Journal of Global Ecology and Environment

Volume 19, Issue 4, Page 40-51, 2023; Article no.JOGEE.12016
ISSN: 2454-2644

Investigating the Acute Cardiac Restoration for Rescuing Daphnia Magna Stressed from Environmental Disturbances Using Antioxidants

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Author's contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

Article Information

DOI: 10.56557/JOGEE/2023/v19i48616

Original Research Article

Received: 26/10/2023
Accepted: 29/12/2023
Published: 31/12/2023

ABSTRACT

Freshwater ecosystems face a significant challenge with the pervasive oxidative stress induced by pollution with global warming and ozone layer depletion. Amidst this concern, various companies assert their products' antioxidant properties to restore them to normal. Elevated oxygen species, identified as a pivotal contributor to heightened cardiac functionality, particularly impact crucial organisms such as Daphnia Magna within aquatic environments. This study proposes a proactive approach to mitigate oxidative stress by introducing antioxidants into water, aiming to offer insights into fortifying the health and resilience of aquatic ecosystems confronting oxidant escalation.
The primary objective of this research was to assess the impact of commercially available "antioxidants" on the heart rates of Daphnia Magna during the recovery phase from oxidative stress.
As methods the investigation unfolds in two phases: an initial toleration study to gauge Daphnia reactions to selected chemicals, followed by a recovery study where Daphnia was exposed to the oxidizing agent H_2O_2 for 30 minutes and subsequently immersed in antioxidant solutions as well as in culturing water for a comparison purpose. The central methodology revolves around comparing

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the heart rates of Daphnia exposed to H_2O_2 and analyzing their recovery in different solutions. Heart rate serves as a chosen metric due to its reliability as an indicator of stress and its non-invasive measurement, ensuring minimal harm to the organisms. A newly defined parameter, relative restoration power (RRP) as mean heartbeat %change, was estimated and compared. Our conclusions revealed that certain antioxidants exhibit a modest yet noteworthy impact on enhancing the recovery of Daphnia magna. Consequently, the study concludes that improving antioxidant uptake or finding less intrusive delivery methods may be viable strategies to mitigate oxidative stress in Daphnia magna.

Keywords: Acute cardiac restoration; antioxidants; environmental heartbeat disturbances; hydrogen peroxide; relative restoration power.

1. INTRODUCTION

In numerous studies, rising temperatures caused by climate change have been identified as a factor contributing to increased oxidative stress in organisms [1]. The phenomenon is explained by the notion that higher temperatures often amplify biological processes, producing reactive oxygen species and the subsequent imposition of cellular oxidative stress [2]. Scientists have presented a direct reduction in phytoplankton production from the damage of ozone depletion-related increases in UVB. UVB radiation has been claimed to trigger damage to the early developmental phases of fish, shrimp, crabs, amphibians, and other marine animals. The repercussions of oxidative stress on aquatic ecosystems, particularly on vital organisms such as Daphnia Magna, have been extensively investigated.

The detrimental effects encompass a spectrum of cellular and physiological changes with severe implications for all aquatic organisms, including but not limited to growth inhibition, compromised immune function, heightened susceptibility to diseases, and oxidative damage to tissues and organs [3]. Work has been done to mitigate this through polymers that attempt to mitigate oxidative stress in a study with "ROS-responsive catechin chemical intermediates minimize the disturbances in the heart rate of Daphnia magna in oxidative media." [4]. In the realm of potential solutions, an intriguing concept surfaces: the prospect of mitigating oxidative stress by dissolving antioxidants in water. This approach, in contrast to mere recovery in water, holds promise as a proactive measure to alleviate the adverse impact of oxidative stress.

As we explore antioxidants and their potential efficacy, this research seeks to contribute valuable insights into safeguarding the health and resilience of aquatic ecosystems in the face of escalating temperatures. Hydrogen peroxide is chosen because it rapidly turns into water and free radical oxygen in water, a major cause of oxidative stress [5,6]. With Fenton's reaction, a reaction between H_2O_2 and Fe2+ ions create the incredibly reactive hydroxyl (OH) radical, which leads to oxidative stress. Furthermore, it is hypothesized that stress has effects on heart rate. The investigation specifically focused on the impact of hydrogen peroxide (H_2O_2) on lowering the heartbeat of Daphnia, drawing from published data on the generation of oxidant species.

Various naturally derived antioxidant formulations, including Multi-Antioxidant Tablet (21 Century Healthcare Inc), denoted as antioxidant A here, which was a proprietary combination of antioxidant-enhanced herbs and plants such as Acai berry extract [7], Golgi Berry [8], Noni [9], Mangosteen [10] and Pomegranate [11], all superfoods claiming to have antioxidative capabilities. And Yu Xing Cao Solution (Honolulu, HI) was included in our study for its restoration power, which Chinese medicine claims to be an Antioxidant. In addition, another antioxidant supplement was investigated. The Super Antioxidant (Natural Herb, Inc) is referred to as Antioxidant B. The antioxidant B was 100% beta-carotene, and a 7500 ug formulation [12] was employed to evaluate their efficacy in restoring normal physiological conditions.

2. METHODS

2.1 Experimental Materials and Chemicals

The multi-antioxidant tablet as antioxidant A was prepared with vitamins A, C, and E, the complete antioxidant nutrient group, combined with the essential minerals; selenium, zinc, copper, and manganese, which might help neutralize free radicals which may cause cellular damage in the body (Asin#B0007PQAOS). Detailed concentrations of each component were not revealed because of its proprietary rights. However, vitamin A of 5,000 IU is listed with cellulose, starch, gelatin, and croscarmellose sodium. The Super Antioxidant (Natural Herb, Inc) is referred to as Antioxidant B. The antioxidant B was 100% beta-carotene of 7500 ug formulation. Yu Xing Cao (Houttuynia cordata) is a herbaceous perennial plant growing to between 20 and 80 cm. The proximal part of the stem is trailing and produces adventitious roots, while the distal part of the stem grows vertically. The leaves are alternate, broadly heart-shaped, 4–9 cm long and 3–8 cm broad. Flowers, growing usually in summer, are greenish-yellow, borne on a terminal spike 2–3 cm long with 4-6 large white basal bracts. it is known as lizard tail, chameleon plant, heartleaf, fishwort, and bishop's weed (ASIN**:** B00P96Q7KM, Manufactured by HawaiiPharm, USA). As the extract solution from the plants, detailed concentrations of the product were not released. However, optimal concentrations for Daphnia have been determined with rigorous preliminary experiments.

2.2 Dual Study Phases

First, study materials were collected as in our experimental protocol, as seen in Fig.1. Our study was performed under clean laboratory conditions regulated by general daphnia handling guidelines. The study took place in two phases; first, a series of toleration studies will be conducted to determine the proper concentration of Antioxidants.
(Phase 1)

(1) For the Antioxidants, a solution of 1.0g/50 ml of water was prepared, and then a 5-fold serial dilution was performed six times to yield six different concentrations.

(2) Then, Daphnia was prepared, their heart rates were measured, and they were incubated for 30 minutes.
(3) Afterwards, heart rates were recorded, and the Daphnia was returned to their culturing tubes. Heart Rate was determined by measuring the time it took for 40 beats and then extrapolated to find the beat per minute (BPM).

(4) The same procedure was repeated with hydrogen peroxide (H_2O_2)

(Phase 2) The second phase of the study determined the effects of the compounds.

(1) The Daphnia first had their heart rates measured, then exposed to H2O2 for 30 minutes.
(2) And they were put in culturing water for another 30 minutes to examine if their heartbeat changed.
(3) Then, another group was exposed to antioxidants A or B for 30 minutes.
(4) Subsequently, they were exposed to the H202 for thirty minutes.
(5) Heart rates were measured and recorded before the experiment, after the first thirty minutes, and finally, at the end of the experiment.
(6) The change in heart rate was plotted, and regression analysis was performed accordingly.

2.3 Heartbeat Count Procedures

The time of forty heartbeats was measured with a stopwatch and a microscope and then typed into the Microsoft Excel Sheet on a PC to convert the heartbeat number into a beat per minute (BPM) unit. There were some cases for estimation of extreme data; for example, the time of forty beats was typed in as 5000 to be estimated for dead Daphnia, while ten beats were counted and multiplied by four when the heartbeats were too slow, such as taking 20 seconds or longer only for 10 counts. Data plotting and regression analysis using the trendline function were performed in Excel.

2.4 Preliminary Study

For all the groups of Daphnia, their heartbeat changes will be measured, and a reliable preparedness of the Daphnia for further study.

1) The study for the choice of optimal pH, temperature, H_2O_2, and glyphosate concentration will be performed.
2) The study for the optimal concentrations of antioxidants will be performed in serially diluted solutions, respectively.

2.5 Control Group

In this case, the control will be the water fleas exposed to H_2O_2 and returned to the water they were cultured in.

2.6 Study Procedures

As seen in Diagram 1 below, four environmental disturbances were determined to be studied:

temperature, pH, H_2O_2, and glyphosate. Branded as Roundup, glyphosate is one of the most frequently sprayed herbicides globally, and published data shows that glyphosate causes oxidative accumulation when under environmental disturbances.

Among these environmental factors, H_2O_2 was mainly used for its stressed effects on Daphnia's heartbeat rate.

2.7 Background Study

For all the groups of Daphnia, their heartbeat changes were measured, and a reliable preparedness of the Daphnia was established for further study.

1) The study was performed to determine the choice of optimal pH, temperature, and glyphosate concentration.
2) The study for the optimal concentrations of antioxidants was performed in serially diluted solutions, respectively.

Some of the data should have been presented here since they were performed only to seek optimal conditions of the main experiment.

2.8 Heartbeat Restoring Evaluation

The preconditioned Daphnia was placed into the antioxidant solutions after heartbeat measurement. The Daphnia's heartbeat was preconditioned with 30 minutes of exposure to the diluted H_2O_2 solution. Their heartbeat rate was reduced mostly reliably, as expected. After 30 minutes of the H_2O_2 exposure, the Daphnia was moved into the test tube filled with antioxidant solutions. Their restoring heartbeat rate was measured every 10 minutes for 30 minutes, monitoring the restoration of the heartbeat to the normal state.

2.9 Data Summary and Analysis

All the data collected in this study was typed into Microsoft Excel Worksheet during the experiment. Most studies have been performed six times and summarized as mean and standard deviation. Regression analysis was performed in Excel as the functionality of the trendline. The regression equation with the highest coefficient was presented on the plots with R squared. We defined a new parameter for facilitating the capability of HB restoration by the antioxidants here as relative restoration power (RRP). We used n=6, added the restored HB% of all the Daphnia, and divided by 6 (Add the HB% of Daphnia1 through Daphnia 6)/6. Intuitively considering this definition, the RRP from exposure to the antioxidant solution should be greater than the RRP from culturing water.

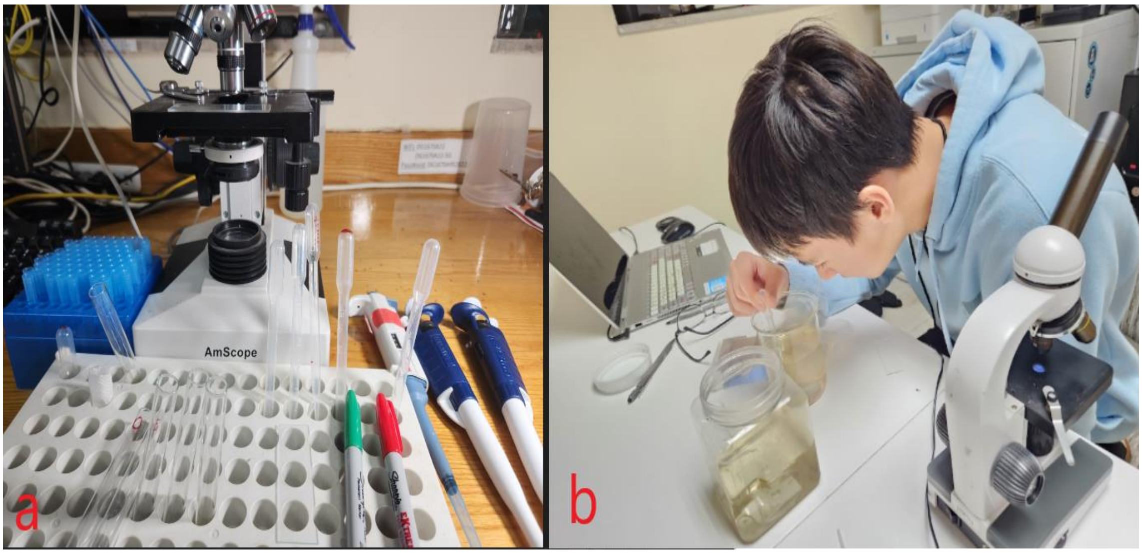

Fig.1. presents our experimental study materials (a) and daphnia handling procedures (b).

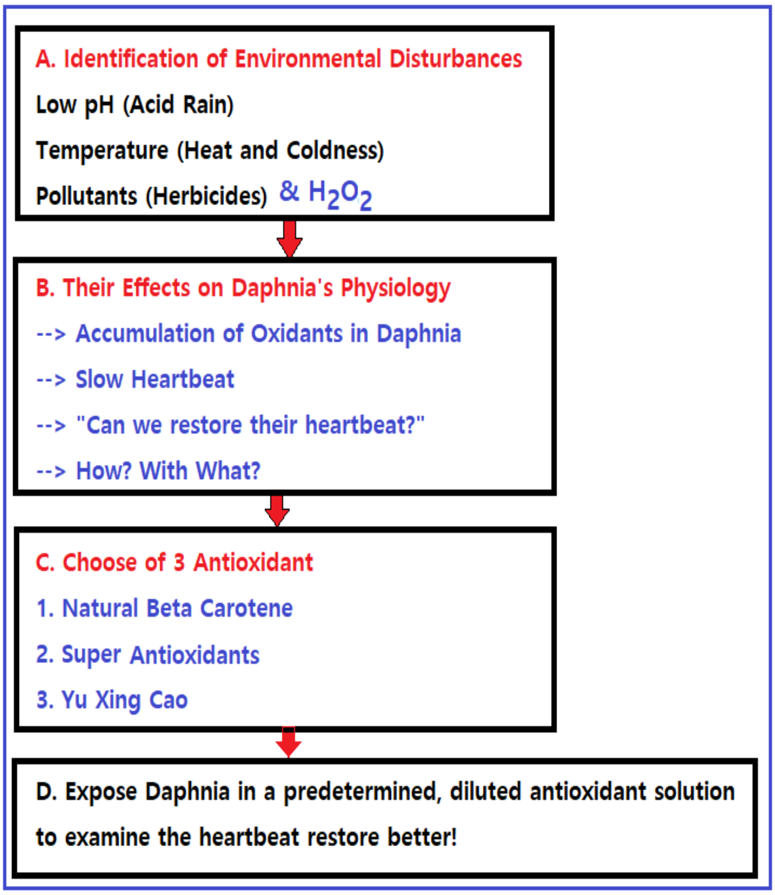

Diagram 1. Above illustrates the study procedures in our study.

Table 1 presents our experimental table in Microsoft Excel. The parameters and %change were calculated automatically while the data was typed in

Drug:	H2O2 Hydrogen Peroxide						
Date:	12/27/2023	= 1:5 Serial dilution		no heartbeat = 3000 sec			
	40 Cts Pre	pre-bpm	takeout		post-bpm		%Change
0	12.06	199.005	30	10.5	228.571	29.5665	-14.8571
5	8.5	282.353	35	8.22	291.971	9.61786	-3.40633
10	8.09	296.663	40	7.94	302.267	5.60446	-1.88917
15	8.03	298.879	45	7.9	303.797	4.91827	-1.64557
20	7.59	316.206	50	7.49	320.427	4.2217	-1.33511
25	8.22	291.971	55	8.11	295.931	3.96015	-1.35635

3. RESULTS

3.1 Baseline Study with Hydrogen Peroxide

To create a reliable and reproducible animal model, it was deemed very important to know how we could produce the HB% depression consistently using H_2O_2 first. A concentration optimization study must be performed before other antioxidant treatments for restoration study. Before experimenting on Daphnia, it was necessary to establish the effects of the substances in isolation on the heart rates. Fig. 2

shows the percent decrease in HR concerning the concentration of hydrogen peroxide. The HR percent decrease has a logarithmic relationship to concentration, with a regression coefficient of 0.8339. This data suggested an ideal concentration for inducing stress without killing should be approximately a 0.75 percent solution of H_2O_2. Based on this precious data, our study continued to prepare the animal to conduct heartbeat restoration capability using the antioxidant compounds.

3.2 Percent Change in HR for Antioxidant a Solution Exposure

One of the researchers presented that Catechin crystallized with ROS-labile polymer displayed accelerated dissolution proportional to the H_2O_2 concentration. The ROS-responsive catechin crystals protected vascular cells from oxidative strikes by activating intracellular glutathione peroxidase expression and, in turn, inhibiting an increase in intracellular oxidative stress. It implied that the heartbeat may be restored faster by the effects of the antioxidant, as seen in Fig.3. It follows a polynomial curve, which means that too much Antioxidant A will slow down the recovery. At the same time, too little is just water, meaning that an ideal concentration for recovery is around 0.04 percent to 0.008 percent.

3.3 Percent Change in HR for Antioxidant B Solution Exposure

Antioxidant B was the mixture of various natural chemical compounds, mainly from plants, that were recognized to possess plenty of antioxidants. As seen below, a concentration to restore heart rate would be around 0.03 percent. Furthermore, this has been tolerated better than Antioxidant A by the Daphnia. Due to the results of this toleration study, I decided to use a 0.03 percent concentration of Antioxidant B for the other experiment as it appears to affect the heart rate without killing it. Furthermore, the polynomial line suggests a cutoff for efficacy, much like the others.

3.4 Percent Change in HR for Antioxidant Yu Xing Cao Solution Exposure

As shown on the graph, there appears to be a linear correlation until a certain point when the concentration becomes irrelevant. Thus, it was decided that a 2 percent concentration of Yu Xing Cao would be used for the experiment.

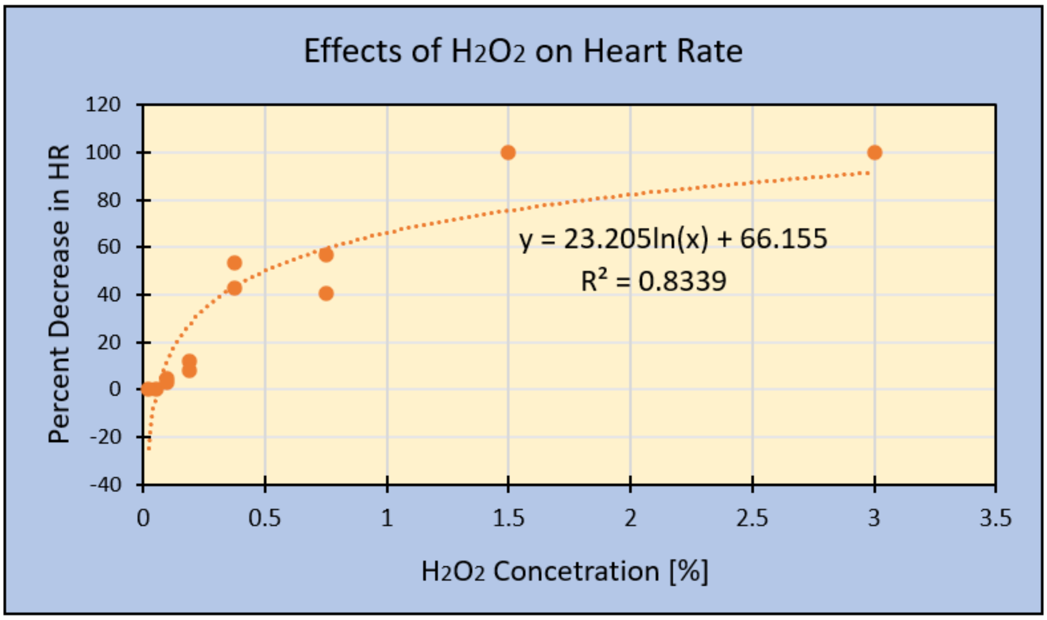

Fig. 2. presents the HR percent change after thirty minutes in the concentration at the bottom of H_2O_2 (n=11). Each dot represents a single daphnia

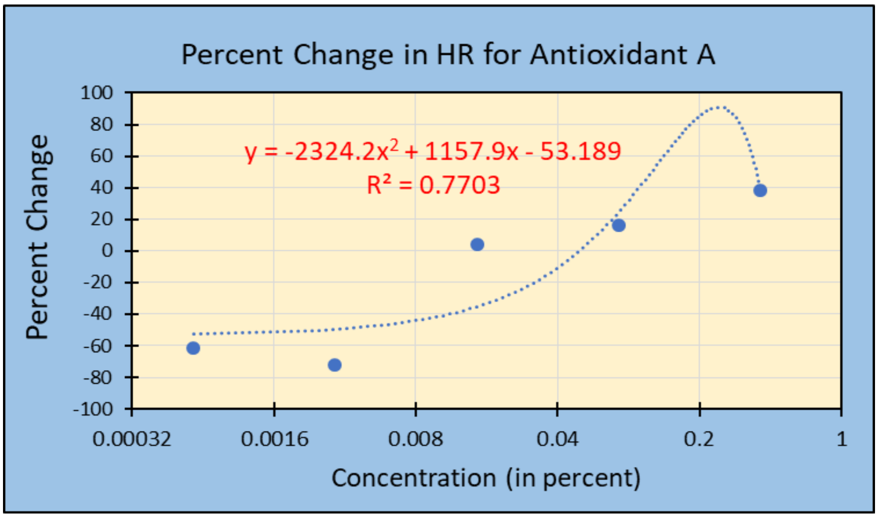

Fig. 3. Presents the relationship between HR percent change and concentration of antioxidant A (n=6).

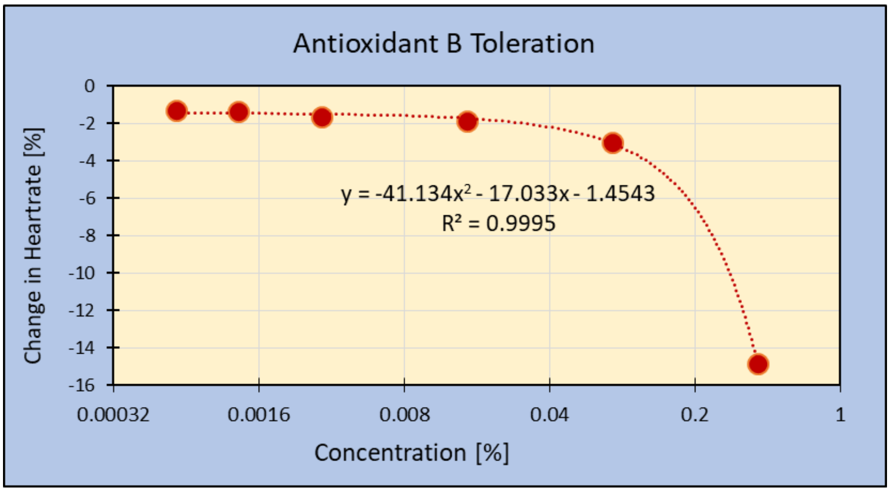

Fig. 4. presents the HR percent changes for the exposure to the antioxidant B (n=6).

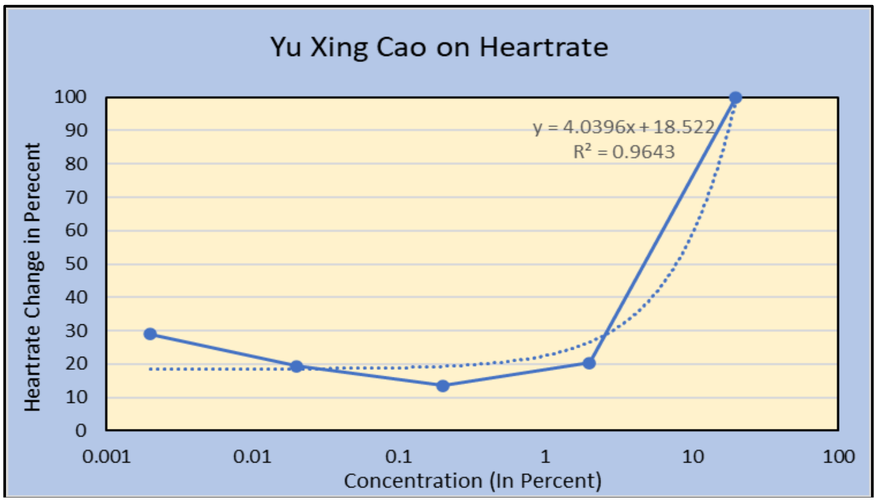

Fig. 5. Depicts the effects of Yu Xing Cao on the Heart Rates of Daphnia Magna

3.5 Comparison of Heart Rate Restoration by Water

Fig. 6 below shows the control on the null hypothesis. In this figure, the blue bars present the HB change after H_2O_2. As you see, their height was far greater than those of the gray bars that show the change after culturing water. While merely returning Daphnia to the water they were cultured in, there was still a decrease in heart rate, and therefore, this allows us to compare this to analyze the efficacy of the other treatments. The bar in Figure three is non-existent because the heart rate was precisely returned to its original rate. This shows a minor effect of water on the restoration of Heart Rate. The RRP was estimated to be 40%.

3.6 Comparison of Heart Rate Restoration by Antioxidant A

Fig. 7 presents the heartbeat rate % change for individual concentration points for ease of comparison for the two groups. As shown, in most cases, Antioxidant A has a significant effect on restoring heart rates of Daphnia exposed to hydrogen peroxide. For the outlier, the first one was pregnant and gave birth during the experiment and thus should not have been included; the baby also died. As the graph shows, in most cases, Antioxidant A had a significant effect on restoring the heart rate of Daphnia, even more than the water, thus demonstrating that antioxidants do have potential. Furthermore, this graph shows that Antioxidant A restores heart rates better than water, albeit to a minor degree. In this case, the RRP was estimated to be 60 percent.

3.7 Comparison of Heart Rate Restoration by Antioxidant B

Fig. 8 presents the heartbeat rate % change for individual concentration points for ease of comparison for the two groups. In most cases, it appears that Antioxidant B has little effect on restoring heart rates of Daphnia exposed to hydrogen peroxide. As the graph shows, in most cases, Antioxidant B has a significant impact on restoring the heart rate of Daphnia, even more than the water, thus demonstrating that Antioxidants do have potential. Furthermore, this graph shows that antioxidant B does not do better than water-restoring heart rates. The RRP was estimated to be 30 percent.

3.8 Comparison of Heart Rate Restoration by Yu Xing Cao

Fig. 9 suggests that the results of Yu Xing Cao are mixed; in some cases, it improved, but in others, it just increased the heart rate. This emphasizes the difficulty of working with biological organisms as many factors may influence the heart rate, and all are slightly different. However, this suggests that Yu Xing Cao is not beneficial for the heart rate and may adversely impact the health of Daphnia Magna. In this case, the RRP was negative, approximately -5 percent.

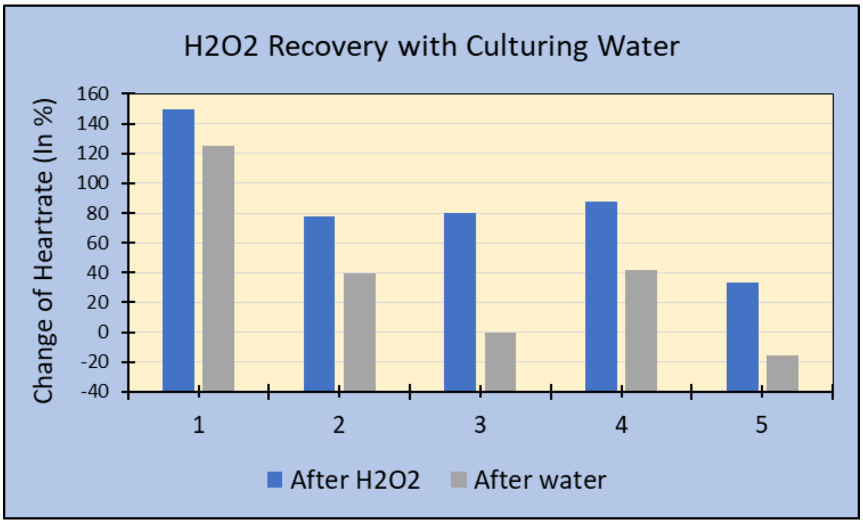

Fig. 6. Depicts the effects of Culturing Water after H_2O_2 Exposure on the Heart Rates of Daphnia Magna

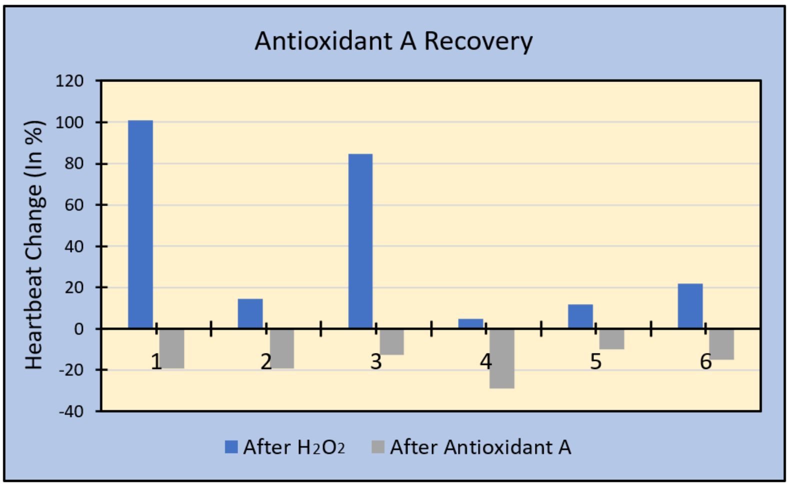

Fig. 7. Illustrates the effects of Antioxidant A after H2O2 Exposure on the Heart Rates of Daphnia Magna

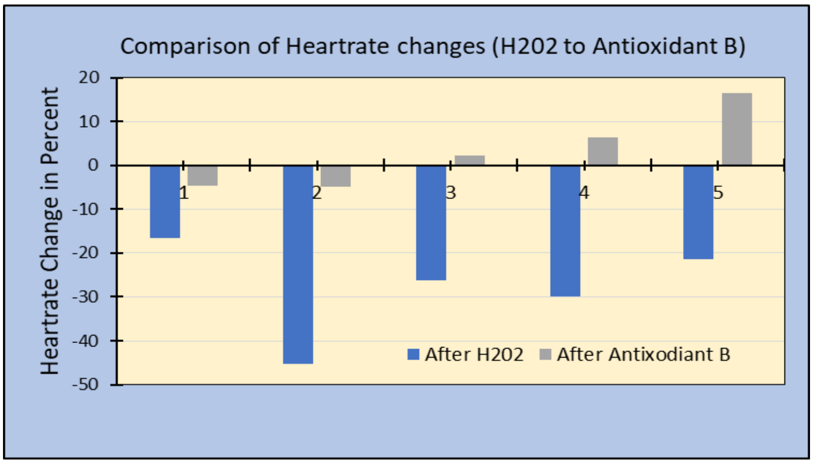

Fig. 8. Illustrates the effects of Antioxidant B after H_2O_2 Exposure on the Heart Rates of Daphnia Magna

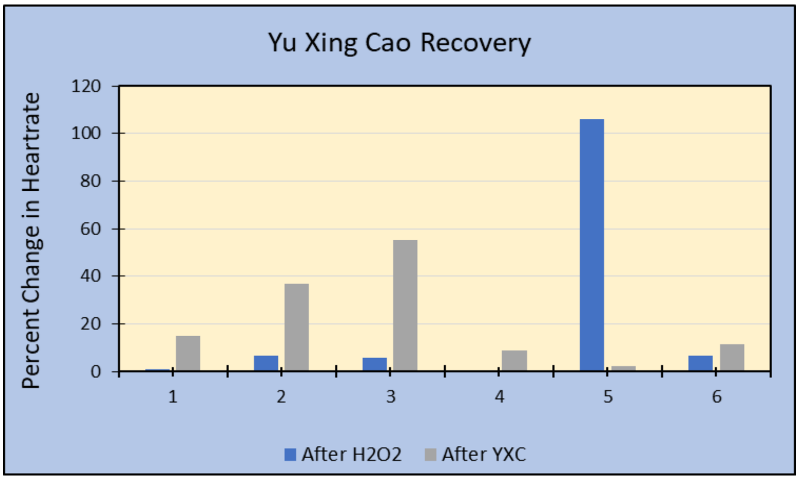

Fig. 9. Illustrates the effects of Yu Xing Cao after H_2O_2 Exposure on the Heart Rates of Daphnia Magna

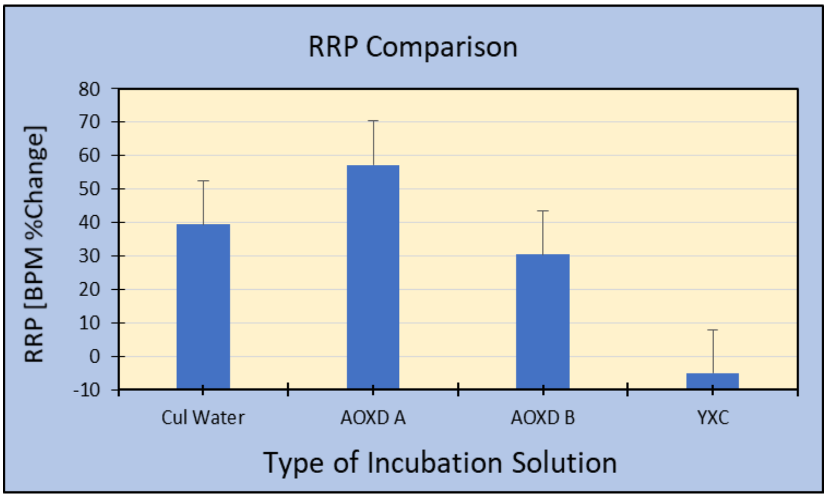

Fig. 10. Presents the RRP for each incubation solution (n=6)

3.9 RRP Comparison

As defined in the method section, RRP was defined as the mean BPM %change difference after incubation in the antioxidant solution. The data in Fig. 10 shows that Antioxidant A was the best for the quantitative presentation of heartbeat restoration. Antioxidant B was less effective in heartbeat restoration than antioxidant A and even in culturing water. Surprisingly, the incubation in the Yu Xing Cao solution gave negative RRP, adversely affecting the heart's functionality. However, even minus RRP estimated in Yu Xing Cao might not mean it would harm Daphnia's heartbeat. The possibility of not using an optimal concentration of Yu Xing Cao should exist. More studies might be needed to find an optimal concentration of Daphnia's incubation.

4. DISCUSSION

The study's findings underscore the significance of antioxidants, specifically vitamins, in influencing heart rates, albeit with a minor impact. This correlation was consistent with existing literature, exemplified by a study conducted by Lobo et al., which emphasized the role of antioxidants in mitigating oxidative stress-induced carcinogenesis through scavenging reactive oxygen species (ROS) and inhibiting cell proliferation. The protective potential of “Superfoods” against cancer was attributed to their antioxidant function, given the ability of oxidative products to cause genetic damage.

However, the inherent complexity of biological organisms introduces many variables that extend beyond oxidative stress. Controlling factors such as temperature, nutritional status of Daphnia, tank crowding, and the stress induced by the heart rate measurement procedure posed substantial challenges. While the initial results present intriguing insights, the need for further investigation becomes apparent to validate and expand upon these preliminary findings.

Future research endeavors should delve into a more comprehensive exploration of the intricate interactions within the experimental setup. Understanding how temperature variations, nutritional conditions, and population density impact the observed effects on heart rates in Daphnia is crucial for a holistic comprehension of the underlying mechanisms. Moreover, meticulous attention to the stressors associated with the measurement procedures will contribute to refining experimental protocols. In this context, a multi-faceted approach will not only enhance the reliability of the study but also contribute valuable pathways to the broader scientific understanding of the impact of antioxidants on cardiac functions in complex biological systems.

5. CONCLUSION

The results above offer compelling evidence supporting the utilization of antioxidants to recover Daphnia from oxidative stress. This finding aligns with the conclusions from various studies within the same domain. While this study lays the groundwork and imparts preliminary merit to introducing antioxidants into water environments, it is imperative to underscore additional testing, particularly concerning the potential ecological impacts.

Moreover, a noteworthy insight from Kim et al. posits that "Hydrogen peroxide is often used to clean water fouled by excessive algae, and this raises concern about how the oxidant may be affecting living organisms in the water." The statement highlights a critical concern regarding using hydrogen peroxide, commonly employed for water treatment, and its potential impact on aquatic life. The researchers propose that their new antioxidant-delivery system could serve as a viable replacement to address the issue of over-oxidized natural waters. A newly defined parameter, relative restoration power (RRP) as mean heartbeat %change, was estimated and compared. Our conclusion revealed that certain antioxidants exhibit a modest yet noteworthy impact on enhancing the recovery of Daphnia magna. Consequently, the study concludes that improving antioxidant uptake or finding less intrusive delivery methods may be viable strategies to mitigate oxidative stress in Daphnia magna.

Given these promising indications in the future, further research and comprehensive testing are imperative to ascertain the efficacy of antioxidant applications and their potential consequences in aquatic environments. By expanding our understanding of the ecological ramifications, we might develop more informed strategies to mitigate oxidative stress and promote the well-being of aquatic organisms.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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