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(RESEARCH ARTICLE)

Histological and antioxidant evaluation of an aqueous extract of hibiscus on the heart of cadmium-induced Wistar rats

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Abstract

This study investigated the protective effects of Hibiscus sabdariffa aqueous extract against cadmium-induced cardiotoxicity in Wistar rats. Twenty-four rats (150–200 g) were divided into four groups (n = 6): Group A (control), Group B (cadmium only), Group C (cadmium + hibiscus), and Group D (hibiscus only). Groups B and C received 14 mg/kg of cadmium via intraperitoneal injection, while Groups A and D were not injected with cadmium. After 72 hours, Groups C and D were administered 100 mg/kg of hibiscus extract orally for four weeks, while Groups A and B received saline as a control. Group B exhibited a significant reduction in body and relative heart weights ($p < 0.05$) compared to the control group, while Group C mitigated these effects, showing no significant difference ($p > 0.05$). Histological analysis revealed disrupted cardiomyocytes in Group B, whereas Groups A, C, and D maintained a normal cardiac structure. Periodic acid-Schiff (PAS) staining displayed abnormal reactivity in cadmium-exposed rats, while the hibiscus-treated groups exhibited normal PAS patterns. Additionally, depletion of reduced glutathione (GSH) and superoxide dismutase (SOD) was observed in the cadmium-only group, whereas the cadmium + hibiscus group showed levels comparable to the control group. In conclusion, the extract of Hibiscus sabdariffa attenuated cadmium-induced cardiotoxicity, preserving heart weight, structure, and biochemical integrity. These findings suggest its potential as a protective agent against heavy metal toxicity.

Keywords: Hibiscus; Cadmium; Reduced glutathione; Superoxide dismutase; Heart

1. Introduction

Cadmium (Cd) is a soft, silvery-white transition metal with an atomic number of 48 [1,2]. It naturally occurs in the Earth's crust at levels ranging from 0.1 to 0.5 mg/kg and is toxic to all organisms, as it has no essential metabolic function [3,4]. Due to its harmful effects on major organs, cadmium toxicity poses a global health concern, contributing to increased morbidity and mortality [5]. Cadmium pollution arises from mining, industrial processes, volcanic activity, and anthropogenic sources such as fertilisers, coal burning, batteries, and steel production [6]. Exposure occurs through contaminated food, water, smoking, and occupational hazards [7]. Once absorbed, cadmium accumulates in tissues, inducing oxidative stress and cellular damage since it cannot be metabolised [8,9]. Hibiscus sabdariffa, a flowering plant of the Malvaceae family, is valued for its medicinal properties [10]. It grows up to 2 meters tall and bears edible calyces rich in anthocyanins, flavonoids, and phenolic acids, which contribute to its therapeutic potential [11,12]. The heart, a fist-sized organ located in the chest cavity, is vital for blood circulation, maintaining blood pressure, and regulating heart rhythm [13]. Given the cardiotoxic effects of cadmium, this study investigates the protective role of Hibiscus sabdariffa aqueous extract on the hearts of cadmium-exposed Wistar rats through histological analysis.

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2. Materials and Methods

2.1. Plant Material Preparation

Fresh Hibiscus sabdariffa flowers were harvested from the Livingstone District of Zambia. The flowers were air-dried, pounded, ground into a fine powder, and then sieved for homogeneity. Aqueous extraction was performed following the method by Alexandro et al. [14].

2.2. Animals and Housing

Twenty-four male Wistar rats (150–200 g) were housed under standard laboratory conditions at the Mulungushi University Department of Anatomy. The rats were divided into four groups (n=6 per cage), with free access to food and water.

2.3. Cadmium Induction

Cadmium was dissolved in citric acid. After an overnight fasting period, Groups B and C received a single intraperitoneal dose of 14 mg/kg [15], while Groups A and D were not induced.

2.4. Experimental Groups

- Group A: Normal control (saline only)
- Group B: Cadmium-only (14 mg/kg intraperitoneal injection) [15]
- Group C: Cadmium + hibiscus extract (100 mg/kg orally)
- Group D: Hibiscus-only (100 mg/kg orally)

2.5. Hibiscus Administration

Seventy-two hours post-cadmium exposure, Groups C and D were administered daily doses of hibiscus extract (100 mg/kg in saline) for four weeks. The control groups (A and B) received 5mls of saline [16].

2.6. Measurement of Body Weight (g)

The body weight of the Wistar rats were recorded for one week (acclimatization period) prior to induction of cadmium and on a weekly basis for a period of four weeks. The weighing scale was used to take the body weights (Venus VT 30 SL); [17].

2.7. The Relative Heart Weight (%)

Using a sensitive weighing balance, the relative heart weight of the Wistar rat was calculated as the product of the weight of its heart and its terminal body weight. The result was expressed as a percentage (%). (SonyF3G brand); [18].

2.8. Histological and Histochemical Processing

Animals were sacrificed by euthanasia at the end of the study. They were laid supine on a dissecting board and pinned through their fore and hind paws. The thorax of each animal was dissected using a scalpel and bone forceps, and each heart was carefully removed and weighed. The hearts were then fixed in 10% formol-saline for 72 hours, processed for paraffin embedding, and stained with Hematoxylin & Eosin (H&E) and Periodic Acid-Schiff (PAS). The tissues for oxidative stress markers (GSH and SOD) were immediately placed in a 0.1 M phosphate buffer solution (pH 7.4) for homogenization. Total GSH was measured using a fluorometric assay, and SOD enzyme activity was measured using activity assay kits

2.9. Photomicrography

Photomicrographs of the histological sections of the heart were taken with an Olympus Microscope (New York, United States of America) coupled with a camera at the Department of Human Anatomy, Mulungushi University School of Medicine and Health Sciences, Livingstone Campus, Zambia.

2.10. Statistical Analysis

Data were presented as mean ± standard error of the mean (mean ± SEM) and analysed using one-way ANOVA. All graphs were drawn using Excel (Microsoft Corporation, U.S.A). P-values less than 0.05 ($p < 0.05$) were considered statistically significant.

3. Results

3.1. Average Body Weight (g) on a Weekly Basis

Figure 1 below shows changes in average body weight (g) on a weekly basis from Week -1 to Week 4. During Week -1 (acclimatization) and Week 0 (induction), body weights did not change significantly. In Week 1 (treatment), there was no significant change in body weight in the cadmium-only group compared to the other groups. From Week 2 to Week 4, there was a significant decrease in body weight in the cadmium-only group compared to the control group, which was statistically significant ($p < 0.05$). In the cadmium + hibiscus group, there was no significant change in body weight compared to the control group ($p > 0.05$). Similarly, in the hibiscus extract-only group, the difference in body weight was not statistically significant ($p > 0.05$) when compared to the control group.

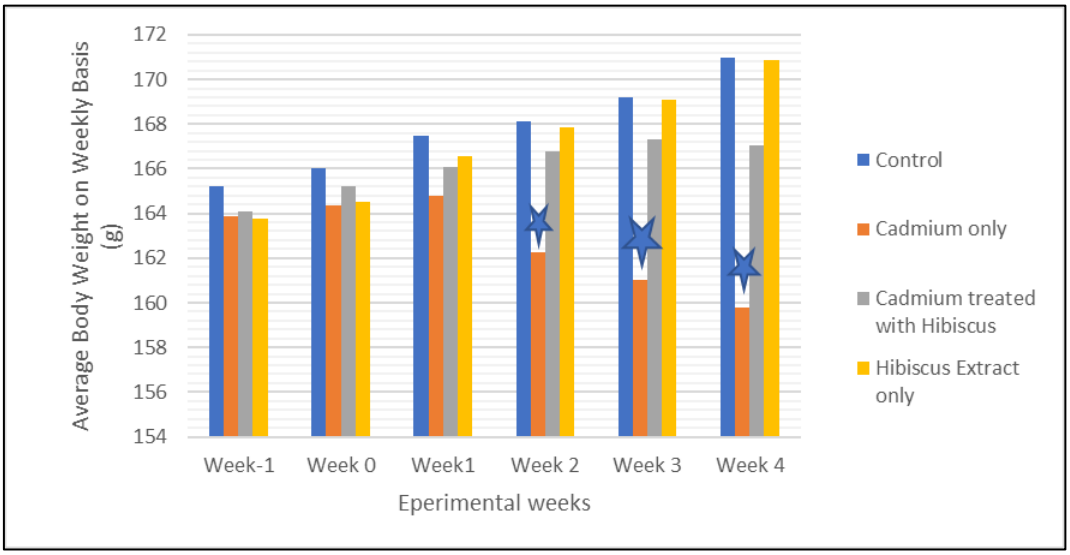


Figure 1 Histogram Chart of Average body weights (g) Data were expressed as mean± SEM ($p< 0.05$)* asterisk means significance at $p<0.05$

3.2. Relative Heart Weight (%)

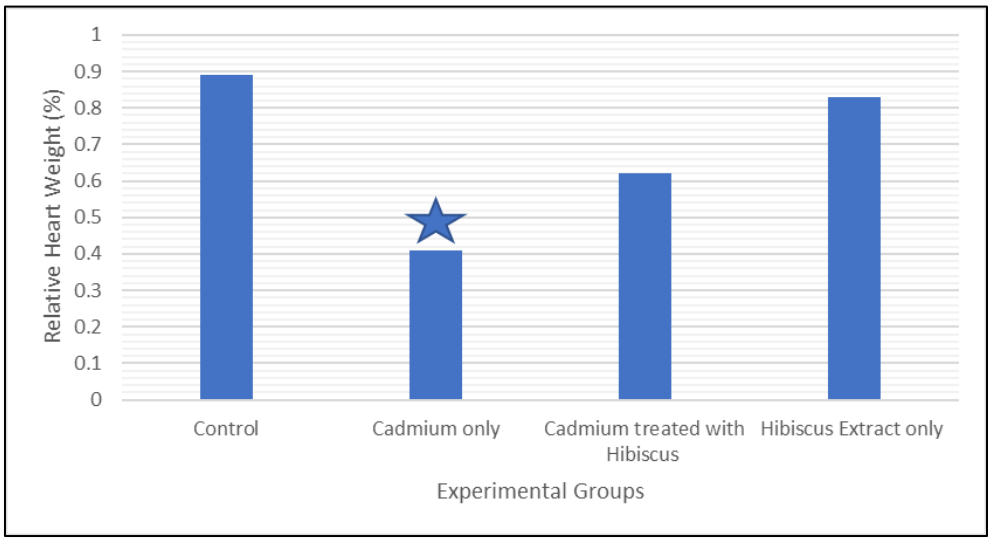


Figure 2 Bar charts of relative heart weight (%). Data were expressed SEM as mean± SEM ($p<0.05$) * asterisk means significance at $p<0.05$

Figure 2 above shows changes in the relative heart weight of Wistar rats in different groups. The cadmium-only group showed a significant decrease in relative heart weight compared to the control group, which was statistically significant

($p < 0.05$). In contrast, the cadmium + hibiscus and hibiscus extract groups showed no significant decrease in relative heart weight compared to the control group ($p > 0.05$).

3.3. Histological findings

3.3.1. Hematoxylin and Eosin (H&E) Stain of the Heart

The hearts in the normal control and hibiscus-only groups exhibited normal histoarchitecture with numerous intact cardiomyocytes (Figures 3A and 3D). The cadmium group showed disrupted histoarchitecture with degenerating cardiomyocytes (Figure 3B). The cadmium + hibiscus group displayed histoarchitecture similar to that of the control group (Figure 3C).

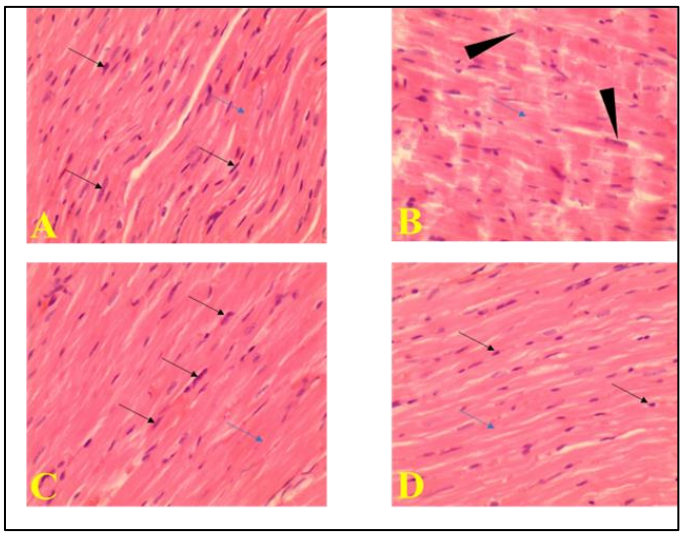


Figure 3 Photomicrograph showing the Heart at day 28. H&E stain X400. A- Control, B – Cadmium, C – Cadmium+ Hibiscus, D – Hibiscus only. Black arrow – cardiomyocyte, Arrow head – degenerating cardiomyocyte, Blue arrow – myocardial fibers

3.4. Periodic Acid-Schiff (PAS) Stain of the Heart

The normal control, hibiscus-only, and cadmium + hibiscus groups showed a normal reaction to the PAS stain (Figures 4A, C, and D). The cadmium group exhibited a positive reaction to the PAS stain (Figure 4.4B).

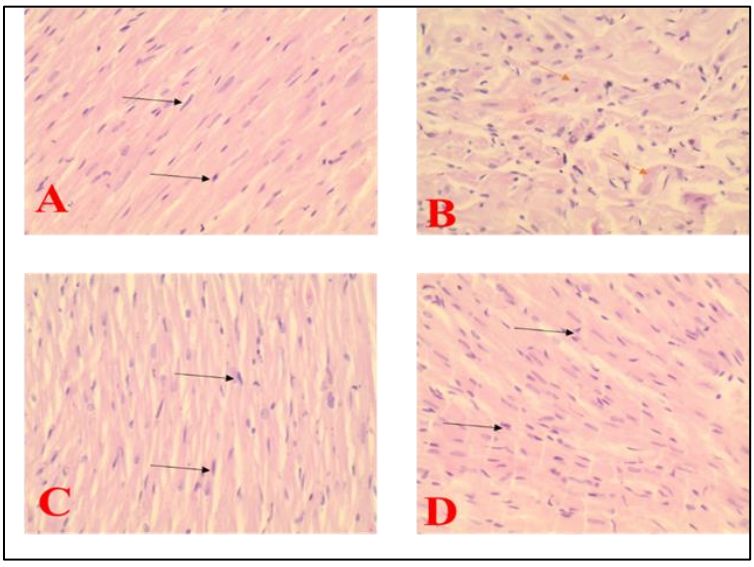


Figure 4 Photomicrograph showing the Heart at day 28. PAS stain X400. A- Control, B – Cadmium, C – Cadmium+ Hibiscus, D – Hibiscus only. Black arrow – cardiomyocyte, Yellow arrow – PAS reaction

3.5. Reduced Glutathione (GSH) Activity in the Heart (µmol/g tissue)

Figure 5 illustrates the variations in reduced glutathione (GSH) levels among the experimental groups. The control and Hibiscus-only groups exhibited significantly higher GSH levels compared to the other groups ($p < 0.05$). In contrast, the cadmium-only group showed the lowest GSH levels, which were significantly reduced compared to all other groups ($p < 0.05$). However, the cadmium + Hibiscus group did not show a significant difference in GSH activity when compared to the control group ($p > 0.05$).

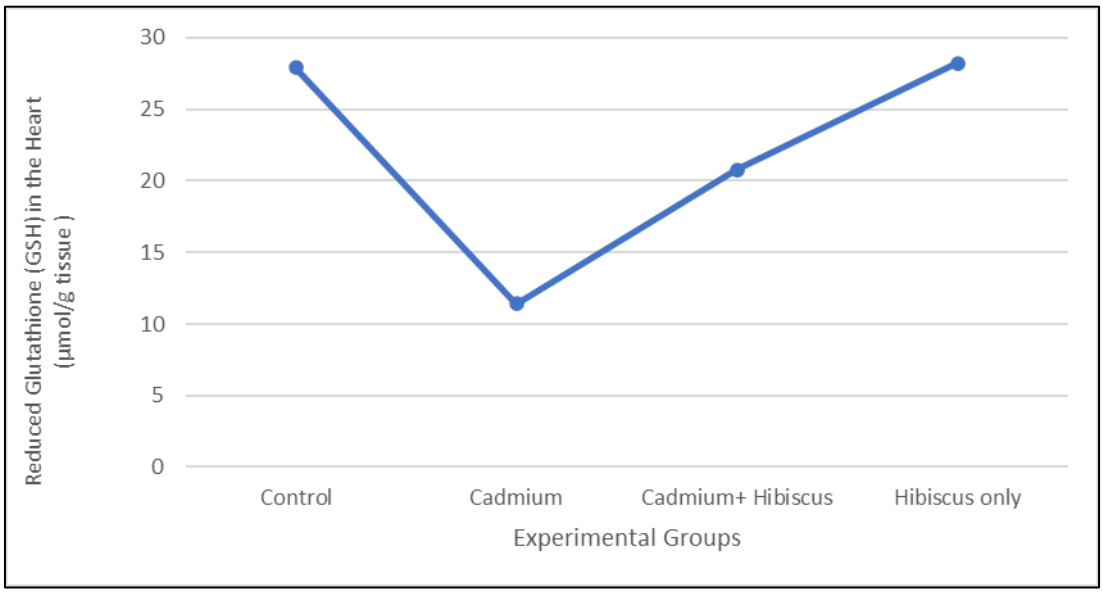


Figure 5 Reduced glutathione (GSH) activity in the frontal cortex (µmol/g tissue). Data Expressed as MEAN±SEM P<0.05

3.6. Superoxide Dismutase (SOD) Activity in the Heart (U/mg protein)

Figure 6 shows the differences in superoxide dismutase (SOD) activity among the experimental groups. The control and Hibiscus-only groups had the highest SOD activity, while the cadmium-only group displayed the lowest activity. The difference in SOD activity between the cadmium-only and cadmium + Hibiscus groups was statistically significant ($p < 0.05$). However, SOD activity in the cadmium + Hibiscus group was not significantly different from that of the control group ($p > 0.05$).

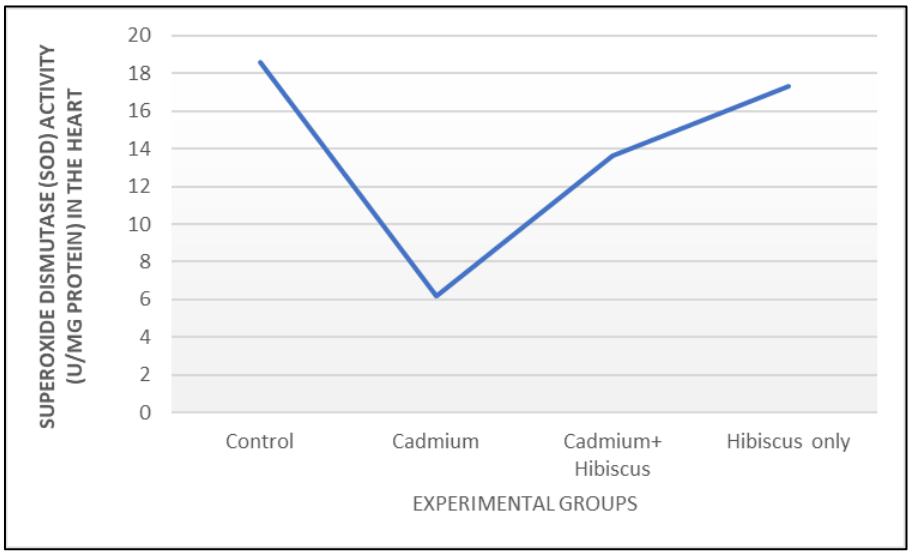


Figure 6 Superoxide dismutase (SOD) activity in the frontal cortex (U/mg protein). Data Expressed as MEAN±SEM P<0.05

4. Discussion

Hibiscus sabdariffa has been reported to possess antioxidant properties [19]. This study aimed to investigate the potential ameliorative effects of hibiscus extract on the hearts of cadmium-induced Wistar rats.

In the present study, Figure 1 demonstrates a significant decrease in body weight in the cadmium-only group from week 2 to week 4 compared to the control group. This reduction is attributed to cadmium toxicity, which oxidises lipids and introduces carbonyl groups into proteins, impairing their function [20]. Consequently, with no available energy source, the body utilises fat and protein stores for energy production [21]. In contrast, the cadmium + hibiscus group maintained body weight compared to the control group, although this change was not statistically significant. This improvement can be attributed to the chemical composition of hibiscus extract, which mitigated cadmium toxicity in body tissues. Key bioactive compounds within hibiscus, such as anthocyanins, phenolic acids, and organic acids, facilitate energy production necessary for cellular growth and metabolism, thereby promoting maintenance of body weight [12]. The hibiscus extract-only group showed a steady increase in body weight, similar to that of the control group. Since this group was not exposed to cadmium toxicity, the observed weight gain is likely due to the extract's enhancement of normal cellular functions [19]. These findings align with previous research by Orororo et al. [22], who reported that *Hibiscus sabdariffa* ameliorates cadmium toxicity. The present study further emphasises the role of hibiscus-derived antioxidants, such as anthocyanins, flavonoids, and polyphenolic acids, in counteracting cadmium-induced damage. The cadmium-only group exhibited a significant decrease in relative heart weight compared to the control group. This reduction was caused by cadmium accumulation in cardiac tissue, which generated reactive free radicals, degraded cellular components, disrupted calcium homeostasis, and triggered apoptosis, ultimately resulting in cardiac atrophy and diminished antioxidant enzyme activity [23]. In the cadmium + hibiscus group, the relative heart weight decreased slightly but not significantly compared to the control group. This protective effect can be attributed to the high antioxidant content of hibiscus extract, which scavenges free radicals [19]. Additionally, bioactive compounds, such as pigments, calcium, and glycosides found in the hibiscus calyx, contribute to tissue protection [24]. The hibiscus extract-only group demonstrated similar relative heart weight compared to the control group. The absence of cadmium exposure, combined with the well-documented benefits of *Hibiscus sabdariffa*, including its antioxidant, diuretic, anti-cholesterol, and anti-obesity properties, likely supported normal cardiac weight maintenance [19]. These results are consistent with Klinova et al. [25], who reported cadmium-induced dystrophic changes in the heart, primarily mediated by apoptosis, leading to reduced heart weight. Figure 3 reveals that the normal control (A) and hibiscus-only (D) groups exhibited intact cardiac histology with healthy cardiomyocytes. The absence of cadmium exposure in the hibiscus-only group preserved normal histoarchitecture. In contrast, the cadmium-only group (B) displayed disrupted histoarchitecture, characterised by degenerating cardiomyocytes and localised necrosis. Cadmium also upregulated matrix metalloproteinases (MMP-2 and MMP-14), contributing to cardiomyocyte death. These pathological changes were driven by oxidative stress, mitochondrial dysfunction, and apoptosis [26]. Cadmium toxicity further impairs cellular proliferation and survival by generating reactive oxygen species, disrupting DNA repair mechanisms, and depleting reduced glutathione (GSH), thereby exacerbating oxidative damage and apoptosis [6]. The cadmium + hibiscus group exhibited near-normal cardiac histoarchitecture, resembling the control and hibiscus-only groups. The restoration of tissue integrity is likely due to flavonoids, alkaloids, cardiac glycosides, and saponins in hibiscus extract, which counteract the cytotoxicity of cadmium [19]. Furthermore, polyphenolic compounds in *Hibiscus sabdariffa* have been shown to inhibit vascular smooth muscle cell proliferation and migration under high glucose conditions [27]. These findings align with Concepcion et al. [28], who reported that cadmium-induced oxidative stress leads to cardiac tissue damage, fibrosis, and irregular matrix networks. Similarly, Prabu et al. [29] observed myocardial fibre degeneration, necrosis, and disorganisation in cadmium-exposed rats, corroborating the present study. Figure 4 demonstrates a normal PAS reaction in the control (A), hibiscus-only (C), and cadmium + hibiscus (D) groups. However, the cadmium-only group showed a positive PAS reaction, indicating the presence of exposed polysaccharides (e.g., glycogen) due to cardiac tissue damage [30]. The cadmium + hibiscus group exhibited a normal PAS reaction, suggesting that the free radical scavenging and antioxidant properties of hibiscus extract mitigated cadmium-induced damage [19]. These results are supported by Aleksandra and Zorc-Pleskovic [31], who used PAS staining to assess glycogen content in cardiac tissue, noting significant glycogen accumulation in damaged myocardium.

The present study evaluated reduced glutathione (GSH) levels and superoxide dismutase (SOD) activity. Our findings demonstrate that the cadmium-only group exhibits significant depletion of cardiac GSH and SOD activity. In contrast, the Cadmium + Hibiscus group mitigates these adverse effects, restoring antioxidant levels close to those of the control group. Cadmium is a well-known pro-oxidant heavy metal that induces oxidative stress by generating reactive oxygen species (ROS) and depleting endogenous antioxidants [32] Our results align with previous studies showing that cadmium exposure reduces GSH levels in cardiac tissue [33]. GSH, a critical intracellular antioxidant, neutralises ROS and maintains cellular redox balance [34, 35]. The significant decrease in GSH in the cadmium-only group suggests severe oxidative damage, as cadmium binds to sulfhydryl groups in GSH, rendering it inactive [36, 37]. Similarly, SOD

activity was markedly reduced in the cadmium-only group. SOD is a primary enzymatic defence against superoxide radicals, and its inhibition exacerbates oxidative injury [38]. Cadmium disrupts SOD activity by displacing essential metal cofactors, leading to enzyme inactivation [39]. These findings corroborate earlier reports of cadmium-induced cardiac oxidative stress and depletion of antioxidants [39]. Notably, the Cadmium + Hibiscus group prevented significant reductions in GSH and SOD activity, bringing their levels close to those of the control group. Hibiscus species, particularly Hibiscus sabdariffa, are rich in polyphenols, flavonoids, and anthocyanins, which exhibit strong antioxidant and metal-chelating properties [40, 41]. The restoration of GSH levels in the cadmium + Hibiscus group may be attributed to direct ROS scavenging by Hibiscus polyphenols, thereby reducing the oxidative burden on GSH [42], as well as upregulation of glutathione synthesis via nuclear factor erythroid 2-related factor 2 (Nrf2) activation, as demonstrated by other plant-derived antioxidants [43, 44]. Similarly, the preservation of SOD activity in the cadmium + Hibiscus group suggests that Hibiscus compounds either protect SOD from cadmium-induced inactivation or enhance its expression. Previous studies have shown that natural antioxidants can restore SOD activity in cadmium-exposed tissues [45, 46]. The control and Hibiscus-only groups exhibited the highest GSH and SOD levels, indicating that Hibiscus alone does not negatively affect cardiac antioxidant defences. Instead, it may provide a mild antioxidative boost, as seen with other dietary antioxidants [47]. The lack of significant difference between the cadmium + Hibiscus and control groups suggests near-complete mitigation of cadmium-induced oxidative damage.

5. Conclusion

Cadmium exposure severely impairs cardiac antioxidant defences, as evidenced by alterations in histoarchitecture and reduced activity of glutathione (GSH) and superoxide dismutase (SOD). However, Hibiscus supplementation effectively counteracts these effects, likely due to its antioxidant and metal-chelating properties. These findings highlight Hibiscus as a potential therapeutic agent against cadmium-induced cardiotoxicity. Further studies should explore the molecular mechanisms, including modulation of the Nrf2/ARE pathway, to fully elucidate the protective effects of Hibiscus.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declare that they have no conflicts of interest.

Statement of ethical approval

Ethical clearance was obtained from the Ethics Committee on Animal Use and Care at the Mulungushi University School of Medicine and Health Sciences (MUSoMHS) under approval number SMHS-MU4-2023-138.

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