Protective potentials of aqueous *Anthocliesta djalonensis* stem bark extract on mercury chloride-induced cardiotoxicity in Wistar rats.

Abstract

Background

Mercury chloride ($HgCl_2$) induces oxidative stress and cardiac damage by generating reactive oxygen species (ROS), disrupting antioxidant enzyme activity, and increasing lipid peroxidation. Conventional treatments often fail to counteract these effects, making natural antioxidants like *Anthocleista djalonensis* a potential alternative.

Aim

This study evaluates the protective potential of *Anthocleista djalonensis* stem bark extract against mercury chloride-induced cardiotoxicity in Wistar rats.

Materials and Methods

Thirty-six Wistar rats were divided into six groups: control, mercury chloride-only, and groups receiving mercury chloride with either low-dose (150 mg/kg) or high-dose (300 mg/kg) *Anthocleista djalonensis* extract. Additional groups received extract alone. Oxidative stress biomarkers (Superoxide dismutase, Catalase, Glutathione peroxidase, Malondialdehyde) were measured after 28 days. Histological and body weight changes were also assessed.

Results

The mercury chloride group showed significantly reduced in the body weight change and antioxidant enzyme activities and increased MDA levels ($p < 0.05$), indicating severe oxidative stress. Histological analysis revealed myocardial fiber disorganization and vascular stenosis, alongside significant weight loss. Treatment with *A. djalonensis* extract dose-dependently

restored antioxidant enzyme activities, reduced lipid peroxidation, improved cardiac architecture, and prevented weight loss.

Conclusion

Anthocleista djalonensis effectively mitigates $HgCl_2$-induced oxidative stress, histological damage, and weight loss, highlighting its potential as a natural cardioprotective agent.

Keywords: *Anthocleista djalonensis*, Oxidative stress, Mercury chloride toxicity, Cardioprotective effects, Antioxidant enzymes**.**

Introduction

Mercury chloride ($HgCl_2$) is a widely recognized toxicant with significant implications for human and environmental health. Its presence in the environment arises from both natural processes, such as volcanic emissions and degassing from the Earth's crust, and anthropogenic activities, including mining, coal combustion, and industrial processes (Burger and Gochfeld, 2011; Cox, 1997). This compound is notorious for its capacity to cause systemic toxicity, particularly targeting the cardiovascular system. The toxic effects of mercury chloride are primarily driven by its ability to generate reactive oxygen species (ROS), leading to oxidative stress and cellular damage. This results in the disruption of antioxidant defense mechanisms, lipid peroxidation, mitochondrial dysfunction, and enzyme inactivation. In the cardiovascular system, mercury chloride is particularly harmful, causing endothelial dysfunction, vascular inflammation, and myocardial injury. Its high affinity for thiol groups impairs key antioxidant enzymes such as superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPx), further exacerbating oxidative damage. (Cappelletti et al., 2019; Valko et al., 2005).

Despite advancements in conventional treatments, these approaches often fail to fully mitigate the underlying oxidative and inflammatory mechanisms, highlighting the need for alternative therapeutic strategies (Cappelletti et al., 2019).

Natural products, particularly those rich in antioxidants, have garnered attention as potential alternatives for addressing mercury-induced toxicity. Among such natural remedies, *Anthocleista djalonensis*, a plant belonging to the Gentianaceae family, has shown promise due to its traditional use in managing cardiovascular diseases and inflammation. This plant is rich in bioactive compounds, including flavonoids, polyphenols, and alkaloids, which possess potent antioxidant and anti-inflammatory properties (Okoli and Iroegbu, 2004; Awah et al., 2011). Studies have suggested that these compounds play a vital role in scavenging free radicals, enhancing enzymatic antioxidant activity, and modulating inflammatory pathways.

This study aims to evaluate the Protective potentials of aqueous *Anthocliesta djalonensis* stem bark extract on mercury chloride-induced cardiotoxicity in Wistar rats.

Materials and Methods

PLANT COLLECTION AND IDENTIFICATION

The stem bark of *Anthocleista djalonensis* used in this research work was gotten from a farm in Benin City. Collection of plant was done in relevance to the guidelines of Edo State Forestry Commission for harvesting wild plants. It was identified and authenticated by a plant taxonomist at the department of Plant Biology and Biotechnology, Faculty of Life sciences, University of Benin, Benin city, Edo State, Nigeria and herbarium number UBH-A594 was allocated to the plant.

EXTRACT PREPARATION

Aqueous extraction of the plant was done by freeze-dry method. *Anthocleista djalonensis* stem bark was washed with tap water, shade dried and then pounded. The powder obtained was soaked in distilled water for 24 hours in a separating funnel with occasional shaking. The solution was then filtered. The filtrate was allowed to settle and then decanted. The filtrate was then freeze dried with freeze drying machine and refrigerated at -6 celsius.

ETHICAL APPROVAL

The research ethics committee's guidelines for animal handling and treatment at the University of Benin's College of Medical Sciences was fully implemented and ethical approval number CMS/REC/2023/340 was assigned.

EXPERIMENTAL DESIGN

Thirty-six (36) adult Wistar rats were randomly assigned into six (6) groups; Groups A – F comprising of six rats per group.

1. Control group (no treatment).

2. Mercury chloride-treated group.

3. Mercury chloride + low-dose *Anthocleista djalonensis* extract (150 mg/kg).

4. Mercury chloride + high-dose *Anthocleista djalonensis* extract (300 mg/kg).

5. *Anthocleista djalonensis* extract only (150mg/kg).

6. *Anthocleista djalonensis* extract only (300 mg/kg).

Oxidative Stress Biomarkers

After 28 days of administration, cardiac tissues were homogenized and analyzed for antioxidant enzyme activities, including

1. Superoxide dismutase (SOD): Adrenaline rapidly oxidises to adrenochrome, the concentration of which can be measured at 420 nm using a spectrophotometer. Adrenaline auto-oxidation is dependent on the presence of superoxide anions. A 0.2 ml amount of plasma was mixed with 2.5 ml of carbonate buffer and 0.3 ml of adrenaline solution, whereas 0.2 ml of distilled water was mixed with 2.5 ml of carbonate buffer and 0.3 ml of adrenaline as a reference sample. These were combined and the absorbance measured at 420 nm (Misra and Fridovich, 1972).
2. Catalase (CAT): Catalase is present in nearly all animal, plant and bacteria cells. It acts to prevent the accumulation of noxious H2O2 which is converted to O2 and H2O. A known volume of plasma (0.5ml) was mixed with 5.0ml of H2O2. This was combined by inversion and left to stand for 30 minutes. The reaction was stopped by adding 6 M H2SO2. The absorbance was taken at 480nm within 30-60 seconds against distilled water. (Cohen et al., 1970).
3. Glutathione peroxidase (GPx): This is based on the oxidation of pyrogallol to purpurogallin by peroxidase activity, resulting to a deep brown colour disposition, read at 430nm. To a 0.2ml aliquot of plasma, 2.5ml of phosphate buffer, 2.5ml of H2O2, 1.5ml of distilled water, and 2.5ml of pyrogallol were added. The reaction was left to stand for 30 minutes at room temperature. A rich brown colour was produced, which was detected at 420nm. (Nyman, 1959).
4. Lipid peroxidation levels were quantified by measuring malondialdehyde (MDA) Malondialdehyde which is a product of lipid peroxidation reacts with thiobarbituric acid

to give a red species. A volume of plasma (1.0ml) was combined with 2.0ml of TCA-TBA-HCL and carefully mixed. The solution was boiled in a water bath at boiling temperature for 15 minutes. After cooling, the flocculent precipitate was removed by centrifugation at 1000 g for 10 minutes. The absorbance was measured at 535 nm against a blank. The concentration MDA was determined using the formula MDA = (unit/mg protein) (Buege and Aust, 1978).

Histological Analysis

Heart tissues were fixed in 10% formalin, processed, and stained with hematoxylin and eosin (H&E) for histological examination under a light microscope. Pathological changes, including interstitial congestion, vascular stenosis, and cardiomyocyte organization, were evaluated.

Results

Weight

The weight results in the study demonstrated notable changes across the experimental groups after 28 days of treatment. The group exposed to mercury chloride exhibited significant weight loss compared to the control group, indicating systemic toxicity likely due to oxidative stress, metabolic disturbances, and reduced appetite caused by the toxicant.

In contrast, groups treated with *Anthocleista djalonensis* extract, particularly *a*t higher doses, showed weight stabilization or gains relative to the Mercury chloride -only group. This suggests the extract's potential role in mitigating the systemic effects of Mercury chloride, possibly through its antioxidative and anti-inflammatory properties that support overall metabolic health.

The extract-only group maintained normal weight, confirming its safety and absence of adverse effects.

These findings align with the hypothesis that *Anthocleista djalonensis* extract helps counteract the toxic metabolic effects of Mercury chloride, promoting recovery and preventing further weight loss.

Oxidative Stress

In the Mercury chloride -treated group, significant reductions in SOD, catalase, and GPx activities were observed compared to the control group, indicating a compromised antioxidative defense system. MDA levels were markedly elevated, reflecting enhanced lipid peroxidation and oxidative damage. Treatment with *Anthocleista djalonensis* extract restored antioxidant enzyme activities and significantly reduced MDA levels in a dose-dependent manner, with high-dose extract showing near-complete normalization of oxidative stress markers.

Histology

The Mercury chloride-treated group displayed severe histopathological changes, including disorganized myocardial fibers, interstitial congestion, and vascular stenosis. Treatment with *Anthocleista djalonensis extract preserved cardi*ac architecture, with low doses showing partial restoration and high doses achieving near-normal histological profiles. Hearts from the extract-only group exhibited normal histological structure, confirming its safety and lack of intrinsic toxicity.

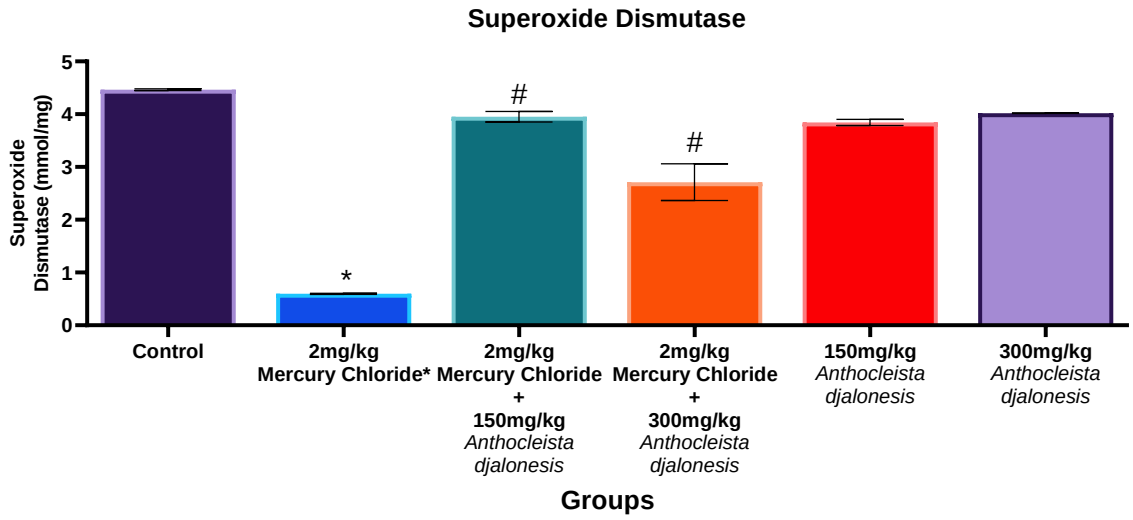
Result of Weight

Table 1: showing body weight, heart weight and cardio-somatic index analysis

Groups/Test	Control	2mg/kg of Mercury Chloride	150mg/kg of *Anthocliesta djalonesis*	300mg/kg of *Anthocliesta djalonesis*	2mg/kg of mercury chloride + 150mg/kg of *Anthocliesta djalonesis*	2mg/kg of mercury chloride + 300mg/kg of *Anthocliesta djalonesis*	p-value
Initial weight	252.7±9.831	193.0±8.157	148.0±6.261	136.8±3.449	142.2±6.954	153.7±5.383	<0.0001
Final weight	292.2±10.360*	*19932±7.778*	*185.2±5.152**	*235.0±7.861**	*184.5±3.926**	*198.5±11.680**	*<0.0001*
Weight Change	39.50±3.019	6.17±0.872*	*31.60±7.019*	*97.80±6.967*	47.50±5.454	44.89±7.846	<0.0001
Heart weight	0.95±0.062	0.78±0.070	0.70±0.084	0.80±0.070	0.78±0.111	1.00±0.158	0.2289
Cardio-somatic index	0.33±0.017	0.397±0.041	0.38±0.047	0.34±0.027	0.42±0.063	0.40±0.095	0.8138

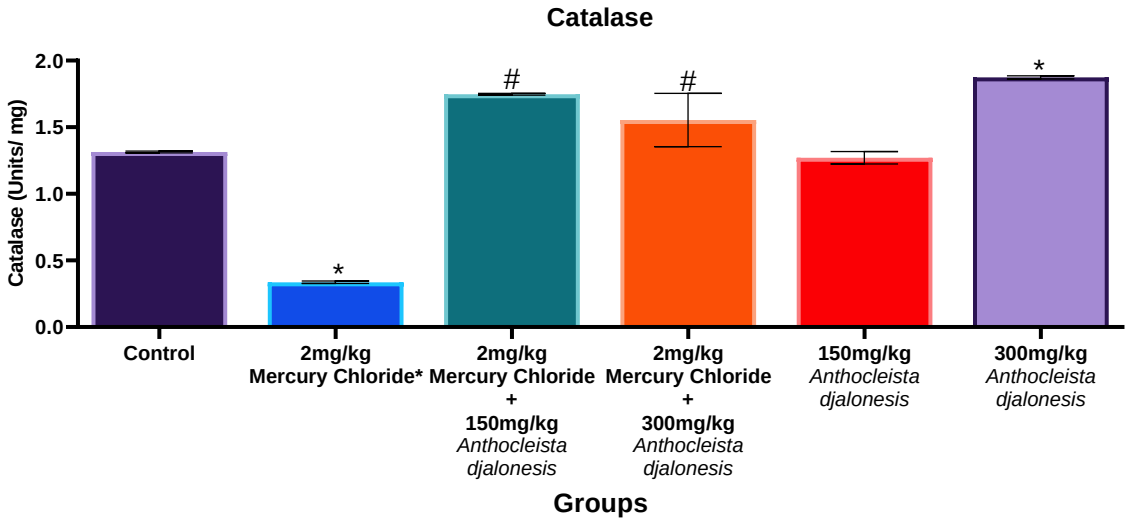
Table is presented as Mean ± Standard Error of Mean (SEM)
**Represent statistically* significant change at p<0.05 of final weight compared to initial weight.

Figure 1 showing Superoxide Dismutase activities in Heart across all groups



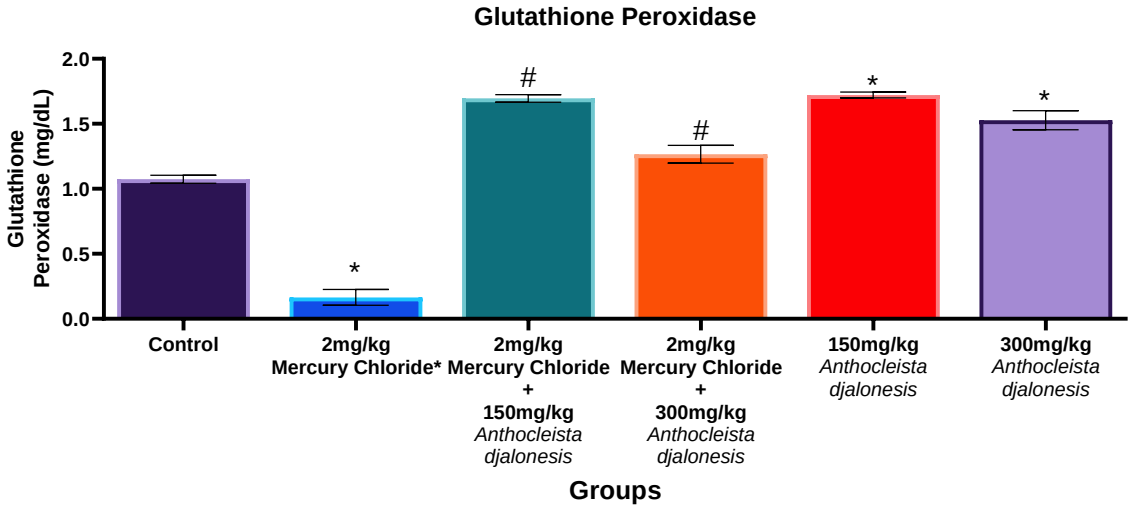
Superoxide dismutase activity in the heart of control and treatment groups after 28 days. Values are given as mean ± SEM. $^{*}p< 0.05$ *compared with th*e control group; $^{\#}p< 0.05$ compared with the Mercury chloride-alone group.

Figure 2 showing Catalase activities in Heart across all groups



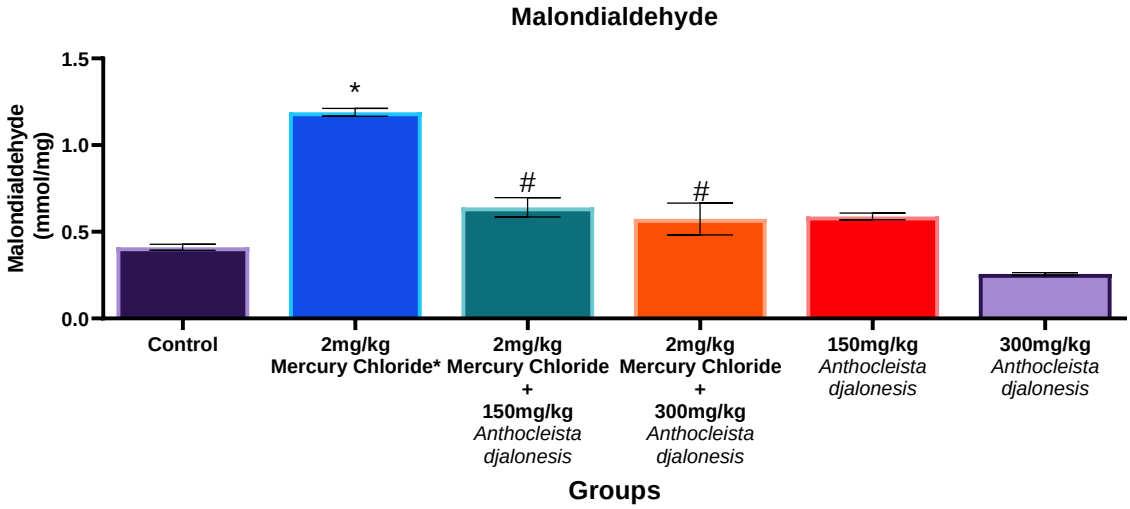
Catalase activity in the heart of control and treatment groups after 28 days. Values are given as mean ± SEM. $^{*}p< 0.05$ *compared with th*e control group; $^{\#}p< 0.05$ compared with the Mercury chloride-alone group.

Figure 3 showing Glutathione Peroxidase activities in Heart across all groups



Glutathione Peroxidase activity in the heart of control and treatment groups after 28 days. Values are given as mean ± SEM. $^{*}\ p < 0.05$ *compared with* the control group; $^{\#}\ p < 0.05$ compared with the Mercury chloride-alone group.

Figure 4 showing Malondialdehyde activities in Heart across all groups



Lipid peroxidation activity in the heart of control and treatment groups after 28 days. Values are given as mean ± SEM. $^{*}p< 0.05$ *compared with th*e control group; $^{\#}p< 0.05$ compared with the Mercury chloride-alone group.

Histology

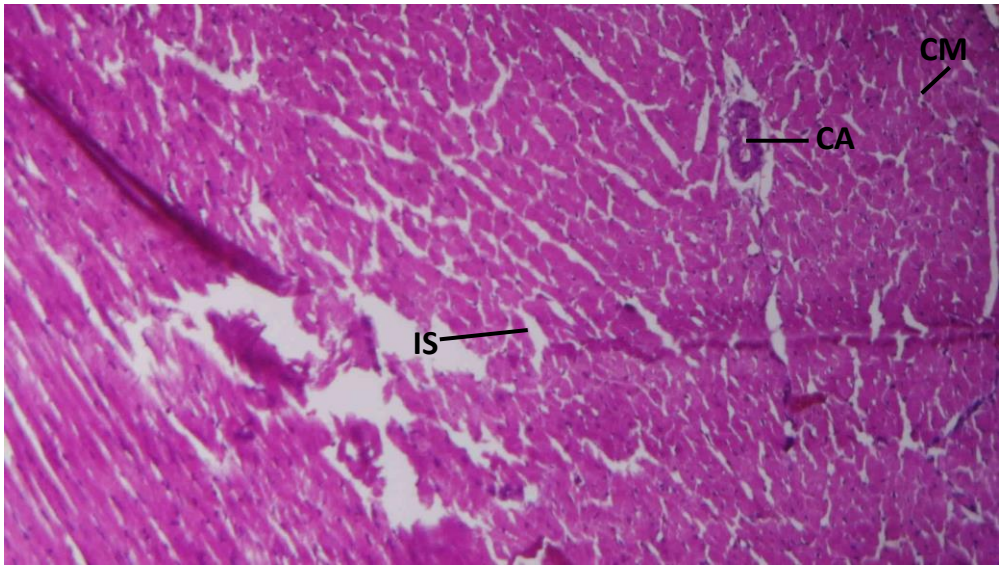


Figure 5. Sections from control rats show: normal architecture: bundles of cardiomyocytes (CM), interstitial space (IS) and coronary artery (CA):HandE x100

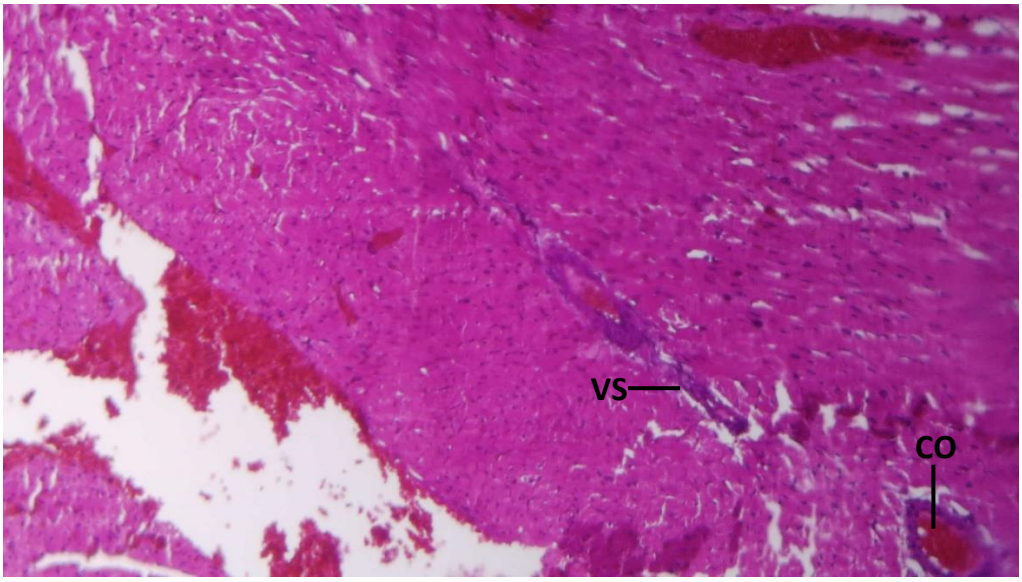


Figure 6. Sections from rats given 2mg/kg $HgCL_2$ only: severe interstitial congestion (CO) and coronary vascular stenosis (VS): HandE x100

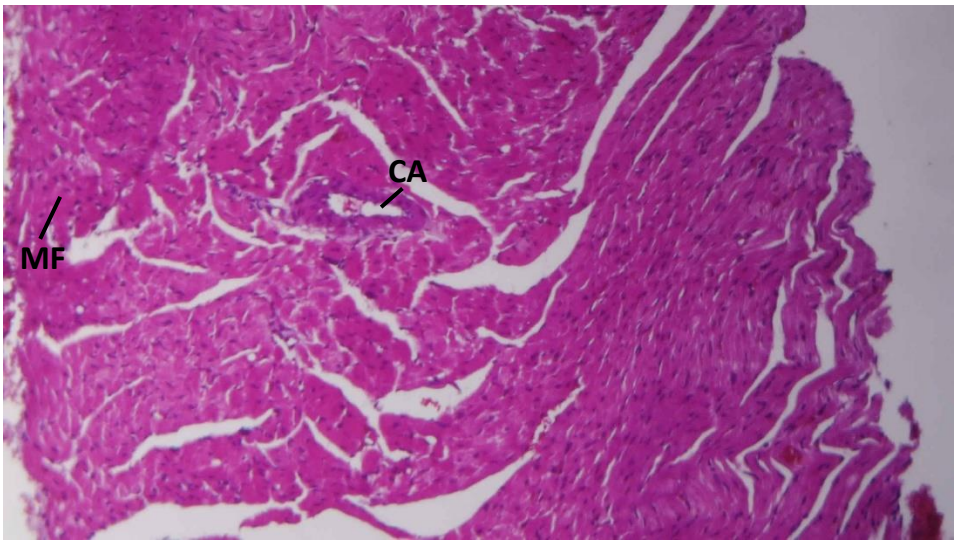


Figure 7. Sections from rats given 2mg/kg $HgCL_2$ + 150mg *Anthocleista djalonensis* shows: normal bundles of myocardial fibres (MF) and coronary artery (CA): HandE x100

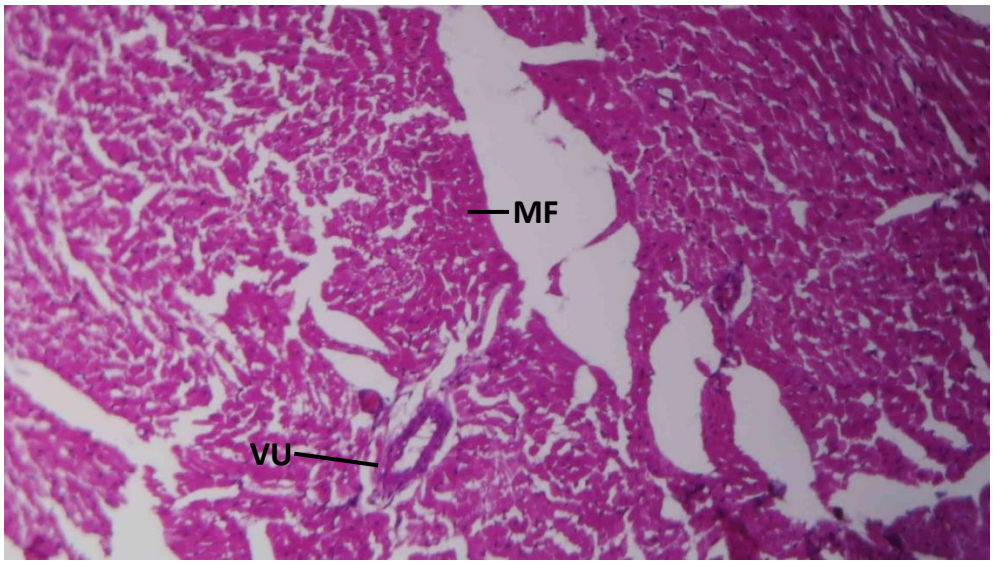


Figure 8. Sections from rats given 2mg/kg $HgCL_2$ + 300mg *Anthocleista djalonensis* shows: normal bundles of cardiomyocytes (MF) and vascular ulceration (VU): HandE x100

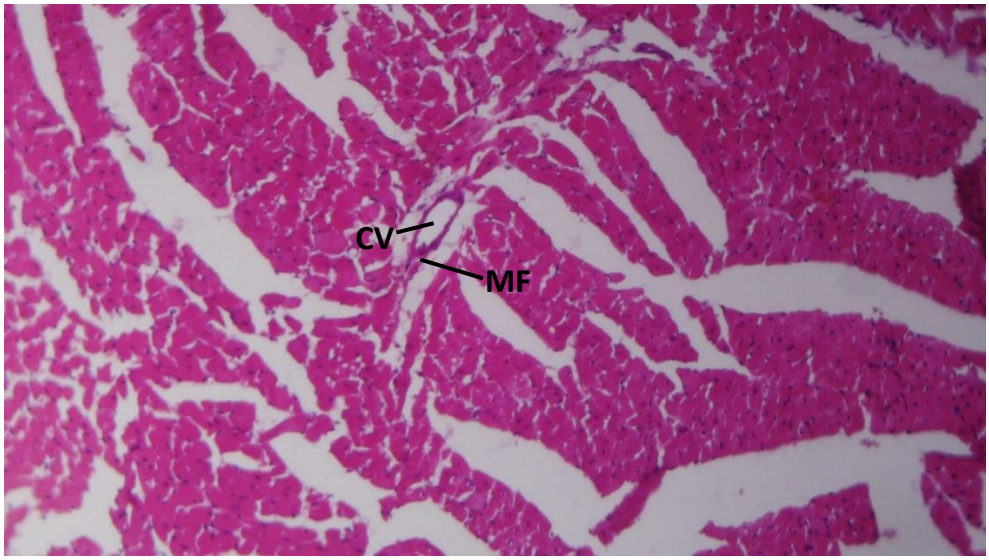


Figure 9. Sections from rats given 150mg *Anthocleista djalonensis* only show: normal cardiac vessels (CV) and bundles of myocardial fibres (MF): HandE x100

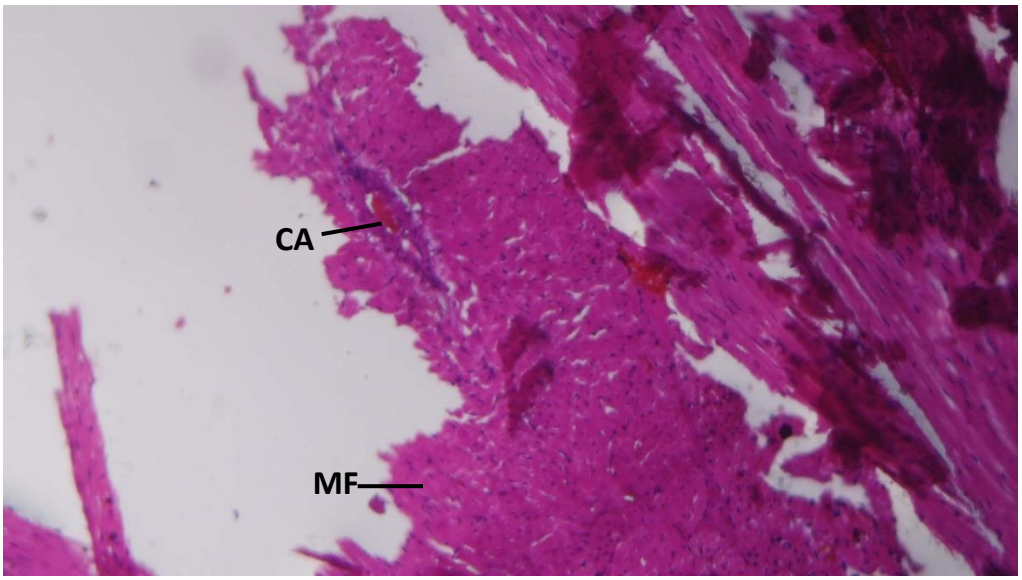


Figure 10. Sections from rats given 300mg *Anthocleista djalonensis* only show: normal bundles of myocardial fibres (MF) and coronary artery (CA): HandE x100

Discussion

Weight

The study's evaluation of body weight changes highlights a foundational measure of systemic toxicity. Significant weight loss in mercury chloride-treated groups reflects cachexia, a condition characterized by severe wasting of muscle and fat due to inflammation and metabolic dysregulation. Cachexia commonly results from exposure to toxins that disrupt appetite-regulating hormones, damage gut integrity, or induce systemic oxidative stress (Evans et al., 2008). The significant weight loss observed in the mercury chloride-treated group aligns with previous studies that highlight the toxic impact of heavy metals on metabolism and nutritional status (Evans et al., 2008). In contrast, the weight stabilization in groups receiving *Anthocleista djalonensis* suggests mitigation of these deleterious processes, likely through anti-inflammatory and antioxidant mechanisms that preserve metabolic function. This aligns with research

demonstrating that flavonoid-rich plant extracts improve weight maintenance in toxicant-exposed models (Valko et al., 2005).

The increase in cardio-somatic index in the untreated toxic group is indicative of cardiac hypertrophy, a pathological enlargement of the heart muscle often driven by stressors such as hypertension or direct toxic insult. In this context, mercury chloride's oxidative effects can trigger compensatory hypertrophy as cardiac myocytes attempt to cope with increased workload and tissue damage (Liu et al., 2018). Treatment with *Anthocleista djalonensis* normalized these indices, pointing to its ability to reduce oxidative stress and inflammation, thereby preventing maladaptive structural changes.

Oxidative Stress

The antioxidant enzyme assays, including superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPx), are central to evaluating oxidative stress. These enzymes collectively constitute the body's primary defense against free radicals. SOD catalyzes the dismutation of superoxide radicals into oxygen and hydrogen peroxide, catalase decomposes hydrogen peroxide into water and oxygen, and GPx reduces lipid hydroperoxides and hydrogen peroxide. Mercury chloride significantly reduces these enzyme activities, signaling oxidative overload, which can lead to lipid peroxidation, protein denaturation, and DNA damage (Valko et al., 2007). The observed depletion of antioxidant enzymes (SOD, catalase, and GPx) in mercury-exposed rats is consistent with findings from previous toxicological studies on heavy metals (Cappelletti et al., 2019). Mercury has a high affinity for thiol-containing proteins, leading to inactivation of antioxidant defense mechanisms, thereby increasing oxidative stress (Valko et al., 2007). The significant restoration of enzyme activities following *Anthocleista djalonensis* extract

administration supports previous reports on plant-derived antioxidants counteracting oxidative stress-induced enzyme suppression (Awah et al., 2011). The flavonoids and polyphenols in *Anthocleista djalonensis* likely function as free radical scavengers, enhancing endogenous enzymatic defense systems, as observed in studies involving other medicinal plants with similar phytochemical compositions (Okoli and Iroegbu, 2004). Malondialdehyde (MDA) levels, a marker of lipid peroxidation, were elevated in mercury-treated groups, reflecting extensive membrane damage. Lipid peroxidation compromises cell membranes, leading to loss of cellular function and necrosis (cell death). Reduction of MDA levels in the treatment groups further emphasizes the extract's role in neutralizing ROS and preventing cellular destruction.

Histology

The histological alterations observed in the mercury-exposed group, including myocardial disorganization, vascular stenosis, and interstitial congestion, are consistent with previous studies demonstrating mercury-induced cardiovascular toxicity (Heusch, 2022). Mercury has been shown to disrupt vascular homeostasis, leading to endothelial dysfunction and inflammatory damage (Valko et al., 2007). Treatment with *Anthocleista djalonensis* significantly improved cardiac architecture, supporting its cardioprotective role. These findings are in agreement with studies on other cardioprotective medicinal plants, where bioactive compounds contribute to vascular integrity and myocardial preservation (Awah et al., 2011)

In conclusion, this study demonstrates that *Anthocleista djalonensis* extract effectively counters oxidative stress and histological damage induced by $HgCl_2$, emphasizing its potential as a natural therapeutic agent for mercury toxicity.

Availability of data and materials

The data can be obtained from the corresponding author upon reasonable request by contacting them through their email ID.

Acknowledgements

None.

Funding

None.

Ethics declarations

Ethics approval and consent to participate

The research ethics committee's guidelines for animal handling and treatment at the University of Benin's College of Medical Sciences was fully implemented and ethical approval number CMS/REC/2023/340 was assigned.

Consent for publication

Not applicable.

Competing interests

None.

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