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EVALUATION OF OXIDATIVE STRESS BIOMARKERS IN POMACEA CANALICULATA (APPLE SNAIL, GASTROPODA: AMPULLARIIDAE) EXPOSED TO CRYSTAL VIOLET

Sangeeta Sinha[1] and Atmaram Vitthal Andhale[2*]

[1]Department of Zoology, MES's Nowrosjee Wadia College of Arts and Science, Pune, affiliated to Savitribai Phule Pune University, Pune, Maharashtra, India

[2]Department of Zoology and Biotechnology, MES's Nowrosjee Wadia College of Arts and Science, Pune, affiliated to Savitribai Phule Pune University, Pune, Maharashtra, India

atmabiotech@gmail.com*

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ABSTRACT

The apple snail (Pomacea canaliculata), a prominent gastropod in tropical regions of Asia and Africa, has emerged as a valuable model organism in ecotoxicology. Its sensitivity to environmental stressors makes it an effective bioindicator for assessing ecosystem health. Dye pollution poses a serious threat to aquatic ecosystems. Crystal Violet (CV), a ubiquitous biological dye, presents a significant risk to the aquatic environment. This study aimed to evaluate the acute toxicity of CV on apple snails and assess the associated oxidative stress response. Acute toxicity tests were conducted to determine the LC_{50} of CV. Oxidative stress parameters, including lipid peroxidation, protein oxidation (carbonyls and sulphydryls), SOD activity, and Catalase activity, were measured in snails exposed to different CV concentrations. LC_{50} of 1.15 ppm CV was observed after 96 hours. Exposure to CV significantly increased lipid peroxidation and protein carbonyls. It also depleted sulfhydryls and altered the activities of antioxidant enzymes. Behavioural abnormalities, such as excessive mucus secretion and abnormal movement, were also observed. These findings demonstrate that CV exposure induces oxidative stress and causes significant harm to apple snails. The release of untreated effluents containing CV into aquatic environments can have detrimental consequences for the health of aquatic organisms and disrupt the ecological balance.

Keywords: *Pomacea canaliculata, Crystal violet, Antioxidant enzymes, Lipid peroxidation, LC_{50}, oxidative stress*

1. INTRODUCTION

Environmental pollution is widely acknowledged as one of the most significant problems that cities face. Heavy metals may be very reactive and, thus, toxic to most living organisms, depending on their degree of oxidation. They originate from an increasing variety of man-made sources, such as industrial processes, transportation, smelting, burning fossil fuels, and certain agricultural practices, suggesting that metal pollution is becoming a bigger problem Hama *et al* [15]. They can persist in soil for long periods and may continue to have detrimental effects on the ecosystem and human health even after the pollution source has ceased operations Briffa *et al* [7].

The generation of reactive oxygen species (ROS) in biological systems, which impact several cellular functions, primarily the membrane system's and are linked to the harmful effects of heavy metals, Santos *et al* [39] and Mani *et al* [24]. Oxidative stress is a common reaction to toxicity brought on by a variety of pollutants. The use of oxidative stress biomarkers may be useful for evaluating the effects of pollutants or seasonal fluctuation in animals Kovacikova *et al* [20] and Migaszewski *et al* [27]**.** Numerous animal species have been seen to exhibit alterations in the antioxidant defence systems in response to various stimuli other than chemical contaminants (heavy metals). They include the physiological stress of freezing Karam *et al* [18] and Lushchak *et al* [23], aestivation Nowakowska *et al* [31], anoxia Joanisse *et al* [17], seasonal changes Valavanidis *et al* [47], long heat stress Mani *et al* [24] and heavy metals Hermes *et al* [16].

According to Regoli *et al* [35], the ecotoxicological reaction of an organism to its surroundings is significantly influenced by the interaction between heavy metals and the elements of its antioxidant defence systems. Crystal violet, a triphenylmethane dye widely employed in laboratories which may negatively impact aquatic life due to its toxicity, persistence, and potential carcinogenic effects, regardless of low concentrations Sousa *et al* [43].

Understanding how a species reacts to environmental toxins is crucial for both its conservation and environmental protection Paital *et al* [32]. Recent research has focused on studying the physiological changes, such as alterations in biochemical or enzymatic activity, that occur in ectothermic invertebrates due to fluctuating environmental and toxicity conditions Maruni *et al* [26] and Stankovic *et al* [44].

Although the toxicological effects of crystal violet have been examined in fish Kovacikova *et al* [20], crustaceans Sun *et al* [45], Sea turtle Morão *et al* [28] and microbial systems Mansoor *et al* [24], research on CV impact on *Pomacea canaliculate,* notwithstanding their recognized sensitivity as bioindicators of environmental pollution, is notably lacking. In particular, behavioral responses, lethal thresholds, and physiological or biochemical alterations, including antioxidant defense alterations under crystal violet stress, remain poorly understood. Addressing this gap is essential to establishing *P. canaliculata* as a bioindicator species for monitoring dye-induced aquatic toxicity. The objective of this study was to assess the harmful effects of crystal violet at varying doses on the mollusc *Pomacea canaliculata*. To further understand the species' detoxification processes and provide insight into its invasion success under environmental stress, bioaccumulation and antioxidant enzyme activities were assessed. To investigate the relationship between the snails' metabolic reactions and their exposure to crystal violet, statistical studies were conducted.

2. MATERIAL AND METHODS

2.1 Experimental animals and culture:

Adults of the invasive *P. canaliculata* were procured from Deeshrimps Company, Nallasopara, Maharashtra, during the Rainy Season 2022. The age of snails was determined by counting the rings on their operculum Bökenhans *et al* [6]. The snails of similar size and age group were selected and acclimated to laboratory conditions for 7 days before experiments. The animals were kept in glass aquaria at ambient room temperature (25 ± 2 °C) and fed daily with lettuce leaves during the acclimation and experimental period. Animals with an average wet weight of 4.50 ± 0.12 mg and shell length of 25.12 ± 0.25 mm were selected to perform acute toxicity tests and bioaccumulation studies. The physical-chemical properties of the test water were established according to Cresswell *et al* [8] and Reátegui- Zirena *et al* [34] and used for culturing and all subsequent experiments.

2.2 Acute Toxicity Study

Crystal Violet (CV) [Hexamethyl pararosaniline chloride] was purchased from Glaxo Laboratories, India. Toxicity tests were performed in which groups of snails were exposed to 0.5 ppm, 1 ppm, 2 ppm, 5ppm, and 10 ppm crystal violet for 96 h. Twenty snails of each group were transferred to each aquarium containing 5 L of test solution and were not fed during exposure. For each species and each concentration, the death was checked at 24, 48, 72, and 96 h, respectively. Dead snails were removed immediately to avoid secondary effects on living snails in the same tank. All experiments were carried out under a water temperature of 25 ± 2 °C and 16-h:8-h light: dark photoperiodic conditions. The test procedures were carried out according to the modified methods of Ng *et al* [30]. Mortality data were expressed as percentages (dead/total × 100). Abbott's correction was not required since no mortality occurred in the control. Median lethal concentration (LC_{50}) was estimated by linear interpolation between doses bracketing 50% mortality (1 and 2 ppm). To provide confidence intervals, a non-parametric bootstrap (10,000 resamples of replicates at 1 and 2 ppm) was applied, and the 2.5th and 97.5th percentiles were taken as the 95% CI.

Tissue samples from the gill, lung, and digestive gland (midgut gland or hepatopancreas) were dissected and stored at −20°C until use.

2.3 Quantifying Oxidative Damage to Biomolecules

2.3.1 Damage to Lipids

Thiobarbituric acid reactive substances (TBARS) were quantified as an index of lipid peroxidation. Tissue samples (~100 mg) were homogenized in 900 µl of 0.1 M sodium phosphate buffer (pH 7.0) and centrifuged (10,000 rpm, 10 min), and the supernatants were kept frozen until TBARS quantification. Oxidative damage to

lipids in apple snails induced by crystal violet at different concentrations was measured in terms of nmol of malondialdehyde equivalents formed Li *et al* [21] and expressed as nmol of TBARS/100 mg tissue.

2.3.2 Measurement of Antioxidant Enzymes

Catalase (CAT, *EC 1.11.1.6)* activity was determined by the method Aebi [1]. Superoxide dismutase (SOD, *EC 1.15.1.1)* was assayed by Beauchamp, C., & Fridovich, I. [5]. Enzyme activity was expressed as U/mg.

2.3.3 Damage to Proteins

Oxidative damage to protein carbonyls is based on the reaction of carbonyl groups with 2, 4-dinitrophenylhydrazine (DNPH) to form a 2,4-dinitrophenylhydrazone, which can be measured at 366 nm. Amount of carbonyls formed was expressed as nmol of protein carbonyls formed/100 mg tissue and protein sulphydryls were quantified using Ellman's reagent (5,5-dithiobis-2-nitrobenzoic acid) and expressed as nmol of protein sulphydryls/100 mg tissue, Ellman [12].

2.3.4 Measurement of protein

Protein was estimated following the method of Lowry [22]. All assays were replicated five times, and the values provided are the averages of these replicates.

2.3.5 Statistical analysis

Mortality differences between treatments and control were assessed using a chi-square test of independence, with $p < 0.05$ considered significant. One-way ANOVA revealed a significant effect of CV concentration on mortality ($F = 915.4$, $p < 0.001$). Pairwise comparisons showed that all tested concentrations caused significantly higher mortality than the control ($p < 0.001$). Differences between treatments of different exposure times and crystal violet concentrations were statistically analyzed by two-way analysis of variance (ANOVA) and LSD multiple comparisons at a 0.05 probability level using SPSS 18.0. An independent sample t-test also determined the comparison between snail species under each treatment. The data were presented as mean ± S.D. of samples at least five times. Differences were considered significant at $p< 0.001$ and $p< 0.05$.

3. OBSERVATIONS AND RESULTS

3.1 Behavioral Responses

Inhibition of *Pomacea canaliculata* by different concentrations of crystal violet (CV) resulted in instantaneous behavioral changes. In minutes, snails displayed stress signals such as efforts to escape from the exposure medium. After 24 hours, organisms in more concentrated solutions (5ppm and 10 ppm) of CV were immobilized in a typical knock-down position, with observable black and white esidues deposited in the surrounding medium.

3.2 Lethal Dose Assessment

Exposure of *P. canaliculata* to increasing concentrations of crystal violet (0.5–10 ppm) resulted in a clear, concentration-dependent increase in mortality. Mean mortality at 0.5, 1, 2, 5, and 10 ppm was 27%, 46%, 63%, 85%, and 100%, respectively, compared with 0% in the control group (Table 1). The median lethal concentration (LC_{50}) was estimated by interpolation between 1 and 2 ppm. The calculated LC_{50} was 1.15 ppm (95% CI: 1.00–1.44 ppm). Mortality rates across treatments were significantly different from the control (χ^2 test, $p < 0.001$). The value of LD_{50} was significantly different from the control group ($p < 0.001$), which reiterates the very high toxicity of CV against *P. canaliculata* (Fig. 1). One-way ANOVA confirmed a highly significant overall effect of concentration on mortality ($F = 915.4$, $p < 0.001$). Pairwise comparisons with the control indicated that even the lowest tested concentration (0.5 ppm) caused a significant increase in mortality ($p < 0.001$). Mortality at higher concentrations was also significantly greater than control: 1 ppm ($p < 0.001$), 2 ppm ($p < 0.001$), 5 ppm ($p < 0.001$), and 10 ppm ($p < 0.001$).

Table 1: Concentration-dependent mortality of *Pomacea canaliculata* exposed to crystal violet.

Different Concentrations (CV)	No. of Snails	Set 1	Set 2	Set 3	Set 4	Set 5	Mean	Percent Mortality
Control	20	0	0	0	0	0	0 ± 0	0
0.5 ppm	20	5	5	6	5	6	5.4 ± 0.5*	27
1 ppm	20	10	8	9	9	10	9.2 ± 0.7*	46
2 ppm	20	12	13	13	12	13	12.6 ± 0.5**	63
5 ppm	20	17	16	17	17	18	17 ± 0.6**	85
10 ppm	20	20	20	20	20	20	20 ± 0**	100

Table 1: Mortality (mean ± SD, n = 5 replicates of 20 snails each) recorded after 96 h exposure to increasing concentrations of crystal violet (CV). Percent mortality was calculated relative to the control group. $^{*}p < 0.05$ and $^{**}p < 0.001$ compared to control. Data are expressed as mean ± SD of five replicates (n = 20) snails per group.

Figure 1: Concentration-dependent Mortality of Snails Exposed to Crystal Violet (CV):

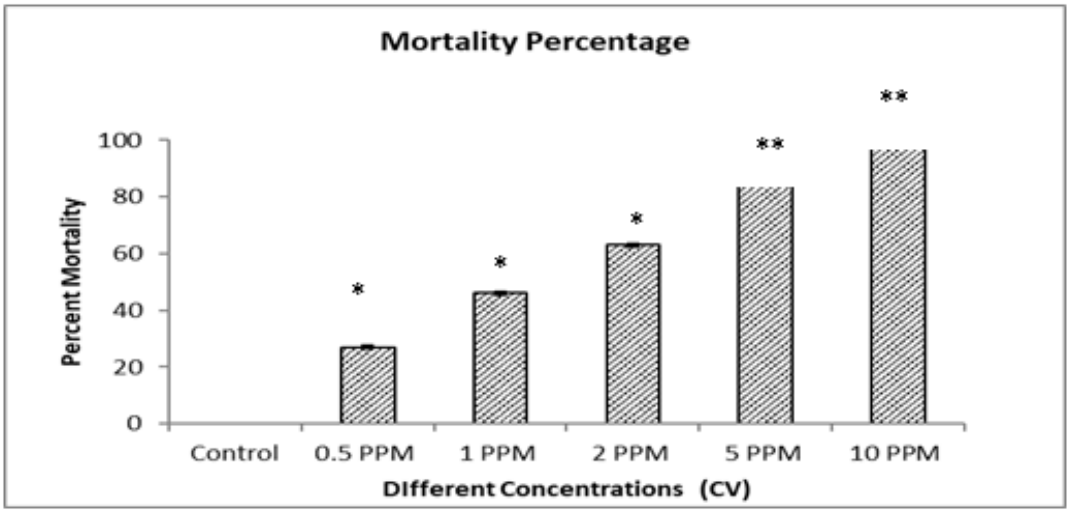

Figure 1: Concentration-dependent Mortality of Snails Exposed to Crystal Violet (CV): Percentage mortality of snails exposed to different concentrations of crystal violet (CV). Data are expressed as mean values from five replicates (n = 20 snails per group).

3.3 Determination of MDA Levels

Exposure of snails to increasing concentrations of the chemical stressor (CV) led to a dose-dependent rise in lipid peroxidation (MDA levels) across all tissues compared to the control (Fig. 2). At the lowest exposure (0.5 PPM), changes were minimal (Digestive gland: 1.1-fold; Gills: 1.0-fold; Lungs: 1.1-fold; Kidney: 1.25-fold). With increasing concentrations, the magnitude of change became more pronounced. At 2 ppm, lipid peroxidation rose sharply (Digestive gland: 2.0-fold; Gills: 2.1-fold; Lungs: 2.2-fold; Kidney: 3.5-fold). The strongest induction was observed at 10 ppm, where the digestive glands exhibited the highest fold increase (8.7-fold; 9.26 ± 0.4 nM), followed by gills (7.5-fold; 6.6 ± 0.2 nM), kidney (5.8-fold; 4.5 ± 0.23 nM), and lungs (4.5-fold; 3.66 ± 0.23 nM) compared to control. Statistical analysis (t-test) confirmed that values from 10 ppm onwards were significantly higher than controls ($p < 0.01$), indicating a strong oxidative stress response.

Figure 2: Tissue-specific lipid peroxidation (MDA levels) in snails under chemical stress

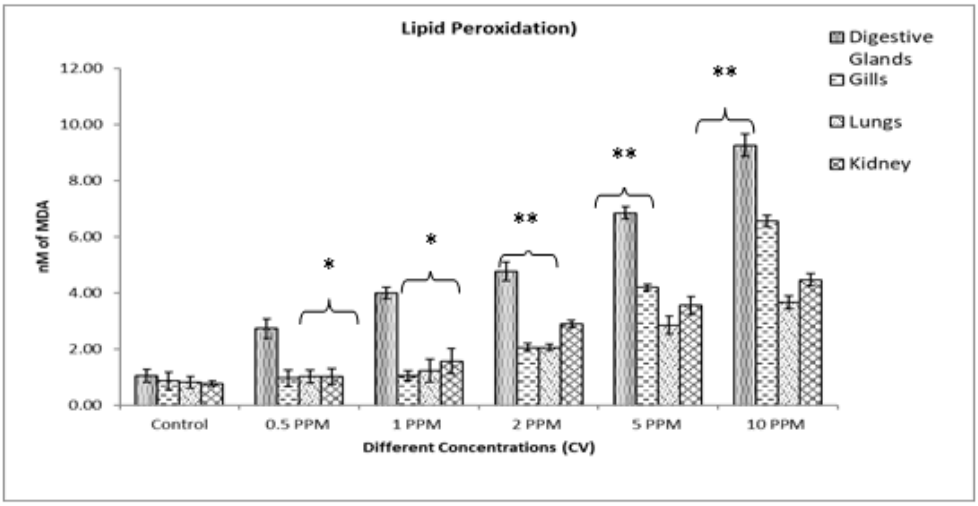

Fig. 2: Lipid peroxidation measured as malondialdehyde (MDA, nM) in digestive glands, gills, lungs, and kidneys of snails exposed to increasing concentrations of chemical stressor (CV: 0.5–10 PPM). $^{*}p < 0.05$ and $^{**}p < 0.001$ compared to control. Data are expressed as mean ± SD of five replicates (n = 20 snails per group.

3.4 Antioxidant Enzyme Activity

The activity of superoxide dismutase (SOD) in snails exposed to different concentrations of crystal violet (CV) showed a significant, concentration-dependent increase compared to the control (Table 2). The results demonstrate that exposure to increasing concentrations of the chemical stressor induced a highly significant rise in superoxide dismutase (SOD) activity across all examined tissues when compared to controls ($p < 0.001$). The digestive gland (hepatopancreas) showed the highest absolute enzyme activity (246 ± 43 U/mg) at 10 ppm, with fold increases ranging two folds (39 ± 8 U/mg) at 0.5 ppm to 12-fold at 10 ppm compared to control (21.4 ± 2.3 U/mg), consistent with its central role in detoxification metabolism. The gills also exhibited a strong induction of six-fold (112.4 ± 22.5 U/mg) at higher concentration (10 ppm) compared to control (18.9 ± 5.4 U/mg), reflecting their direct contact with CV, while the lungs and kidney displayed the smallest relative increases up to five-fold, despite starting from lower baseline levels (Fig. 3).

Figure 3: Tissue-specific Catalase (SOD) activity in snails under chemical stress

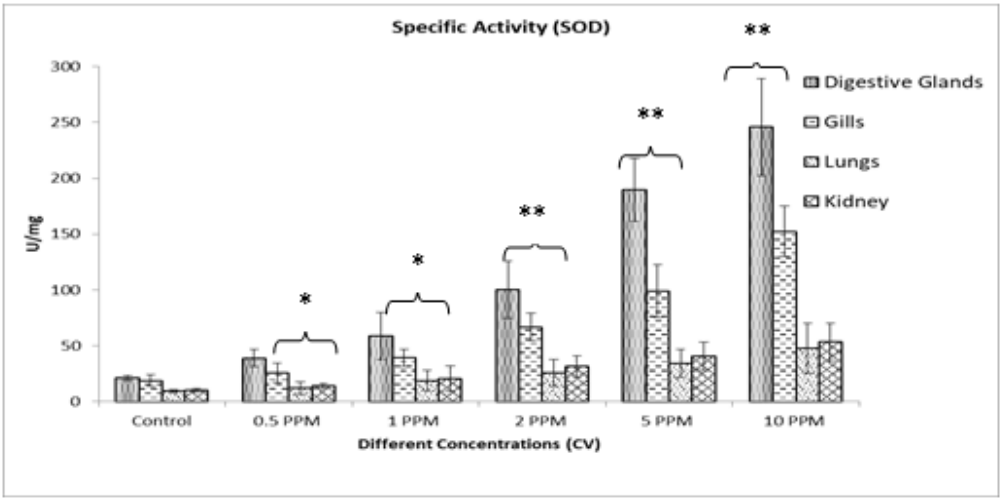

Fig. 3: Superoxide Dismutase (SOD) specific activity (U/mg protein) measured in digestive glands, gills, lungs, and kidneys of snails exposed to different concentrations of chemical stressor (CV: 0.5–10 PPM). $^{*}p < 0.05$ and $^{**}p < 0.001$ compared to control. Data are expressed as mean ± SD of five replicates (n = 20) snails per group

Catalase (CAT) activity across snail tissues under chemical stress showed statistically significant increases ($p < 0.001$) relative to the control group at all tested concentrations (Table 2). In the digestive gland, CAT activity rose progressively, reaching an 8.6-fold increase at 10 ppm (200.5 ± 22.8 U/mg), compared to the control (23.1 ± 3.4 U/mg). Similarly, gills exhibited strong induction, with activity elevated 7.7-fold at 10 ppm (134.5 ± 25.9 U/mg), while the kidney (57.3 ± 12.9 U/mg) and lungs (74.3 ± 21.5 U/mg) displayed approx. 6-fold increase at the same concentration (Fig. 4). Even at lower concentrations (0.5–1 ppm), significant elevations were evident, particularly in the gills (up to 2.6-fold at 1 ppm) and digestive gland (2.5-fold at 1 ppm). Overall, the digestive gland showed the highest magnitude of CAT induction, followed closely by gills, whereas lungs and kidneys responded more moderately.

Figure 4: Tissue-specific Catalase (CAT) activity in snails under chemical stress

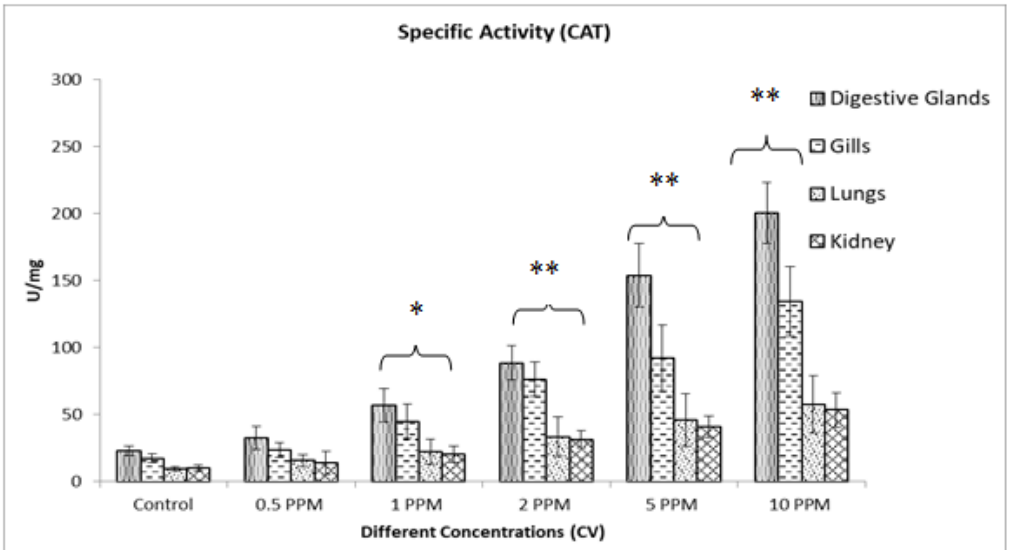

Fig. 4: Catalase (CAT) specific activity (U/mg protein) measured in digestive glands, gills, lungs, and kidneys of snails exposed to different concentrations of chemical stressor (CV: 0.5–10 PPM). $^{*}p < 0.05$ and $^{**}p < 0.001$ compared to control. Data are expressed as mean ± SD of five replicates (n = 20 snails per group).

Exposure to increasing concentrations of CV induced a clear dose-dependent elevation of SOD and CAT activities across all tissues, with the digestive gland showing the strongest response (Table 2). At 10 PPM, digestive gland SOD increased nearly 12-fold (246 U/mg) compared to control (21 U/mg), while CAT rose almost 9-fold (201 U/mg vs. 23 U/mg). Gills also exhibited strong induction, with 8-fold increases in SOD and CAT. Kidney responses were moderate, with SOD rising 5-fold and CAT 6-fold at the highest concentration, whereas lungs showed the least but still significant increase (4.8-fold for SOD and 5.7-fold for CAT).

Table 2: Superoxide Dismutase (SOD) and Catalase (CAT) activities in snail tissues under Chemical stress (CV exposure)

Different Concentrations (CV)	Digestive Gland		Gill		Lung		Kidney	
	SOD	CAT	SOD	CAT	SOD	CAT	SOD	CAT
Control	21 ± 2	23 ± 3	19 ± 5	17 ± 3	10 ± 1	13 ± 2	10 ± 2	9 ± 2
0.5 ppm	39 ± 8	32 ± 9	26 ± 9	23 ± 5	12 ± 6	17 ± 4	14 ± 2	16 ± 9
1 ppm	59 ± 21*	57 ±12*	39 ± 8*	45 ± 13*	19 ± 9*	22 ± 10*	20 ± 11*	22 ± 6*

2 ppm	100 ± 26**	88 ± 13**	67 ± 12**	76 ± 13**	26 ± 12**	35 ± 15**	32 ± 9**	33 ± 6**
5 ppm	189 ± 28**	154 ± 24**	99 ± 24**	92 ± 25**	34 ± 13**	65 ± 20**	41 ± 13**	46 ± 8**
10 ppm	246 ± 43**	201 ± 23**	152 ± 23**	135 ± 26**	48 ± 22**	74 ± 22**	53 ± 17**	57 ± 13**

Table 2: Enzymatic activities of superoxide dismutase (SOD, U/mg protein) and catalase (CAT, U/mg protein) in digestive gland, gill, lung, and kidney tissues of snails exposed to increasing concentrations of chemical stressor (CV: 0.5–10 PPM). $^{*}p < 0.05$ and $^{**}p < 0.001$ compared to the control. Data are expressed as mean ± SD of five replicates (n = 20 snails per group).

3.5 Measurement of Protein Damage

Protein carbonyl (PC) levels increased in all tissues in a concentration-dependent manner compared to controls (Fig. 5). In the Digestive gland PC rose from 1.9 ± 0.2 (control) to 12.9 ± 2.9 at 10 ppm, showing a 6.8-fold increase with strong significance ($p<0.05$ across all treatments). In gills, the levels increased from 1.6 ± 0.1 to 10.9 ± 1.3 at 10 ppm, a 6.8-fold rise, significant from 0.5 ppm onwards ($p<0.05$, $p=0.0002$ at 10 ppm). In Lungs, PC value was significantly increased from 1.3 ± 0.1 (control) to 8.9 ± 1.7 at 10 ppm, showing 6.8-fold elevation, with significance observed at ≥1 ppm ($p=0.0221$–0.0015). In kidneys, the levels increased moderately, from 1.4 ± 0.2 to 6.5 ± 0.7 at 10 ppm, a 4.6-fold rise, with significant differences emerging at ≥1 ppm ($p=0.0182$–0.0003).

Figure 5: Protein Carbonyl Levels in Different Tissues of Snails Exposed to Varying Concentrations of CV

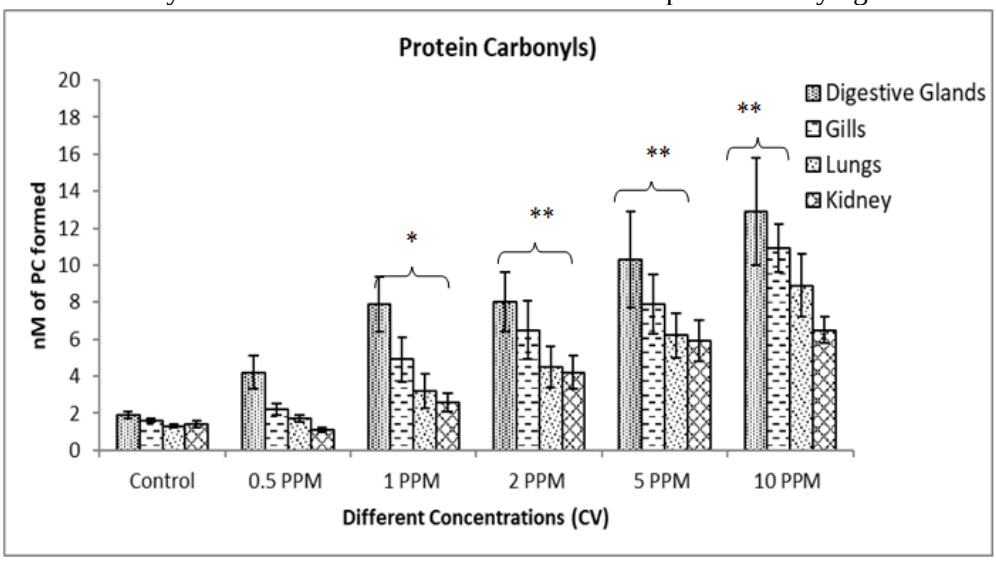

Fig.5: Values represent mean ± SD (nM PC) of protein carbonyls in snail digestive gland, gills, lungs, and kidneys under increasing concentrations of CV (0.5–10 PPM). Statistical significance was determined against control values (n=20), with $^{*}p < 0.05$ and $^{**}p < 0.001$.

Protein sulfhydryl (PS) levels across different tissues under stress revealed a progressive and statistically significant decline compared to the control group. In the digestive gland, PS dropped from 7.67 ± 1.4 nM in controls to 0.56 ± 0.1 nM at 10 ppm ($p < 0.05$ from 1 ppm onward), showing a marked reduction consistent with oxidative modification. In the gills, values decreased from 5.1 ± 0.5 nM to 0.3 ± 0.1 nM at 10 ppm, with significant differences observed even at low exposure ($p = 0.021$ at 0.5 ppm and < 0.01 from 1 ppm onward). Similarly, lung tissues exhibited a decline from 4.6 ± 0.6 nM in controls to 0.7 ± 0.1 nM at 10 PPM, reaching statistical significance at 0.5 ppm ($p = 0.037$) and becoming highly significant at higher doses ($p < 0.01$). The kidney showed a comparable trend, with PS decreasing from 5.2 ± 0.3 nM to 0.9 ± 0.1 nM, statistically significant from 1 ppm onwards ($p = 0.001$). These results clearly demonstrate tissue-specific oxidative stress, with gills and lungs being more sensitive at lower doses, while the digestive gland and kidney show pronounced depletion at higher concentrations (Fig. 6).

Figure 6: Protein Sulphydryl Content in Snail Tissues under Different Concentrations of CV Exposure.

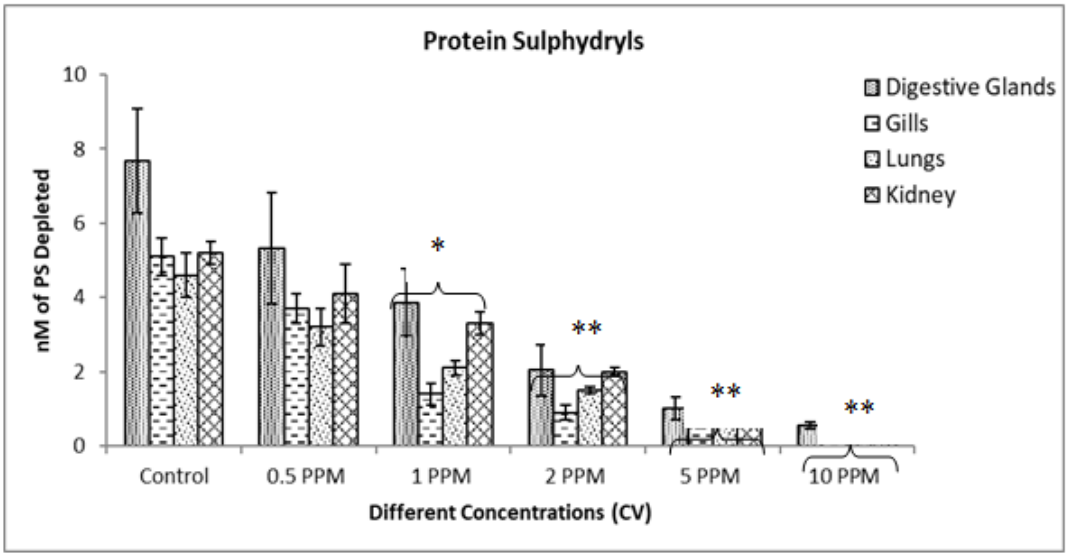

Fig 6: Values represent mean ± SD (nM PS) in digestive gland, gills, lung, and kidney tissues of snails exposed to increasing concentrations of CV. Significant decreases compared to control are indicated by p-values, $^{*}p < 0.05$ and $^{**}p < 0.001$.

Table 3 shows that protein carbonyl and sulphydryl levels in different snail tissues revealed statistically significant alterations relative to controls. In the digestive gland, protein carbonyls increased progressively with CV exposure, rising from 1.9 ± 0.2 nM in controls to 12.9 ± 2.9 nM at 10 ppm ($p < 0.001$), while sulphydryl levels showed a marked decline from 7.67 ± 1.4 nM to 0.56 ± 0.1 nM ($p < 0.001$), suggesting strong oxidative damage. In gills, carbonyls similarly increased from 1.6 ± 0.1 to 10.9 ± 1.3 nM ($p < 0.001$), while sulphydryls dropped significantly from 5.1 ± 0.5 to 0.3 ± 0.1 nM ($p < 0.001$), indicating protein oxidation and thiol depletion. Lung tissue also showed a steady rise in carbonyls from 1.3 ± 0.1 to 8.9 ± 1.7 nM ($p < 0.001$), accompanied by a decrease in sulphydryls from 4.6 ± 0.6 to 0.7 ± 0.1 nM ($p < 0.001$). In the kidney, carbonyl levels increased significantly from 1.4 ± 0.2 to 6.5 ± 0.7 nM ($p < 0.001$), whereas sulphydryls declined from 5.2 ± 0.3 to 0.9 ± 0.1 nM ($p < 0.001$)

Table 3: Protein carbonyl and sulphydryl content (nM ± SD) in different tissues of snails exposed to varying CV concentrations.

Different Conc. (CV)	Digestive Gland		Gills		Lung		Kidney	
	Carbonyl	Suplhydryl	Carbonyl	Suplhydryl	Carbonyl	Suplhydryl	Carbonyl	Suplhydryl
Control	1.9 ± 0.2	7.7 ± 1.4	1.6 ± 0.1	5.1 ± 0.5	1.3 ± 0.1	4.6 ± 0.6	1.4 ± 0.2	5.2 ± 0.3
0.5 ppm	4.2 ± 0.9	5.3 ± 1.5	2.2 0.3	3.7 ± 0.4	1.7 ± 0.2	3.2 ± 0.5	1.1 ± 0.1	4.1 ± 0.8
1 ppm	7.9 ± 1.5*	3.9 ± 0.9*	4.9 ± 1.2*	1.4 ± 0.3*	3.2 ± 0.9*	2.1 ± 0.2*	2.6 ± 0.5*	3.3 ± 0.3*
2ppm	8.0 ± 1.6**	2.0 ± 0.7**	6.5 ± 1.6**	0.9 ± 0.2**	4.5 ± 1.1**	1.5 ± 0.1**	4.2 ± 0.9**	2 ± 0.1**
5 ppm	10 ± 2.6**	1.0 ± 0.3**	7.9 ± 1.6**	0.6 ± 0.1**	6.2 ± 1.2**	0.9 ± 0.1**	5.9 ± 1.1**	1.3 ± 0.1**
10 ppm	13 ± 2.9**	0.6 ± 0.1**	11 ± 1.3**	0.3 ± 0.1**	8.9 ± 1.7**	0.7 ± 0.1**	6.5 ± 0.7**	0.9 ± 0.1**

Table 3: Values represent means ± SD (n = 20). Statistical comparisons were made between exposed groups and controls using Student's t-test. $^{*}p < 0.05$ and $^{**}p < 0.001$indicates a significant difference from the control.

4. DISCUSSION

The present study demonstrates that crystal violet (CV) exerts pronounced toxicological effects on *Pomacea canaliculata*, as reflected in behavioral, biochemical, and physiological responses. Snails exposed to increasing concentrations of CV showed acute stress behaviors and concentration-dependent mortality. The estimated LC_{50}

of 1.15 ppm for crystal violet against *Pomacea canaliculata* indicates a relatively high level of acute toxicity, consistent with its known persistence and bioactive properties. The narrow 95% CI (1.00–1.44 ppm) reflects the steep mortality response between 1 and 2 ppm. Similar dose–response patterns have been reported for other synthetic dyes with insecticidal or ecotoxic effects, suggesting that crystal violet may exert toxicity through conserved cellular mechanisms Santos *et al* [39], Silva *et al* [41], and Sousa *et al* [43]. The significant increase in mortality compared with controls ($p < 0.001$) highlights its potential risk to non-target organisms in both aquatic and terrestrial ecosystems. The LD_{50} values observed in this study are consistent with earlier ecotoxicological assessments where industrial dyes induced high mortality in freshwater invertebrates Aziz *et al* [3] and Kögel *et al* [19].

Lipid peroxidation is a well-established marker of oxidative stress, reflecting the damage caused by reactive oxygen species (ROS) to polyunsaturated fatty acids in cellular membranes Halliwell & Gutteridge [14]. The lipid-peroxidation data (MDA) show a clear, dose-dependent oxidative injury across all tissues, with the digestive gland (hepatopancreas) displaying the highest absolute MDA at each concentration, and the gills and kidney showing large relative increases at ≥2 PPM. This pattern is biologically coherent for gastropods: the hepatopancreas is the principal metabolic–detoxification organ, where Phase I/II biotransformation and metal sequestration (e.g., metallothioneins) generate and cope with reactive oxygen species (ROS), so it typically exhibits both strong antioxidant responses (SOD, CAT, GST/GPx) and high oxidative damage when exposure overwhelms defenses Regoli & Giuliani [35]. Gills are in continuous contact with the dissolved toxicant and oxygen, making them a sensitive site for ROS generation through mitochondrial and membrane redox cycling; correspondingly, molluscan gills often show rapid rises in peroxidation and antioxidant enzymes under waterborne contaminants Valavanidis *et al* [47]. The kidney's marked increase in MDA at higher concentrations is consistent with its role in concentrating and excreting xenobiotics and metals, a process that can intensify Fenton-type chemistry and lipid damage when antioxidant capacity is exceeded Regoli & Giuliani [35]. The lungs show smaller absolute changes, but still a significant upward trend, indicating systemic spillover of oxidative stress. The present findings demonstrate that CV exposure induces dose-dependent lipid peroxidation in the experimental organism, as evidenced by elevated MDA levels.

SOD is a key enzymatic antioxidant responsible for the dismutation of superoxide radicals (O_2^-) into hydrogen peroxide and oxygen, thus serving as a first-line defense mechanism against reactive oxygen species (ROS) Altobelli *et al* [2]. The significant increase in SOD and CAT activity at low concentrations ($p < 0.05$) suggests an early adaptive response of the antioxidant defense system to counteract CV-induced ROS generation. However, at higher concentrations ($p < 0.001$), the sharp rise in SOD and CAT activity reflects excessive ROS production, which may overwhelm the antioxidant capacity, leading to oxidative damage. This pattern indicates that while the digestive gland is the primary site of antioxidant defense due to its detoxifying capacity, peripheral tissues such as lungs, gills, and kidneys also undergo substantial oxidative stress responses. These findings align with previous studies showing that the hepatopancreas is the most responsive organ in terms of absolute antioxidant activity Regoli & Giuliani [35] and Nasri *et al* [29], whereas gills and lungs provide sensitive biomarkers of pollutant exposure due to their rapid relative induction Giraud-Billoud *et al* [13] and Soliman *et al* [42]. Overall, the results suggest that both digestive and respiratory/excretory tissues play complementary roles in the oxidative stress response of snails, with the hepatopancreas serving as the main detoxification hub and the other organs acting as early-warning indicators of chemical stress. This is in line with reports that exposure to synthetic dyes and other environmental toxicants enhances oxidative enzyme activities in aquatic organisms Regoli & Giuliani [35]. Together, these results demonstrate tissue-specific patterns of antioxidant defense, highlighting that the digestive gland and gills are the most responsive to chemical stress in snails, with SOD and CAT activity providing a sensitive biomarker of oxidative stress exposure

Protein carbonyls, a well-known biomarker of oxidative stress, indicate oxidative protein damage. The digestive gland exhibited the greatest increase, consistent with its role as the primary detoxification organ in molluscs. Gills also showed high susceptibility, likely due to their direct exposure to environmental contaminants and high

oxygen turnover Regoli & Giuliani [35]. Lungs and kidneys displayed moderate but significant increases, suggesting systemic oxidative impacts. These findings align with previous studies reporting elevated protein oxidation in mollusks under pollutant stress Sureda *et al* [46]. Protein carbonyl accumulation reflects irreversible oxidative modifications, impairing enzyme activity and cellular homeostasis Dalle-Donne *et al* [9]. Protein sulphydryls are critical redox regulators and antioxidant buffers, particularly vulnerable to oxidative modifications under stress conditions Dalle-Donne *et al* [10]. The observed progressive depletion reflects covalent oxidation and thiol loss, a hallmark of oxidative stress caused by pollutant exposure. Previous studies in aquatic invertebrates and mollusks have similarly reported reductions in protein thiols under heavy metal or pesticide stress, linking it to impaired protein function and cellular damage Dhara *et al* [11] and Maruni *et al* [26]. The greater sensitivity of gills and lungs at early exposures may be explained by their direct interface with the environment, leading to rapid oxidative interactions, while the digestive gland and kidney accumulate oxidative burden at higher doses due to their metabolic and detoxification roles. Overall, the results support the concept that PS depletion is a reliable biomarker of oxidative protein damage in mollusks under xenobiotic stress. Overall, these results confirm that protein carbonylation and sulphydryl depletion are sensitive biomarkers of CV-induced oxidative damage in snail tissues.

These data support earlier evidence that CV causes cytotoxicity and genotoxicity in various organisms ranging from bacteria, fungi, algae, to higher animals Santos *et al* [39], Salimi *et al* [38], and Batista *et al* [4]. Interestingly, the extreme mortality and biochemical disquiet at extremely low concentrations underscore the environmental hazard of CV contamination in aquatic ecosystems. At the ecosystem level, declines in snail survival and physiological functioning could impact nutrient cycling, algal control, and trophic interactions Panda *et al* [33] and Sharan *et al* [40]. Considering that *P. canaliculata* is a globally distributed species and part of numerous freshwater food webs, the cascading ecological effects of CV exposure are justified. In addition, CV's bioaccumulative and biomagnification potential Rodrigues *et al* [36], Wang *et al* [49], and Zhang *et al* [50] extends its risk profile, as higher trophic-level predators could have even higher toxic loads.

Though this research presents strong evidence of CV toxicity, additional studies are required to elucidate the molecular pathways involved in oxidative damage, to evaluate chronic exposure impacts, and to identify possible bioremediation alternatives. Coupling ecotoxicological information with engineering solutions for wastewater treatment can represent useful ways to counteract hazards.

5. CONCLUSION

This study demonstrates that crystal violet (CV) induces severe oxidative stress and toxicity in *Pomacea canaliculata*, evidenced by mortality, lipid peroxidation, antioxidant enzyme activation, and protein damage. The digestive gland and gills were identified as the most sensitive tissues, reflecting their detoxification and environmental interfaces. Protein carbonyl accumulation and sulphydryl depletion confirmed irreversible oxidative modifications and impaired redox regulation. The low LC_{50} and strong biochemical responses highlight the ecological risk of CV even at trace concentrations. Overall, CV poses a significant hazard to aquatic ecosystems, warranting stricter regulation and remediation efforts. In conclusion, the synergy between acute toxicity, oxidative damage, and protein disruption in *P. canaliculata* underscores the necessity to assess and regulate the environmental discharge of industrial dyes under the umbrella of applied environmental engineering and sustainable ecosystem management.

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