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Original Article

Evaluating Heart Tissue Changes in Iron Chloride-exposed Rats: Impact of *Chasmanthera dependens* Methanol Leaf Extract Treatment

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Abstract

BACKGROUND AND AIM: Iron is essential for body metabolic activities but excessive iron exposure poses a significant threat to cardiovascular health due to its role in oxidative stress and tissue damage. Natural iron-chelating agents from plants offer a promising alternative to synthetic chelators with potentially fewer side effects. The efficacy of *Chasmanthera dependens* as a plant-based cardioprotective agent chelating agent has not been fully explored. This study evaluates the changes in iron chloride-exposed rats and the impact of MLECD treatment.
METHODOLOGY: Twenty-five adult Wistar rats averagely weighing between 185-225g were used for this study. They were divided into five rats per groups with daily administration for treatment for twenty-eight days. Group A served as the control group. Group B was given 2mg/kg body weight of $FeCl_2$ only, groups C, D and E with 200, 400, and 800mg/kg body weights of MLECD in addition to 2mg/kg body weight of $FeCl_2$ solution respectively. Rats were euthanized under chloroform and heart harvested and fixed in neutral buffered formalin for hematoxylin and eosin histological staining procedure, and histological slides were examined using light microscope.
RESULTS AND CONCLUSION: Histopathological findings on $FeCl_2$ administration showed myocardial degeneration, coronary vascular ulceration and perivascular inflammation and oedema but MLECD, especially at the lowest dose (200mg/kg MLECD), protected against $FeCl_2$ induced iron-toxicity, consequently proving cardioprotective evidence of MLECD against excessive iron exposure.

Keywords:
Iron exposure, *Chasmanthera dependens*, cardiotoxicity, Cardioprotective, Wistar rats

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INTRODUCTION

Iron is an important element required for life (Yee and Tolman, 2014). It participates in a wide variety of metabolic processes, including oxygen transport, deoxyribonucleic acid synthesis, and electron transport. However, excessive accumulation of iron in the body, particularly in vital organs such as the heart, liver, and pancreas can form free radicals, its concentration in body tissues must be tightly regulated because in excessive amounts, it can lead to tissue damage (Abbaspour *et al*., 2014). Treatment of iron overload-induced diseases entails iron removal using iron chelation therapy (Innih *et al*., 2020). Despite advances in iron chelation therapy, current treatments have limitations, including adverse effects and incomplete efficacy, underscoring the need for alternative therapeutic strategies (Reddy *et al*., 2022). Iron (II) chloride also known as ferrous chloride with chemical formula $FeCl_2$ forms greenish tetrahydrate when crystallized from water (Alcaraz et al. 2021) and it has been established that iron chloride consumed in excess can induce tissue damage and increase serum iron level (Wallace 2016; Innih *et al*., 2020).

Plant-derived substances that bind to excess iron present a hopeful substitute to artificial iron-binding compounds, with the potential for reduced adverse effects. *Chasmanthera dependens* is used in Nigerian traditional medicine as a therapy for various diseases like red-eye infections, also in the treatment of abdominal pain, sprained joints and bruises (Olukoya *et al*., 1993). In Democratic Republic

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of Congo, the leaf sap is applied as first aid to stop bleeding of wounds (Mosango, 2008). The leaf possesses anti-inflammatory, analgesic and antifungal activity (Morebise *et al*., 2001), *C. dependens* leaf has also proven to be nephroprotective (Fatokun *et al*., 2016), the leaves, roots and stem possess anti-plasmodial and in-vitro antioxidant potentials (Enenebeaku *et al*., 2022) and tannin-rich extract of the leaves possess hepatoprotective potentials (Abiola *et al.,* 2021). *C. dependens* has been proven to possess antioxidant properties (Madueke *et al.,* 2020) but there is no existing knowledge of its cardioprotective potential hence this study.

The heart is a muscular organ responsible for pumping blood throughout the body, contracting and relaxing rhythmically to maintain blood circulation, it achieves this through a coordinated electrical signal that triggers each heartbeat, known as the cardiac cycle (Khonsary, 2017)

According to Ravingerová *et al*., (2020), iron overload can severely impact cardiomyocytes by disrupting mitochondrial function, promoting fibrosis, triggering apoptosis, and impairing contractility. In particular, cardiomyopathy is the most common cause of morbidity and mortality in patients with iron overload disorders (Murphy and Oudit 2010; Wood *et al*., 2005), the accumulation of excess iron within cardiomyocytes leads to increased production of reactive oxygen species, which damages cellular components and compromises cell integrity. Additionally, mitochondrial dysfunction and fibrosis further exacerbate cardiomyocyte dysfunction, contributing to reduced cardiac function (Sirinart and Chattipakorn, 2022). This study is aimed at evaluating the histological alterations of cardiac histology caused by excessive iron exposure focusing on the impact of Methanol leaf extract of *Chasmanthera dependens* treatement

MATERIALS AND METHOD

Procurement and Preparation of Methanolic Leaf Extract of Chasmanthera dependens

Fresh *Chasmanthera dependens* leaves were sourced and collected from local forest reserves in Ugbowo, Ovia North-East Local Government Area, Benin City, Edo State and identified in the herbarium unit of the Department of Plant Biology and Biotechnology, University of Benin, Benin City and was assigned with herbarium specimen voucher number UBH-C387.

C. dependens leaves were air-dried at room temperature and milled into fine powder with a British milling machine Viking Exclusive Joncod (Type YL112M-2). The resulting powder was weighed to be 110g and soaked in absolute methanol for 48 hours and then filtered using cellulose filter paper. The filtrate was evaporated at 40ºC using a water bath resulting in 33.6g yield of the extract with yield percentage of 30.5%. The residue was stored in a refrigerator at the Department of Anatomy, University of Benin, and 10g of MLECD was dissolved in 100ml of distilled water for use during the experiment.

$$\boldsymbol{Yield}(\%) = \frac{\boldsymbol{Final\ weight\ of\ MLECD\ (g)}}{\boldsymbol{Weight\ of\ powdered\ C.dependens\ leaves\ (g)}}\ \boldsymbol{x\ 100}$$

10g of Iron Ⅱ chloride crystals (500g) manufactured by MOLYCHEM® with catalog number 13478-10-9 was dissolved in 100 ml of distilled water to form Iron chloride solution and administered.

Experimental Design

Twenty-five (25) adult Wistar rats averagely weighing 185-225g were used for this study and randomly divided into five groups – A, B, C, D and E with five rats in each group. Group A served as control while B, C, D and E served as experimental groups. They were acclimatized for two weeks. All groups received feed (growers mesh) and water *ad libitum* and daily administrations were done orally using orogastric tube.

Group A (control group) received 1ml of distilled water. Group B (negative control) received 2mg/kg body weight of $FeCl_2$ only. Group C: 200mg/kg body weight of MLECD + 2mg/kg body weight of $FeCl_2$. Group D: 400mg/kg body weight of MLECD + 2mg/kg body weight of $FeCl_2$. Group E: 800mg/kg body weight of MLECD + 2mg/kg body weight of $FeCl_2$

Histological Assessment

At the end of the administration on the 28th day, rats were euthanized under chloroform and heart was harvested and fixed in 10% buffered formalin for 72 hours, and processed using hematoxylin and eosin staining technique according to Drury and Wallington, (1980). Processed tissue slides were examined under Leica DM750 research microscope with a digital camera (LeicaICC50) attached. Digital photomicrographs of the tissue sections were taken at 400x magnifications.

RESULTS

The control group presented normal bundles of cardiomyocytes, interstitial spaces and blood vessels (Figure 1) whereas group B given $FeCl_2$ only showed coronary vascular ulceration, perivascular oedema, and mild perivascular infiltrates of inflammatory cells (Figure 2).

Rats given 200mg/kg of MLECD and $FeCl_2$ showed well aligned bundles of cardiomyocytes, normal interstitial spaces, no polymorphs or mononuclear cells were seen in and around blood vessels (Figure 3).

Groups E and F given 400 and 800mg/kg of MLECD and $FeCl_2$ all showed myocardial degeneration, mild perivascular inflammatory infiltrates (Figures 4 and 5) respectively.

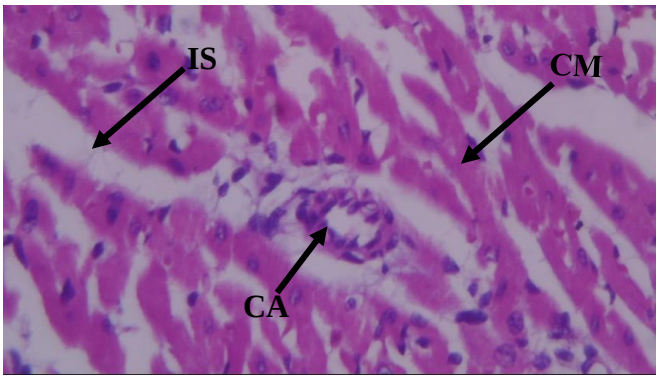

Figure 1: Photomicrograph of Rat's Cardiac histology in Control group Composed of normal architecture: bundles of cardiomyocytes (CM), interstitial space (IS), coronary artery (CA): H&E; 400x

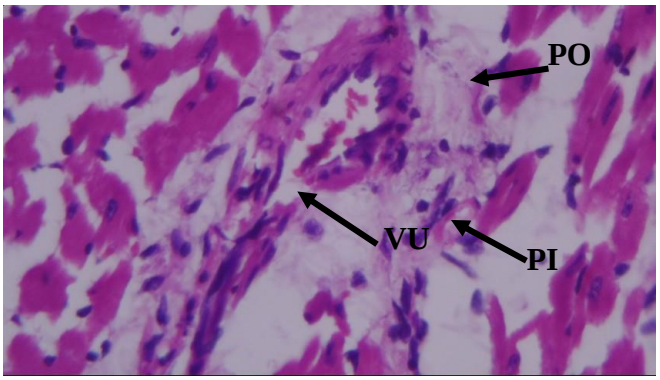

Figure 2: Photomicrograph of Rat's Cardiac histology in Group B (2mg/kg $FeCl_2$ only) showing coronary vascular ulceration (VU), perivascular oedema (PO), mild perivascular infiltrates of inflammatory cells (PI): H&E; 400x

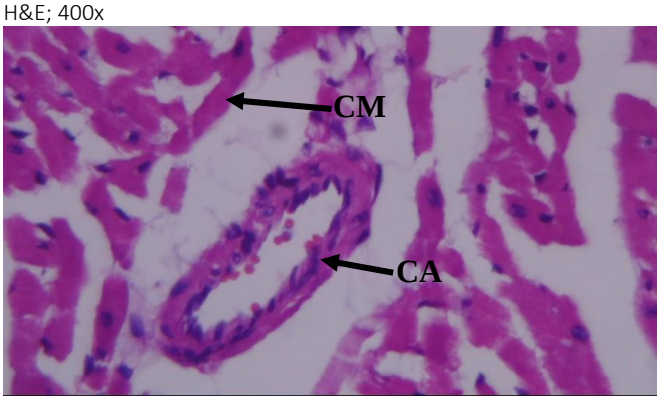

Figure 3: Photomicrograph of Rat's Cardiac histology in Group C (2mg/kg $FeCl_2$ and 200mg/kg MLECD) showing: bundles of cardiomyocytes (CM), coronary artery (CA), all normal: H&E; 400x

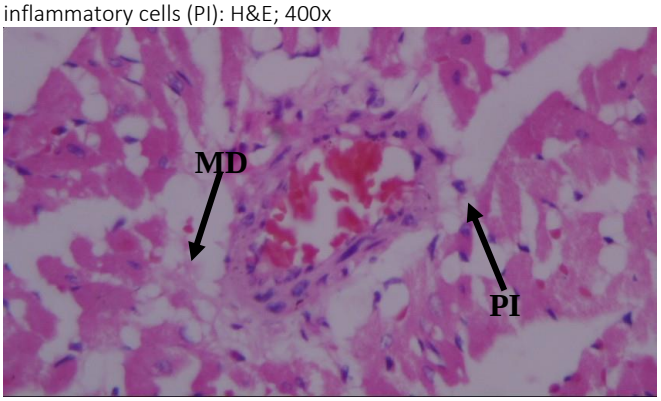

Figure 4: Photomicrograph of Rat's Cardiac histology in Group D (2mg/kg $FeCl_2$ and 400mg/kg MLECD) showing myocardial degeneration (MD), mild perivascular inflammatory infiltrates (PI): H&E; 400x

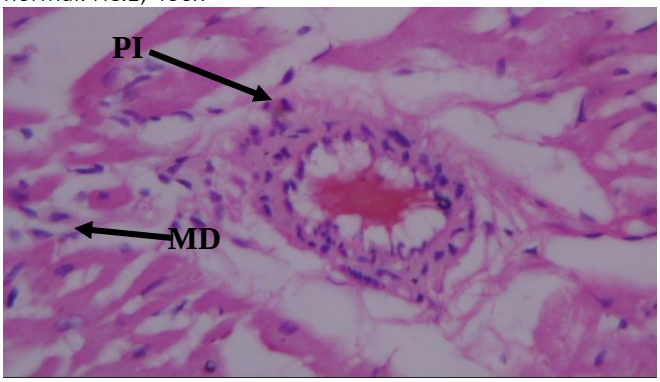

Figure 5: Photomicrograph of Rat's Cardiac histology in Group D (2mg/kg $FeCl_2$ and 800mg/kg MLECD) showing myocardial degeneration (MD), mild perivascular inflammatory infiltrates (CA): H&E; 400x

DISCUSSION

Excessive iron exposure presents a grave threat to health, unleashing a cascade of complications that can ravage the body (Kohgo *et al.,* 2008). The relentless accumulation of iron in crucial organs like the liver, heart, and endocrine glands triggers a vicious cycle of damage fueled by reactive oxygen species (Karim *et al.,* 2022). This onslaught manifests in liver afflictions such as cirrhosis and hepatocellular carcinoma (Kouroumalis *et al*., 2023), cardiac devastation in the form of cardiomyopathies, and endocrine turmoil leading to diabetes and hypothyroidism (Harrison *et al.,* 2022). Moreover, the insidious grip of iron overload leaves individuals vulnerable to infections, crippling their immune defenses and amplifying the risk of frther afflictions (Walker and Walker, 2000; Porto, 2007).

Cardiomyocytes are rich in mitochondria and consumes large amounts of oxygen (Gammella *et al.*, 2015). However, cardiomyocytes have decreased levels of antioxidant enzymes compared with other organs (Doroshow *et al*., 1980) Therefore, increased demands for oxygen along with inappropriately low levels of antioxidant enzymes make the heart highly susceptible to oxidative injury.

Histologically, accumulated iron deposits on the myocardium results in cardiomyopathy, inducing severe cardiac complications. This condition triggers oxidative stress, inflammatory responses, and cellular damage within the heart muscle, culminating in structural and functional abnormalities (Gammella *et al*., 2015). Histological findings in this study showed coronary vascular ulceration, perivascular oedema, and mild perivascular infiltrates of inflammatory cells in rats given iron only. Iron-induced vasculopathy and myocarditis were observed in groups with 400 and 800mg/kg of MLECD treatment, showing inflammations and loss of myocardial integrity and no sign of cardioprotection from excessive iron chloride exposure.

MLECD at 200mg/kg body weight proved to be effective in protecting the heart from the adverse effects of excessive iron exposure. This agrees with the therapeutic activities of *C. depenedens* in accordance with the studies "Anti-ulcerogenic effect of the methanol extract of *Chasmanthera dependens"* by Tijani *et al.* (2018), "The Effect of Chloroform Extract of *Chasmanthera dependens* on Carbon Tetrachloride (CCL_4) Induced Hepatoxicity" by Ogbozor and Anosike, (2020) and "Effect of Tannin-Rich Extract of *Chasmanthera dependens* on Piroxicam-induced Liver Damage in Male Wistar Rats" by Abiola *et al*. (2021). The 200mg/kg dose of MLECD was the most effective and was sufficient to ensure protection, this implies that there is a dose-dependent relationship, where the 400 and body weights of MLECD treatment failed to provide additional benefits and proved to be less protective suggesting that at higher doses, MLECD itself is cardiotoxic or inefficient in preventing iron accumulation in cardiac tissue. The mechanism of action to this effect is yet to be understood.

CONCLUSION

The findings proved that excessive iron exposure induced significant damage to heart tissue, however treatment with the graded doses of MLECD protected the heart from adverse effects with the 200mg/kg MLECD dose of the treated groups as the optimal dosage.

RECOMMENDATION

We recommend further research to elucidate the underlying mechanisms of action and to evaluate the long-term effects of *C. dependens* treatment on cardiovascular health.

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