Evaluation of Sodium Chloride and Hydrogen Peroxide as Sustainable Therapeutic Agents for Controlling Saprolegniasis in Ornamental Aquarium Fish in Algeria

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Abstract

Introduction: Saprolegniasis, caused by oomycete pathogens such as Saprolegnia, poses a major challenge to the global ornamental fish industry, resulting in economic losses and raising animal welfare concerns. Restrictions on traditional chemical treatments have prompted the search for safe, effective, and eco-friendly alternatives. This study aimed to assess and compare the effectiveness of two biodegradable substances, sodium chloride (NaCl) and hydrogen peroxide (H_2O_2), in controlling natural saprolegniasis infections in ornamental aquarium fish.

Materials and Methods: Sixty-two naturally infected fish were selected for two trials. In the first trial, 30 fish were exposed to sodium chloride baths at concentrations of 0, 2, 4, 6, and 8 g/L for 12 hours each day. The second trial involved 32 fish exposed to hydrogen peroxide baths (30% solution) at 0.5, 1, 1.5, and 2mL/L for 30 minutes daily. The primary measures of effectiveness were lesion healing and survival rates.

Results: Sodium chloride showed a concentration-dependent effect, with 4 and 6 g/L reducing lesion severity and mortality, but higher concentrations (8 g/L) were toxic. Hydrogen peroxide at 2.0 mL/L provided the best results, significantly improving lesion regression and survival without causing adverse effects.

Discussion: Both treatments were effective in controlling saprolegniasis, though hydrogen peroxide at 2.0 mL/L outperformed sodium chloride, providing a faster, more potent treatment. Sodium chloride's mechanism is osmotic, whereas hydrogen peroxide is cytotoxic, which explains its superior efficacy. However, both treatments have limitations in completely eradicating the infection.

Conclusion: Using 2mL/L of hydrogen peroxide during 30-minute daily baths provides a sustainable and effective way to treat saprolegniasis in ornamental fish, offering aquarists an environmentally friendly alternative.

Keywords: Aquaculture, Hydrogen Peroxide, Ornamental Fish, Saprolegniasis, Sodium Chloride.

1. Introduction

The global aquarium trade is a multi-billion-dollar industry [1], and in Algeria, it constitutes a substantial and growing segment of the pet industry. However, maintaining healthy populations of ornamental fish is often complicated by various health problems, particularly infectious diseases, which can cause significant damage [2]. These diseases not only result in substantial economic losses for importers, retailers, and hobbyists but also raise important ethical questions about animal welfare. In this context, saprolegniasis emerges as a major fungal threat, particularly in temperate regions and during colder seasons [3,4].

Saprolegniasis is a severe aquatic disease caused by common, opportunistic water molds mainly from the genera *Saprolegnia* and *Achlya* (Oomycetes). These organisms are not true fungi but are often treated as such in aquatic pathology because of their similar filamentous growth and ecological roles [5]. The disease is common in freshwater environments and often occurs under stressful conditions, such as poor water quality, handling, transportation, or be secondary following physical injury or bacterial disease [6]. The most characteristic clinical sign is the appearance of white or gray cotton-like mycelial patches on the skin, fins, gills, eyes, or mouth of affected fish. These patches are attached to necrotic tissue, and as the infection progresses, it causes severe damage, including hemorrhagic ulcerations, epidermal erosion, and destruction of muscle and fin tissues. Without treatment, the infection can lead to osmoregulatory failure, respiratory distress, and eventually mass mortality, often wiping out entire aquarium populations [3].

A key factor influencing the outbreak and severity of saprolegniasis is water temperature. The disease is significantly promoted by low water temperatures, usually below 15°C, which can

weaken the fish's immune system while still supporting the healthy growth of water molds [4]. This presents a major seasonal challenge for aquarists in Algeria and similar climates. In the past, controlling these pathogens primarily relied on chemical treatments, such as malachite green and formalin. However, the use of these chemicals has become increasingly restricted or banned in many countries due to their teratogenic, carcinogenic, and environmental persistence [7,8]. This regulatory change has created an urgent need for safe, effective, affordable, and environmentally friendly alternatives [9,10]. This need has spurred significant research into sustainable health management strategies, including dietary immunostimulants [11], probiotics and postbiotics [12,13], and stress-reducing additives [14] to improve fish resilience and provide alternatives to conventional chemotherapy.

In response to this need, the scientific community has focused on the potential of biodegradable and generally recognized as safe substances for disease management in aquaculture. Two promising options are sodium chloride (common salt) and hydrogen peroxide. Both are low-cost, easily accessible, and break down into harmless components, salt into sodium and chloride ions, and hydrogen peroxide into water and oxygen [15,16]. Salt acts as a therapeutic agent by creating an osmotic imbalance that stresses freshwater pathogens, such as *Saprolegnia*, causing them to lose water and die, while also supporting the fish's osmoregulatory function and mucus production [15]. Hydrogen peroxide, a powerful oxidizing agent, is lethal to many pathogens by generating highly reactive free radicals that damage cellular structures [16]. While the antifungal properties of both NaCl and H_2O_2 are individually documented in aquaculture, a direct comparative assessment of their efficacy against natural saprolegniasis infections in a multi-species ornamental aquarium context is lacking. Furthermore, optimized, practical treatment protocols for hobbyists, particularly in regions such as Algeria, where local data are scarce, remain undefined. This constitutes a significant gap between laboratory studies and field application.

Therefore, this study was designed directly compare the efficacy of these two biodegradable substances, sodium chloride and hydrogen peroxide, to systematically identify the optimal therapeutic concentrations for effectively controlling saprolegniasis in aquarium fish. By establishing safe and effective treatment protocols, this research aims to provide Algerian aquarists and the broader ornamental fish industry with practical, sustainable, and environmentally responsible tools to reduce losses caused by this widespread disease.

2. Materials and Methods

2.1. Experimental Fish and Acclimatization

A total of 62 freshwater aquarium fish representing various ornamental species, all displaying clear clinical signs of natural saprolegniasis infection, mainly characterized by white, cotton-like mycelial lesions and skin ulcers [17], were selected for this study (Figure 1). While definitive diagnosis requires mycological culture or molecular methods, the presented signs are universally accepted for initiating treatment in clinical settings. Before beginning the experiments, all fish underwent a 48-hour acclimation period in a controlled laboratory setting to reduce stress. During this time, they were housed individually in aerated 20-liter glass aquariums. Each tank was filled with dechlorinated tap water, and water quality was carefully

monitored. Key parameters, including temperature (20 ± 1°C), pH (7.2 ± 0.2), and ammonia levels (<0.05 mg/L), were checked and recorded daily using standard aquarium test kits to ensure stable, optimal conditions throughout the study.

Figure 1. Platy fish infected with Saprolegniasis showing cotton wool-like masses on the lateral surface of the body

2.2. Therapeutic Trial Design: Sodium Chloride

The first therapeutic trial aimed to evaluate the effectiveness of sodium chloride. Thirty infected fish were randomly divided into five experimental groups (6 fish per group) using simple random sampling to minimize bias. Each group received a daily static bath in isolated 5-liter containers. Baths were prepared with commercially available non-iodized salt dissolved in dechlorinated water to achieve target concentrations: 0 g/L, 2 g/L, 4 g/L, 6 g/L, and 8 g/L. The concentration range (2-8 g/L) was selected to bracket the commonly recommended therapeutic range (1-5 g/L) for freshwater fish [15], enabling identification of an optimal dose and the upper toxicity threshold for ornamental species. The fish were immersed in their respective salt baths for up to 12 hours daily. After each treatment, all fish were carefully netted and returned to their original, clean, medication-free aquaria for recovery. This protocol was repeated consistently over 10 consecutive days. a duration commonly used to assess therapeutic response in saprolegniasis treatment trials [18]. Fish in the 0 g/L group served as the untreated control and were later used as the shared control for the subsequent hydrogen peroxide trial, undergoing identical handling and observation without exposure to either therapeutic agent.

2.3. Therapeutic Trial Design: Hydrogen Peroxide

The second independent trial tested the effectiveness of hydrogen peroxide. Here, 32 infected fish were randomly split into four equal groups of 8. Each group received a short bath treatment with a 30% analytical-grade hydrogen peroxide stock solution. The solution was carefully diluted to 0.5 mL/L, 1.0 mL/L, 1.5 mL/L, and 2.0 mL/L in separate 5-liter containers. These concentrations were chosen based on established safe and effective ranges (approximately 50-100 ppm, equating to ~1.7-3.3 mL/L of 30% H_2O_2) reported for treating external oomycete

infections in various fish species [16], aiming to identify the minimal optimally effective concentration for sensitive fish. The baths lasted no more than 30 minutes each day to avoid potential toxicity. After each daily treatment, the fish were quickly returned to their recovery tanks. This process was repeated for 10 days, consistent with the NaCl trial and standard therapeutic evaluation periods.

2.4. Data Collection and Assessment

Throughout both trials, all fish were observed multiple times daily for behavioral signs of stress (e.g., lethargy, loss of equilibrium, rapid opercular movement), signs of improvement (e.g., fastest healing, activity, appetite, relief from discomfort from fungal lesions), and clinical changes in their fungal infections. The primary efficacy metrics were lesion regression and survival. Lesion regression was quantified as the percentage change in lesion area over time using a standardized photographic analysis method (ImageJ software) [19], where the healing rate was calculated as the proportion of the healed area relative to the initial lesion area. Fish showing clear signs of intoxication, such as loss of balance, erratic swimming, or immobility, were considered dead for mortality records. These observations were systematically documented to determine the optimal therapeutic concentration for each compound, balancing efficacy with safety.

Statistical analysis

The results were statistically analyzed using SPSS version 23.0 (IBM Corp., Armonk, NY, USA) and GraphPad Prism version 10.1 (GraphPad Software Inc., San Diego, CA, USA). A one-way analysis of variance (ANOVA) was performed to compare the effects of different concentrations of sodium chloride and hydrogen peroxide on lesion reduction and survival rates. Tukey's post hoc test was used for pairwise comparisons. The differences between groups were considered statistically significant if the p-value was less than 0.05.

3. Results

3.1. Efficacy of Sodium Chloride Treatment

The trial evaluating sodium chloride baths showed a concentration-dependent effect on the treatment of saprolegniasis in infected aquarium fish (Figure 2). During the ten-day treatment period, most fish in the treatment groups exhibited significant improvement. This was reflected in increased activity, better appetite, and less visible discomfort from the fungal lesions. However, it is important to note that the external fungal signs, especially the white, cotton-like growths, did not completely disappear in any fish by the end of the trial. This suggests that while salt baths helped manage the infection and improved fish welfare, they did not entirely eliminate it under these experimental conditions.

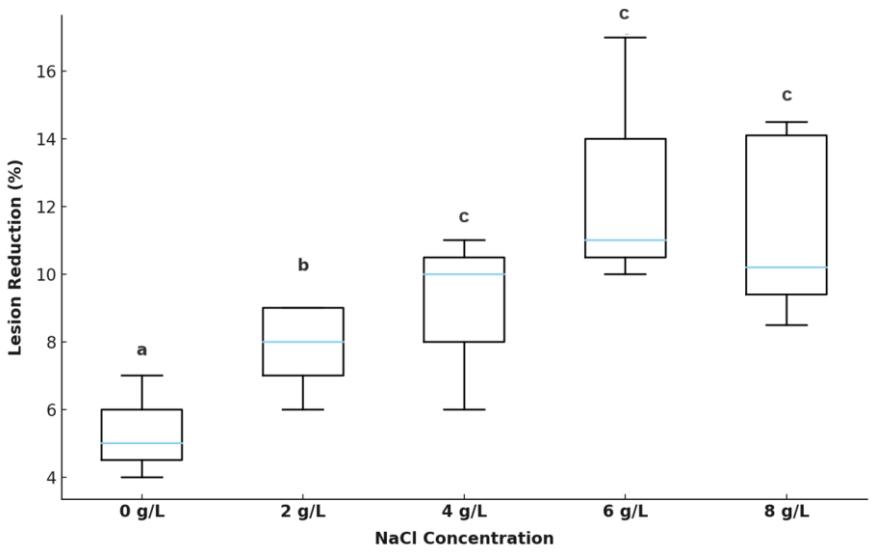

Figure 2. Effect of sodium chloride concentration on lesion reduction (%)

A one-way ANOVA showed that sodium chloride concentration significantly impacted lesion reduction. Post hoc Tukey tests indicated that 4 g/L and 6 g/L concentrations resulted in significantly greater lesion reduction than the control group ($p < 0.01$). An important event occurred during the trial, highlighting potential treatment stress. One fish in the 8 g/L group was removed about 8 hours after the bath began, on the ninth day. This fish showed severe agitation followed by a quick onset of listlessness, requiring immediate removal to prevent death. This suggests a toxicity threshold at this concentration, especially with prolonged exposure. Also, although the 8 g/L concentration was higher than the control ($p < 0.05$), it was less effective than the 6 g/L concentration in reducing mortality, as shown in Figure 3, highlighting its toxicity at these higher levels.

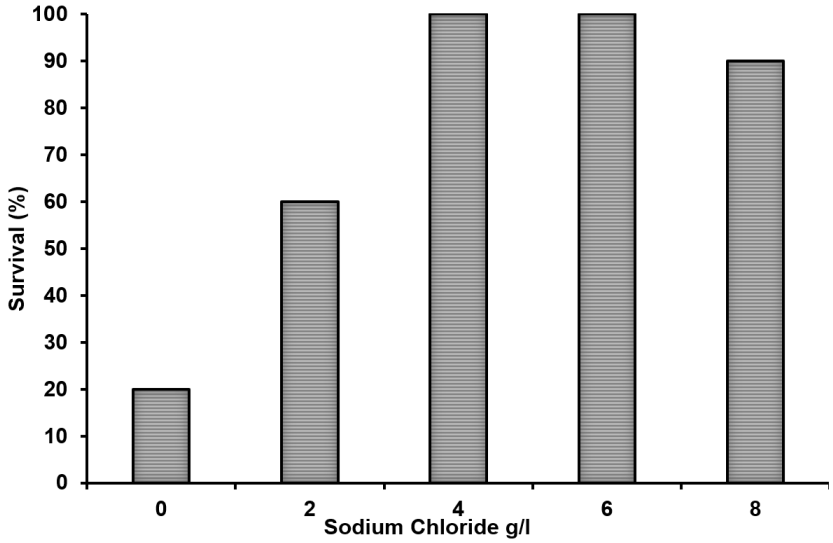

Figure 3. Survival rates of infected fish treated with sodium chloride (%)

Monitoring physiological parameters indicated that concentrations of 4 g/L and 6 g/L offered the best balance between treatment effectiveness and safety, with notable improvements in overall condition and no signs of osmotic stress.

3.2. Efficacy of Hydrogen Peroxide Treatment

The second trial assessed the effects of short-term hydrogen peroxide baths at various concentrations. The survival rate of the treated fish was measured at the end of the 10-day protocol. The results demonstrated a clear relationship between hydrogen peroxide concentration and treatment success (Figure 4). Similar to the sodium chloride trial, the peroxide baths did not completely eradicate the fungal infection in all groups. However, there was a significant and noticeable improvement in lesion severity and overall fish health. Among all tested concentrations, the group treated with 2 mL/L hydrogen peroxide showed the best outcomes. Fish in this group exhibited the most significant reduction in external fungal growth, the fastest healing of ulcerated tissues, and the highest survival rate (Figure 5). Lower concentrations (0.5 mL/L and 1.0 mL/L) caused milder effects, while 1.5 mL/L was effective but did not surpass 2 mL/L. No adverse effects or signs of chemical stress were observed in any group during the carefully controlled 30-minute exposure periods, indicating that the treatment was well-tolerated at these concentrations.

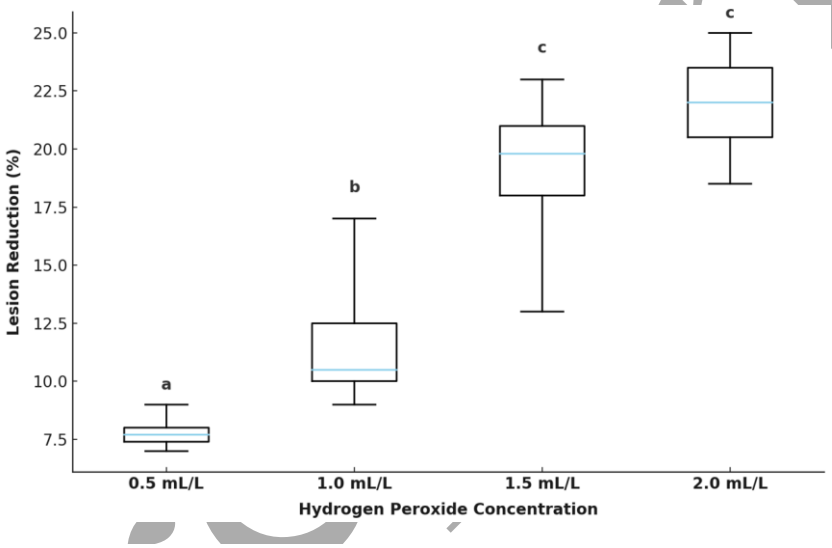

Figure 4. Effect of hydrogen peroxide concentration on lesion reduction (%)

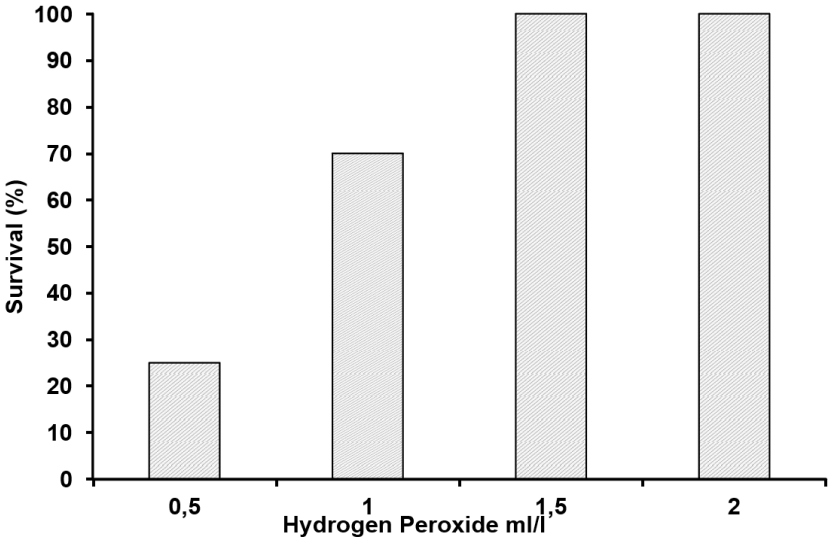

Figure 5. Survival rates for infected fish treated with hydrogen peroxide (%)

4. Discussion

The present study provides a comparative evaluation of two environmentally friendly treatments, sodium chloride and hydrogen peroxide, for controlling saprolegniasis in ornamental aquarium fish. The findings demonstrate that both substances can significantly reduce infection severity in a concentration-dependent manner; however, they differed notably in effectiveness, mechanisms, and practical applications.

Although the antifungal properties of sodium chloride and hydrogen peroxide have been individually reported in aquaculture, direct comparative studies under natural saprolegniasis conditions in ornamental aquarium fish remain scarce. Most previous investigations have focused on single species, induced infections, or food-fish systems, leaving a gap in evidence-based guidance for aquarium settings. This study directly addresses this gap by evaluating both agents side by side under identical, realistic conditions.

The effectiveness of sodium chloride baths aligns with its well-known role as a supportive treatment in freshwater aquaculture [20,21]. Our results indicate that concentrations of 4 g/L and 6 g/L strike the best balance, significantly decreasing lesion scores and mortality without causing osmotic stress. The therapeutic action of salt is primarily osmotic, creating a hyperosmotic environment that harms freshwater-adapted pathogens, such as *Saprolegnia spp*. [22]. This osmotic pressure causes plasmolysis in the hyphal tips, hindering growth and reproduction [4,23]. At the same time, the salt bath supports the fish's osmoregulatory functions, lowering the metabolic effort needed to maintain ionic balance in compromised skin and gills, and may encourage the production of a protective mucus layer [7]. However, the inability of salt baths to entirely eliminate the cotton-like mycelial growths suggests a fungistatic rather than a fungicidal effect at the tested concentrations and exposure times. This finding aligns with observations in other fish species, where salt alone was insufficient to control advanced saprolegniasis [18]. The toxicity observed at 8 g/L, shown by severe agitation and lethargy, emphasizes the importance of concentration and exposure time. It also indicates that although the therapeutic range for sodium chloride is broad, it has an upper limit, especially for sensitive ornamental species [24]. These results refine existing knowledge by defining practical, safe concentration limits for prolonged salt baths in ornamental fish. This group has received far less experimental attention than food fish or hatchery species.

In contrast, hydrogen peroxide showed superior and faster effectiveness, with the 2.0 mL/L concentration yielding the best results for lesion regression and survival. Its strong oxidizing ability, which produces hydroxyl radicals that damage lipids, proteins, and DNA in microbial cells non-selectively, explains hydrogen peroxide's broad-spectrum antimicrobial activity. [25,26]. This direct, cytotoxic mechanism likely accounts for its more pronounced effect on visible mycelial structures than the osmotic stress induced by sodium chloride. The lack of any adverse effects observed during the 30-minute baths supports other studies indicating that short-term exposure to hydrogen peroxide at concentrations up to 100 ppm (~3 mL/L of a 30% solution) is well tolerated by many fish species [16,27].

Its effectiveness against *Saprolegnia* has been documented across various aquaculture settings, from salmonid eggs [28] to channel catfish [29]. The present study extends these findings to naturally infected ornamental aquarium fish, thereby bridging the gap between experimental efficacy and practical aquarium-based disease management. A key advantage of hydrogen peroxide is its transient nature; it breaks down into harmless water and oxygen, leaving no persistent toxic residues in the aquarium. This makes it especially appealing from an environmental and safety standpoint, particularly for display tanks housing sensitive invertebrates or biological filter media, though caution is still recommended.

When comparing the two treatments, several practical considerations emerge. Salt therapy, although gentle and providing excellent osmoregulatory support [17], required prolonged daily baths (up to 12 hours) and did not completely eliminate the infection at the concentrations tested [30]. The stress response at 8 g/L underscores the importance of species-specific tolerance. Hydrogen peroxide, in contrast, offered a more potent and rapid antifungal effect with a much shorter daily exposure time of only 30 minutes. The 2 ml/L concentration was well-tolerated and represented the most effective protocol in this study. However, its potency requires precise dosing to avoid damage to the fish's delicate gill lamellae [31]. This direct comparison addresses an important knowledge gap by providing aquarists and researchers with evidence-based guidance on the relative advantages and limitations of two widely accessible, biodegradable treatments under identical experimental conditions.

Several limitations of this preliminary study must be acknowledged. The use of a multi-species group, while reflecting a real-world aquarium setting, introduces variability in species-specific susceptibility and treatment tolerance. Furthermore, the absence of histopathological analysis to confirm hyphal penetration into deeper tissues limits our understanding of why a complete cure was not achieved. The experimental design also lacked a group treated with a combination of both agents, which could potentially produce a more effective synergistic outcome.

Future studies should address these gaps by incorporating species-specific analyses, histological assessment, and combination or sequential treatment protocols further to optimize sustainable control strategies for saprolegniasis in ornamental fish.

5. Conclusion

This study confirms the effectiveness of both sodium chloride and hydrogen peroxide as treatments for saprolegniasis in aquarium fish. Hydrogen peroxide at 2 mL/L, administered in 30-minute daily baths, was the most effective single treatment under these conditions. Future research should aim to identify species-specific tolerance levels, evaluate the effectiveness of combined treatment methods, and investigate histological changes during infection and healing to improve treatment protocols for complete disease eradication.

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Authors' Contribution

M M collected the data, conceived, designed, and performed the analysis; M B wrote the paper.

Ethics

All procedures involving animals were carried out in accordance with the highest standards of animal care and welfare. This assay was performed in accordance with the European Communities Council Directive (2010/63/EU) on the protection of animals used for scientific purposes. The experimental study took place at the animal house of the Institute of Veterinary and Agricultural Sciences, University of Batna 1, and the protocol was designed and implemented by veterinarians. The protocol aimed to minimize suffering and stress. Fish showing severe signs of intoxication or distress were immediately removed from the study to prevent further suffering. The study used naturally infected fish with saprolegniasis to test

298 treatments that could alleviate the disease, potentially benefiting both the subjects and the
299 species.

300 **Conflict of Interest**

301 The named authors have no conflict of interest, financial or otherwise.

302 **Data Availability**

303 Data will be available upon request to the corresponding author.

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