclavik et al., 2020; Stara et al., 2020a, b; Sula et al., 2020; Vajargah et al., 2021). Chloramine-T at concentrations 25 and 30 ppm led to hypertrophy and hyperplasia gills of goldfish (*Carassius auratus*) 24 h after exposure (Altinok, 2004). The histopathological results showed a concentration of less than 60 ppm of Betadine (povidone-iodine solution 10%) didn't lead to significant tissue damages; in addition, the gills lesions were mild in a concentration less than 140 ppm of Betadine. The fish exposed to nominal lethal concentrations of Betadine showed some clinical signs such as fast swimming, anxiety, a tendency to swim near the surface, and finally death with an open mouth.

Tissue damage of fish can be caused by the oxidative activity of free iodine.. Free iodine after passing through of cell membrane and connection to S-H and N-H groups in amino acids (i.e. cysteine, lysine) can lead to denaturation of structural and enzymatic proteins in cells. In addition, iodine can react to fatty acids and nucleotides and deform structures of DNA and RNA (Zawada et al., 2014). However, according to published information about toxicity categories for terrestrial and aquatic organisms by the United States Environmental Protection Agency (U.S. EPA, 2017), Betadine (povidone-iodine solution 10%) was particularly non-toxic for goldfish (Table 4).

Table 4
Ecotoxicity categories of materials for aquatic organisms; listed by their 96h LC_{50}.

Toxicity category	Concentration (ppm)
Very highly toxic	96h-LC_{50} < 0.1
highly toxic	0.1 < 96h-LC_{50} < 1
moderately toxic	1 < 96h-LC_{50} < 10
slightly toxic	10 < 96h-LC_{50} < 100
practically nontoxic	96h-LC_{50} > 100
Note. All data of acute concentrations of pollutants are from U.S. EPA (2017)	

The result clearly showed that Betadine (povidone-iodine solution 10%) was particularly non-toxic and its half-life is very short. It is very easy to use cheap and available. The concentrations less than 100 ppm of Betadine didn't lead to death and concentrations less than 60 ppm didn't induced any gills damages. Due to the results of the present study and previous studies about Betadine antimicrobial activity, we can recommend it as a useful disinfectant choice in aquaculture. However, knowledge of Betadine affectivity on common aquatic parasites needs more studies, together with Betadine possible effects on molecular, biochemical and physiological levels can be aim of future studies.

Declarations

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Figures

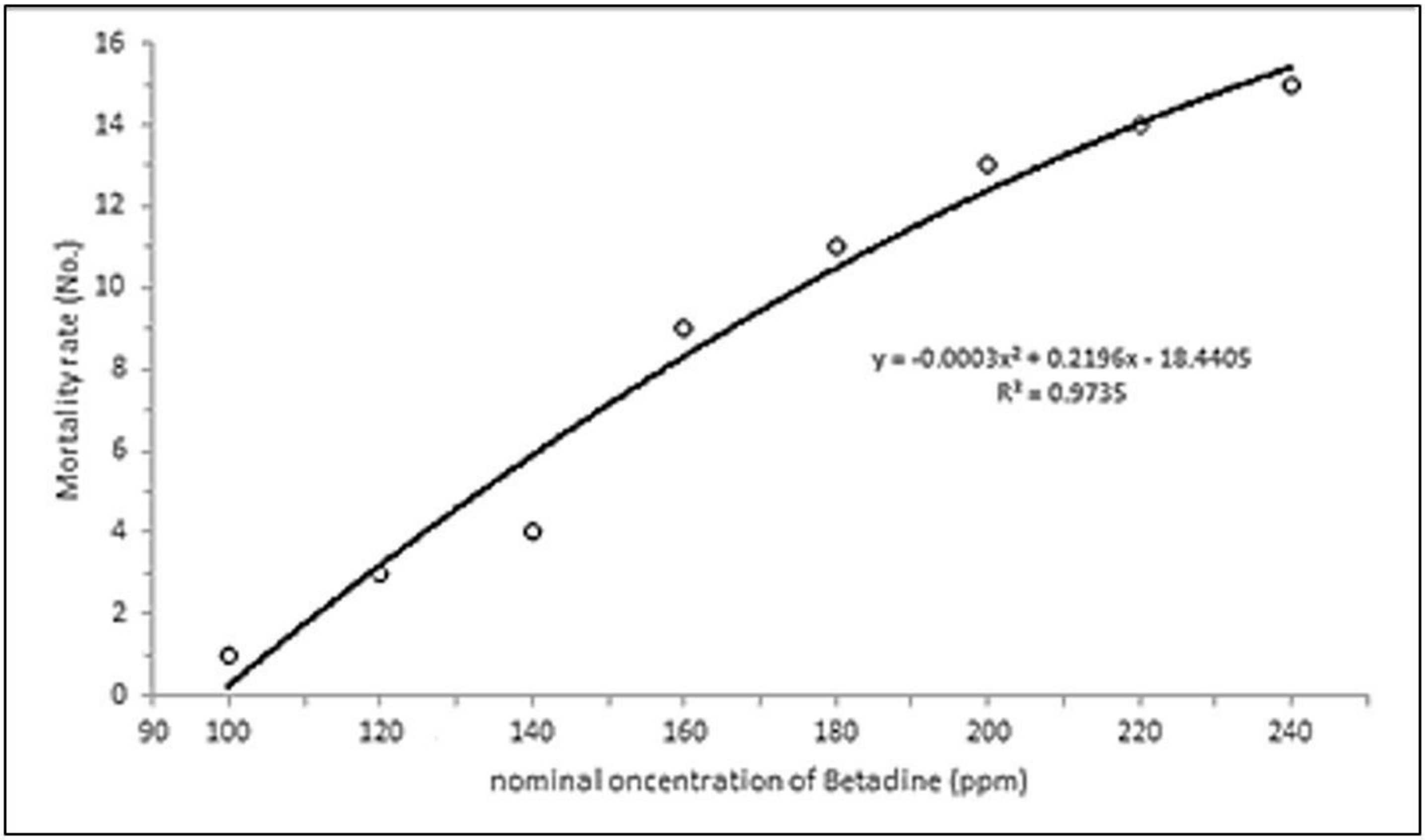

Figure 1

The correlation between nominal lethal concentrations of Betadine (povidone-iodine solution 10% - Darudarman Salafchegan Co., Tehran, Iran) with mortality rate of fingerling Oranda goldfish, Carassius auratus ($p<0.01$).

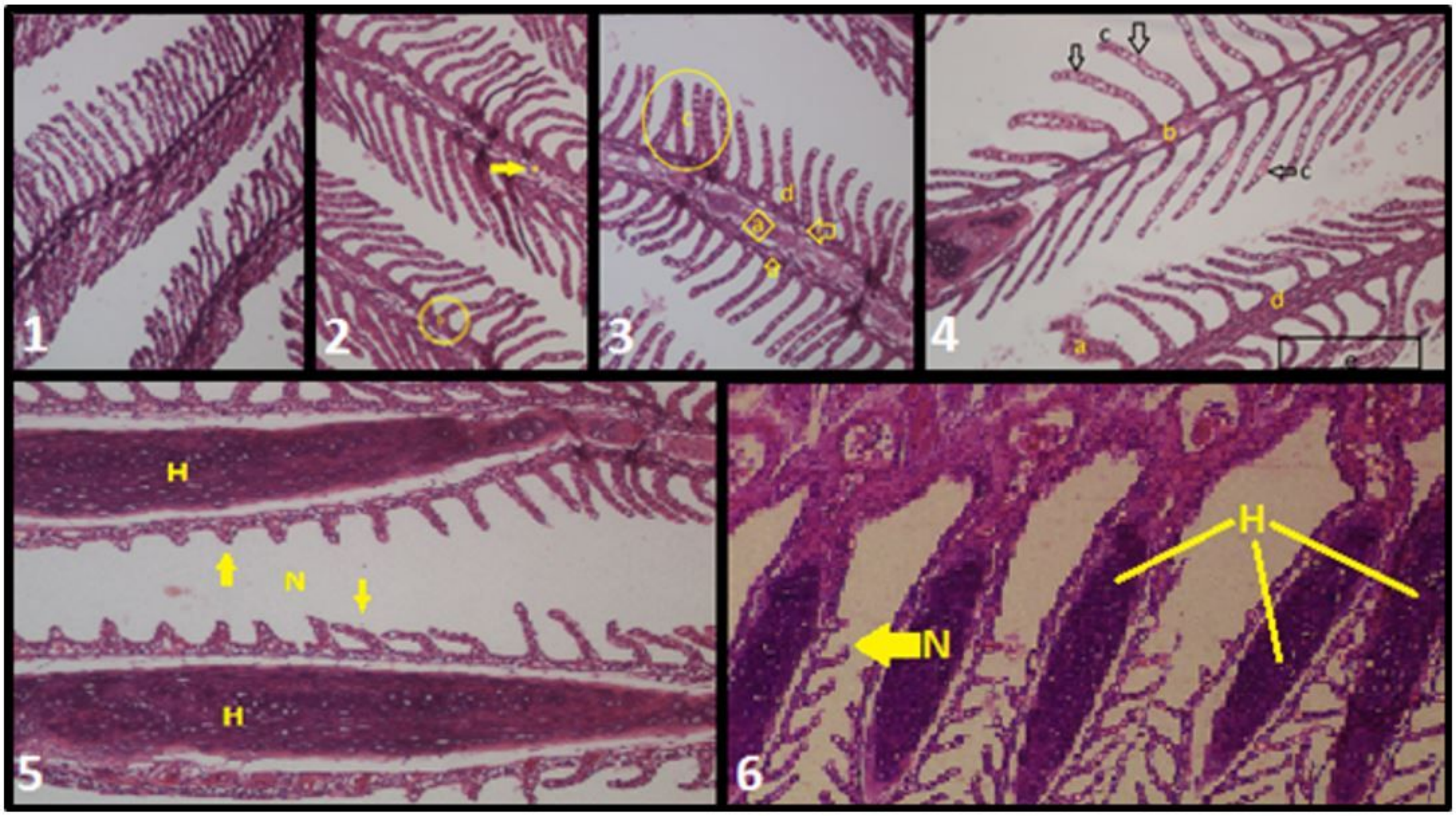

Figure 2

the fingerling Oranda Goldfish (Carassius auratus) Histopathological changes by Betadine (povidone-iodine solution 10% - Darudarman Salafchegan Co., Tehran, Iran); 1. The control group (0 ppm): there did not detect significant tissue damages; 2. Hyperemia (a) and hyperplasia (b) of the gills (140 ppm); 3. Hyperemia (a), hypertrophy (b), secondary lamella adhesion (c) and hyperplasia (d) the gills (160 ppm); 4. Cell necrosis (a), hyperemia (b), Hypertrophy (c), hyperplasia (d) and secondary lamella adhesion (e) of gills (180 ppm); 5. Hemorrhage (H) and necrosis (N) of the gills (220 ppm); 6. Hemorrhage (H) and necrosis (N) of the gills (240 ppm) - All pictures are magnified ×10.

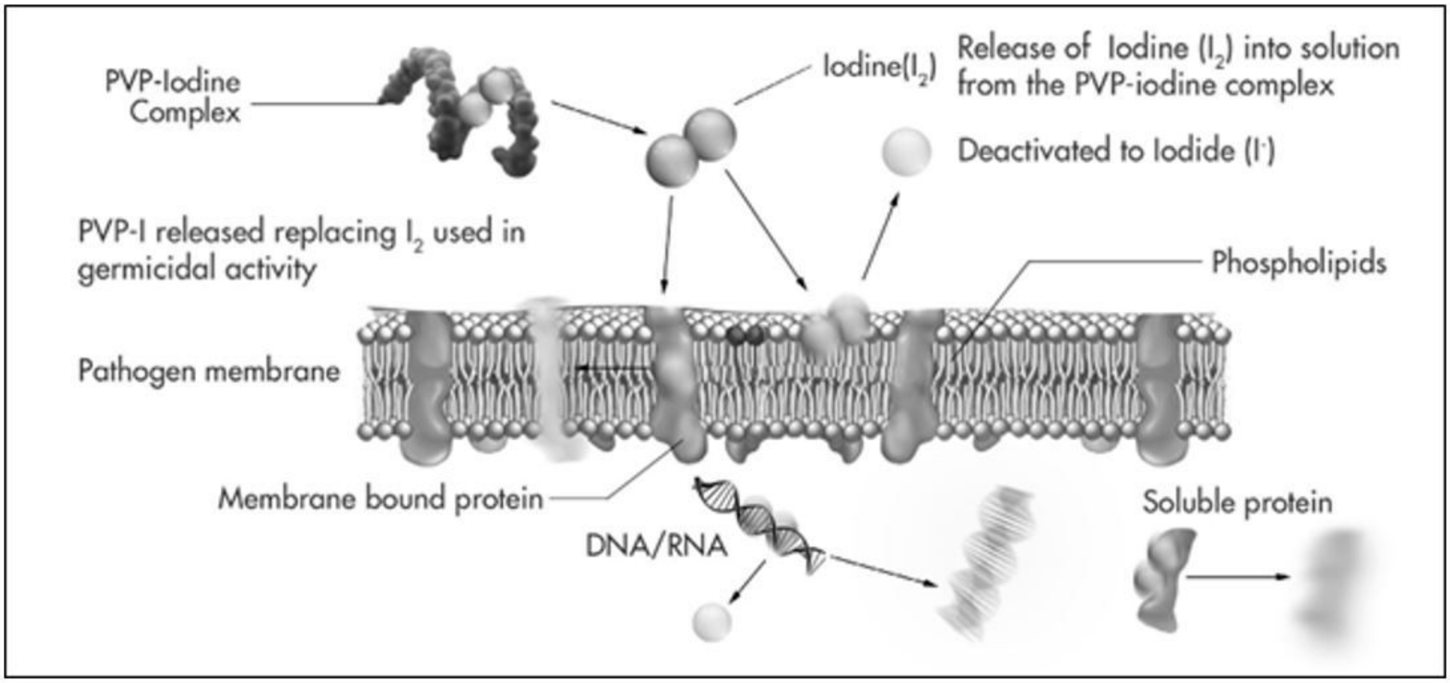

Figure 3

Mechanism of action of povidone iodine in equilibrium with free iodine. Free iodine inactivates DNA or RNA of pathogens through Oxidative reactions. This figure adapted from Bigliardi et al. (2017).