

Research Article

## Effect of dryness on antioxidant enzyme activities in Gastropod *Pomacea canaliculata* from Shatt Al-Arab River, Basrah, Iraq

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### Abstract

It is well known that humidity affects the fertility and reproductive activity of gastropods, inducing an imbalance in oxidative stress that can lead to cell damage and restricted normal activities. The present study investigated the effects of dryness on antioxidant enzymes as a marker in the Gastropod *Pomacea canaliculata* collected from the Shatt Al-Arab River. The experiment was conducted in three successive sets, in addition to the control (Set I). Set II, animal kept for 6 days in constant high humidity (HH). Set III, animal kept for 3 days in low humidity, followed by 3 days in high humidity (DH), Set IV, animal kept for 6 days in low humidity (DD). After 6 days, the antioxidant enzymes, superoxide dismutase (SOD), catalase (CAT), and glutathione S-transferase (GST) in the digestive gland were assessed. Moreover, Thiobarbituric Acid Reactants (TBARS) was also assessed as an indicator of oxidative damage. The results showed that the activity of SOD, CAT, and GST did not change throughout the period (HH). While in the periods of DH and DD, the activity of the three antioxidant enzymes increased. And the highest increasing of SOD (31 U/mg), CAT (383 U/mg) and GST (1812 mU/mg) showed in the period of DD. The same harmonic increase in oxidative damage was also observed during the periods of DH and DD, with the highest TBARS level (24 nmol/g) found at DD. In conclusion, the dryness induced oxidative stress in the digestive gland of *Pomacea canaliculata*. This study is the first to investigate the effect of dryness on this snail from the Shatt Al-Arab River.

**Keywords:** Antioxidant enzymes, Dryness, Oxidative stress, *Pomacea canaliculata*, Shatt Al-Arab River

## INTRODUCTION

Many organisms live under conditions of harsh environment. So, they evolved several strategies to treat with these conditions. Specially through situations of hypometabolic which result in an imbalance of oxidative stress leading to damage of cells and tissues. Preparation for oxidative stress (POS) is one of the physiological strategies that occurs in many animals as a protective measure against oxidative stress (Hermes-Lima *et al.*, 2015; Giraud-Billoud *et al.*, 2019; Giraud-Billoud *et al.*, 2022; Rodriguez *et al.*, 2023). DNA methylation, regulation of transcription and post-translational modifications of antioxidant enzymes involved in the strategy of POS (Giraud-Billoud *et al.*, 2019). Specifically, cytoprotective genes are upregulated in response to high levels of reactive oxygen species (ROS) to reduce damage to intracellular macromolecules, which could otherwise lead to cell death. The situation of hypoxia causes upregulation of REDOX-sensitive transcription

factors, which elevate endogenous antioxidants to counteract ROS overproduction (Ensminger *et al.*, 2021). The gene of forkhead box protein O (FOXO) upregulates target genes in a hypometabolic situation, increasing the concentrations of mRNA for superoxide dismutase (SOD) and regulating the expression of catalase (CAT) (Ponugoti *et al.*, 2013; Giraud-Billoud *et al.*, 2022). The transcription factor nuclear factor erythroid 2-related factor 2 (Nrf2) is regulated negatively by Kelch-like ECH-associated protein 1 (Keap1). The ROS stress effect on cysteine residues of Keap1 which stimulate the Nrf2 leading to transcriptional regulation of antioxidant enzymes including Superoxid dismutase (SOD), Catalase (CAT), glutathione S-transferase (GST) in addition to other proteins responsible for scavenging of ROS and reduce biosynthesis of glutathione (GSH) (Kovac *et al.*, 2015).

Apple snails are amphibious snails that display capacities for broad adaptation to extreme environmental conditions, such as drought, salinity, temperature, or cold.

*Pomacea canaliculata* (Lamarck, 1819) exhibits an unusual form of POS when subjected to environmental stress, such as estivation or hibernation (Giraud-Billoud, 2018; Yao *et al.*, 2024). The native habitat of this species is South America, but it has spread widely to other geographical areas, including North America and Asia, due to agricultural practices and aquarium applications (Yoshida *et al.*, 2016; Yang *et al.*, 2018). Due to its ability to tolerate the harsh conditions of its environment and consume a wide variety of food, the snail has achieved a worldwide distribution. As for Iraq, the species was recorded in Baghdad (Al-Jassany and Al-Hassnawi, 2017) and Shatt Al-Arab River (Al-Khafaji *et al.*, 2015). However, it was indicated that the animal existed in Babylon during the period (1990-1991) in the small rivers of agricultural farms. Then, a large number of the animal transported to the Basrah city and invasive of Shatt Al-Arab River (Ali *et al.*, 2020). Over recent decades, the Shatt Al-Arab River has been subjected to significant environmental changes, including rising salinity, increasing temperatures, and longer drought seasons (UNEP, 2001). For the first time, this study investigates the effect of dryness on the Gastropod *Pomacea canaliculata* collected from the Shatt Al-Arab River, using antioxidant enzymes (SOD, CAT, and GST) as well as oxidative damage as biomarkers.

**MATERIALS AND METHODS**

**Sampling**

In this study, the adult Gastropods *Pomacea canaliculata* with an average shell length 50 mm, were collected by hand from Garmat Ali at Shatt Al Arab River, Basrah (30.621279°N 47.761149°E), as shown in Fig. 1. The snail was identified according to Mahilum and Demayo (2014). Sampling collection was made in September,

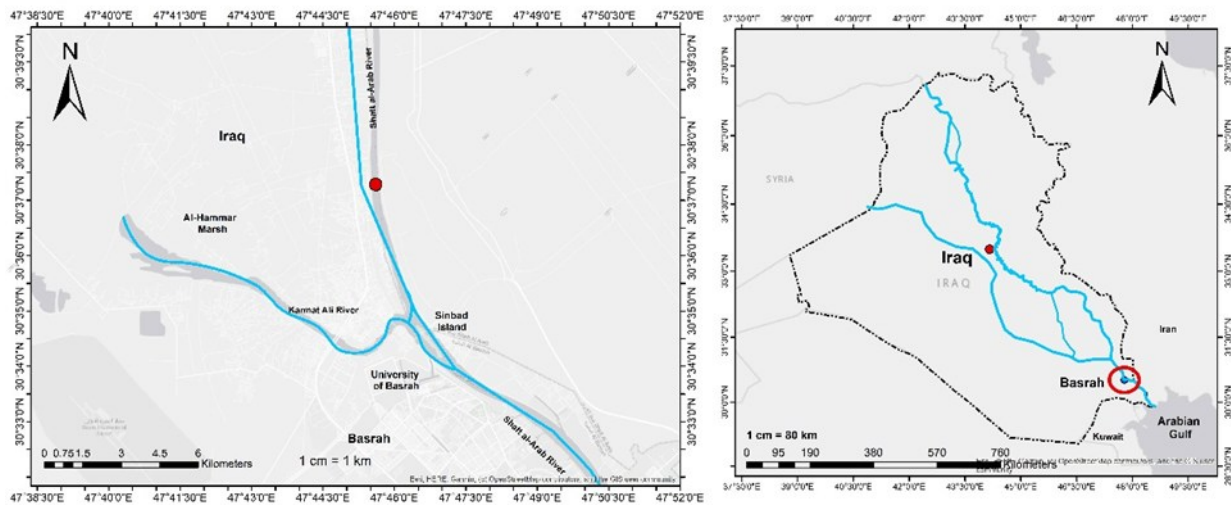
2023. The snails were located in submerged plants of reeds, which were tall, wetland grass-like plants, and Typha, a genus of cattails or bulrushes.

**Experiment**

The experiment included three additional sets, in addition to a control set (Set I), each consisting of 15 snails. Samples of digestive gland (midgut gland or hepatopancreas) were dissected from Set I immediately; individuals of this control set are not subjected to humidity or dryness, and kept under freezing till use. As for other snails, individuals of each set were kept for 6 days in a plastic container with a dimension of length (14 cm), width (10 cm) and height (6 cm), lined with tissue paper. The sets were distributed into three experimental Sets: Set II or (HH), subjected to conditions of high constant humidity; Set III or (DH), subjected to a short dryness condition for 3 days followed by 3 days of humid conditions; Set IV or (DD), subjected to conditions of constant dryness. The containers were kept at room temperature. To achieve the desired humidity condition, containers were sprayed with water periodically. As for dryness condition, containers left with no water spray (Sulikowska-Drozd and Maltz, 2014). The containers were kept in the laboratory under natural lighting. Lettuce, as small pieces, were added as a food. After 6 days, all snails were dissected to collect samples of the digestive gland. Each sample included the digestive glands of three *P. canaliculata*, which were kept frozen until use.

**Antioxidant enzymes measurement**

To measure the activity of SOD, CAT, and GST enzymes, the method of Aouini *et al.* (2019) was used with minor modifications. Samples of the frozen tissue were homogenized (1: 3 w/v) in a (10) mM of phosphate buffer solution (pH 7.4) using glass homogenizer,



**Fig 1.** Showing study site: Garmat Ali, Shatt Al Arab River, Basrah, Iraq

Kimble-Sigma Aldrich. The buffer consisted from (150) mM KCl, (1) mM EDTA, (250) mM sucrose, 0.5% Triton™ X-100, (1) mM DTT and supplemented with (1) µl/mL of Protease Inhibitor (Thermo Fisher). Then centrifuge (4 °C) was done for a 5 min at (14000 x g) and supernatant was collected and kept frozen till using for determination of antioxidants enzymes activities. As for SOD, the enzyme activity measured by using SOD Activity Assay Kit from Merck KGaA-Sigma Aldrich, Germany. While the activities of CAT and GST were assayed using Kits provided by Elabscience, USA. To read the values of absorbance, a microplate reader from Hangzhou Allsheng Instruments Co., Ltd., China, was used.

**Oxidative damage measurement**

To evaluate the level of oxidative damage (lipid peroxidation), a Thiobarbituric Acid Reactive Substances (TBARS) parameter assay kit from Biotechne R&D System, Inc., China, was used. Instructions from the manufacturer were applied to the samples of the frozen supernatants, and the absorbance was read using a microplate reader.

**Statistical analysis**

Software of SPSS Statistics 27 was used to analyses all the data. Comparison of different parameters were evaluated by one-way ANOVA. Least significant difference (LSD) test used to determine the post hoc comparisons. Significant difference was identified at the probabilities < 0.05. Values are expressed as mean ± SEM.

**RESULTS AND DISCUSSION**

After laboratory experiment is over (6 days), all the parameters, SOD, CAT, GST and TBARS, in the digestive glands of the snail in the all sets of the experiment were evaluated. After 6 days of exposure to high, constant humidity, the results revealed no significant differences (p > 0.05) in the activity of SOD, CAT, and GST between the control set and the HH set. Moreover, no

significant differences in oxidative damage are noted between the control set and the HH sets. In contrast, after being subjected to dryness for different periods, increases in the activity of SOD, CAT, and GST (P < 0.05) were observed in the DH and DD sets compared to the control. While the DD set gave the highest increasing (p < 0.05) in activity of the studied antioxidant enzymes (Table 1). As for oxidative damage, an elevation (P < 0.05) in the TBARS existed in the DH and DD sets, and the greatest elevation was in the DD set compared to the control (Table 1).

Many studies were used snails as a biomarker for pollution in the Iraqi waters system (Karim *et al.*, 2011; Salih *et al.*, 2021; Karim, 2022). At the same time, studies by Aldoghachi and Alabdul Aziz (2023) and Aldoghachi *et al.* (2024) used fishes as a biomarker for water quality. However, using snails from the Iraqi waters system as a biomarker for assessing the effect of dryness has not been studied. This study aims to achieve that. The River of Shatt Al Arab undergoing from hydrological dryness results from an increase in the high temperatures, rates of evaporation, wind strength in synchronism with shortages of rain and dampness as well as escalating of water requirements in the basin of Tigris Revier as a result of water shortages and retrogradation its quality (Abdaki *et al.*, 2021; Kadhim, 2023; Al-Mahmood and Al-Rashed, 2024). And, depending on the mentioned studies, the animal in this study undergoes from hydrological dryness. As is known, snails, including *P. canaliculata*, inhabit the near-shore zone of the water ecosystem, which were strongly influenced by fluctuations in water levels. These changes in water levels, as well as seasonal variations, morphometry of the basin, and climate change, strongly increase the intensity and frequency of dryness in the river basin, reflecting their effects on communities of invertebrates located near the shore zone (Poznanska *et al.*, 2015; Schweizer *et al.*, 2019). The present findings of this study showed that the antioxidant enzymes and oxidative stress in the digestive glands of *P. canaliculata* do not affect them under a condition of high, constant humidity for six days (Set II, HH), and the values of the

**Table 1.** Effect different periods of dryness on the activity of antioxidant enzymes and oxidative damage, represented by TBARS, in the digestive glands of the snail *Pomacea canaliculata*

Enzymes	Set I (Control)	Set II (HH)	Set III (DH)	Set IV (DD)
SOD (U/ mg)	13 ± 1.4	11 ± 1.3	19 ± 3.2*	31 ± 4.8*
CAT (U/ mg)	85 ± 15.3	103 ± 20.2	219 ± 38.9*	383 ± 76.8*
GST (mU/ mg)	246 ± 40.1	223 ± 35.1	914 ± 102.2*	1812 ± 198.9*
TBARS (nmol/g)	7 ± 1.1	8 ± 1.4	17 ± 1.2*	24 ± 2.1*

SOD: Superoxide dismutase. CAT: Catalase. GST: Glutathione S-Transferase. TBARS: Thiobarbituric Acid Reactants. HH: 6 days under high constant humidity. DH: 3 days under dryness condition followed by 3 days of humid condition. DD: 6 days under constant drought. Values are shown as mean ± SEM (N = 15 per set); Significant differences with control indicated by asterisks

parameters stay within normal ranges compared to the control. However, when the animal was subjected to the fluctuating period of dryness (Set III, DH), the antioxidant enzymes and oxidative stress were affected and fluctuated as an attempt by the animal to adapt to the harsh circumstances. And this is clearly evident when the animal is subjected to six days of constant dryness (Set IV, DD). The antioxidant enzymes, SOD, CAT and GST, recorded the highest values (31 U/ mg, 383 U/ mg and 1812 mU/ mg, respectively) compared to the control while the TBARS reached to (24 nmol/ g) as an indicator of increased lipid peroxidation and oxidative stress resulted from the harsh dryness condition. Whereas the elevation of antioxidant enzymes reflected an induction of physiological strategies that protect the animal from death. Many studies worldwide have been conducted on the assessment of dryness, as well as conditions related to dryness, on the antioxidant enzymes and oxidative stress in the snail *P. canaliculata*, whose results support the current study. Studies by Xu *et al.* (2014, 2019) mentioned the elevation of oxidative stress and antioxidant enzymes when *P. canaliculata*, collected from the field of *Zizania latifolia* in Yuyao City, China, was subjected to low or high temperatures, which caused an enhancement of POS. Also, other study supported finding of this study, it showed that when the hibernating period of *P. canaliculate*, from ponds of South China Agriculture University, extended to 120 days during the winter, the content of bound water and glycerol dropped by more than half while the oxidative stress in the digestive glands was increased accompanied by an elevate in the activity of the antioxidant enzymes to counteract that rising in the oxidative stress (Yao *et al.* 2024). Moreover, Giraud-Billoud *et al.* (2022) reached similar results to our study, finding that expanding the period of estivation in *P. canaliculata* causes an increase in the expression of tissue FOXO3, resulting in an increase in the synthesis of antioxidant enzymes to diminish the effects of oxidative stress. Furthermore, the study of Rodriguez *et al.* (2023) also supported the current study. They implied an increase in the antioxidant enzymes in the digestive glands of *P. canaliculata* from the laboratory Rosedal strain during its hibernation period. No study comes with findings different from those of the present study. Also, no previous study in Iraq has assessed the effect of dryness on antioxidant enzymes and oxidative stress using the snail *P. canaliculate*.

## Conclusion

In conclusion, it was helpful to use the snail *P. canaliculata* from the Shatt Al Arab River for the first time to assess the effects of dry conditions. An increase in oxidative stress of the digestive glands occurred when the animal was exposed to fluctuating periods of dryness,

as indicated by elevated TBARS levels (the highest being 24 nmol/g). This has induced the POS strategy to counteract the effects of ROS and reduce macromolecule damage by increasing the activity of antioxidant enzymes, SOD, CAT, and GST (with the highest values of 31 U/mg, 383 U/mg, and 1812 mU/mg, respectively).

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## Conflict of interest

The authors declare that they have no conflict of interest.

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