Original Article

Histological Studies of the Heart and Biochemical Changes Due to the Perinatal Consumption of *Hibiscus sabdariffa* (Flavonoid-rich Extract) to Feed-restricted Rats on Offspring

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How to Cite This Article Chukwu, O. O., Emelike, C. U., Konyefom, N. G., Ibekailo, S. N., Ekakitie, O. O., Ghasi, S., et al. (2023). Histological Studies of the Heart and Biochemical Changes Due to the Perinatal Consumption of *Hibiscus sabdariffa* (Flavonoid-rich Extract) to Feed-restricted Rats on Offspring. *Iranian Journal of Veterinary Medicine*, *17*(1): 37-46. http://dx.doi.org/10.32598/ijvm.17.1.1005272

doi http://dx.doi.org/10.32598/ijvm.17.1.1005272

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ABSTRACT

Background: Experiments using animal models have indicated that maternal diet during the perinatal period can drastically impact the life-long susceptibility of offspring to chronic disease.

Objectives: This study aims to determine the impact of perinatal consumption of *Hibiscus sabdariffa* to feed restriction on offspring heart development.

Methods: In this study, we used 25 pregnant rats. They were grouped as follows: group 1 (normal control), group 2 (70% feed restriction), group 3 (70% feed restriction and 5mg/kg body weight of *H. sabdariffa* administration), group 4 (70% feed restriction, and 10mg/kg body weight of *H. sabdariffa* administration), and group 5 (70% feed restriction and 20mg/kg body weight of *H. sabdariffa* administration). Dams were only allowed to nurse 8 pups. Pups were weaned and observed daily for puberty onset. Blood samples were collected to determine serum levels of creatine kinase, hydrogen peroxide, and malondialdehyde. The hearts were excised, weighed, and prepared for histological examination.

Results: The result showed a significant increase in absolute and relative weights of the heart with alterations in the histology of the heart. There was a significant change in serum levels of creatine kinase and malondialdehyde.

Conclusion: Maternal consumption of *H. sabdariffa* during pregnancy and lactation may increase the development of cardiovascular diseases in offspring during postnatal growth.

Keywords: Body weight, Ethnopharmacology, Histology, Nutraceutical, Serum parameters

Article info:
Received: 01 Jun 2022
Accepted: 03 Sep 2022
Publish: 01 Jan 2023

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1. Introduction

The hypothesis of developmental origins of the health and disease proposes that impaired nutrition during critical stages of fetal development results in permanent changes in the structure and function of the physiological systems (programming) (Mandy and Nyirenda, 2018). The resulting phenotype offers the maximum survival chances at the available nutrition by preserving brain growth at the cost of other visceral systems (thrifty phenotype). It proposes that the same phenotype predisposes the individual to chronic medical conditions such as chronic non-communicable diseases (NCDs) when exposed to "surplus" nutrition later in life. This programming hypothesis is known as the Barker hypothesis. It states that "a mismatch between the intrauterine and extrauterine environment results in the birth of a child, is inadequately adapted for function in the extrauterine environment, and is at risk for later development of chronic medical conditions such as NCDs" (Morton & Brodsky, 2016).

The intrauterine environment is key in the etiology of the diseases of the heart and blood vessels in old age. Malhotra et al. (2019) observed the connection between birth weight and surroundings as a unique part of the etiology of cardiovascular disorders. The study revealed that the developmental programming of adult illness arises due to inequality during intrauterine. The fetal requirement and nutrient supply result in fetal undernutrition. The impairment in fetal maturation is characterized by decreased birth weight and fetal maturation limitation, which occurs as intrauterine transformations leading to anatomical and physiological adaptative modifications. These transformations permit intrauterine endurance and substantial rhyme in the physiology and anatomy of the cardiovascular organs (Malhotra et al., 2019). Food limitation during gestation causes growth-limited infants demonstrating that the attained development occurred in more rotund grown-up progeny, with moderately declined poundages of the body organs (Zohdi et al., 2015).

In Nigeria, the general consumption of *Hibiscus sabdariffa* by humans, particularly gestational and lactating mums, is certainly not for therapeutic intentions but the replacement of beverages, no doubt considering the body's normal function (Chukwu et al., 2021). Few studies have shown that consumption of *H. sabdariffa* extracts during gestation and lactation improves the postnatal poundage increase, absence of the start of sexual maturation, and raised body mass index at puberty (Enwerem et al., 2016; Chukwu et al., 2021; Sattar Ali, 2022). Flavonoids exist as polyphenolic phytochemicals noticed in foodstuffs and beverages. They are marked by various chemical compounds and physiological belongings (Formaggio et al., 2015; Tungmunnithum et al., 2018; Chukwu et al., 2021; Mohammed et al., 2021). The study of flavonoids is noteworthy. At high doses, the negative consequences of flavonoids may outrank their advantages. The current research sought to determine the impact of perinatal consumption of *H. sabdariffa* to feed restriction on offspring heart development (histological and biochemical changes).

2. Materials and Methods

Plant materials

H. sabdariffa (ripped calyces) were bought from the Margaret Umahi International Market, Abakaliki, Ebonyi State. A taxonomist affirmed the plant samples at the Department of Plant Science and Biotechnology, University of Nigeria. The plant was deposited in the herbarium with a voucher specimen (UNH No.75a).

Preparation of extract

The methanol extraction of *H. sabdariffa* was done following the standard method (Chukwu et al., 2021). The extract was screened for the existence of phytochemicals using the methods mentioned in the studies (Gul et al., 2017; Emelike et al., 2021). The flavonoid content was determined using the procedure of Formagio et al. (2015).

Experimental design

In this study, 25 mature inbred healthy virgin female rats were obtained from the Animal House, Department of Physiology, Faculty of Basic Medical Sciences, College of Medical Sciences, the Alex Ekwueme Federal University Ndufu Alike Ikwo (AE-FUNAI), Ebonyi State. The animals were maintained in standard environmental conditions (25°C±2°C, 12:12 h dark:light cycle). Animal studies were done after ethical approval by the Faculty of Basic Medical Sciences Research Ethics Committee, AE-FUNAI, which was in line with the ethical guidelines for the care and usage of laboratory animals as given by the National Institute of Health.

Initiation of pregnancy

Mating and pregnancy were done using the method described by Ajayi and Akhigbe (2020) and Chukwu et al. (2021).

Grouping of animal

Dams were housed individually throughout pregnancy and lactation. The dams in groups 2 to 5 were placed on a 70% feed-restriction diet with oral gavage administration of the extract throughout gestation and lactation.

Study groups

Group 1 consisted of normal rats fed with 0% feed restriction and water ad libitum. Group 2, or feed-restricted control, consisted of rats with 70% feed restriction and water ad libitum. Group 3 comprised rats with 70% feed restriction and water ad libitum plus 5mg/kg body weight of *H. sabdariffa* (flavonoid-rich) administration. Group 4 consisted of rats with 70% feed restriction and water ad libitum plus 10 mg/kg body weight of *H. sabdariffa* (flavonoid-rich) administration. Group 5 consisted of rats with 70% feed restriction and water ad libitum plus 20 mg/kg body weight of *H. sabdariffa* (flavonoid-rich) administration.

To eradicate malnourishment or overeating in the pups, only 8 pups were allowed to stay with the dam in each group after delivery. After 21 days, the pups were weaned to 0% feed restriction and water ad libitum. The pups took groups of their dams. The female pups were housed in different cages from the male pups.

Samples collection

The rats were monitored daily for vaginal opening in females and balano-preputial separation in males since puberty set in after weaning around 21 days (Chukwu et al., 2021). At the onset of puberty, two male and two female pups were randomly selected from each group. The body weights of the rats were recorded. The rats were anesthetized with 2% sodium pentobarbital (75mg/kg) intraperitoneally. Venous blood was obtained via the orbital (Salami and Raji, 2015) and poured into plain tubes, and allowed to clot. Sera were obtained from the clotted sample after centrifuging at 3000 rpm for 10 min and stored at -20°C until further use to analyze biochemical parameters. The rats were later sacrificed by cervical dislocation, and their hearts were harvested and weighed (Emelike et al., 2020).

Biochemical assessment

Serum levels of creatine kinase (CK) enzyme activities were measured using the CK activity assay kits (CK-20; Sigma Chemical Co.) as described for CK activity in serum by Kucherenko et al. (2015). The serum levels of malondialdehyde (MDA) were measured using the thiobarbituric acid assay following Khoubnasabjafari (2015) method. The amount of hydrogen peroxide (H_2O_2) in serum samples was measured following Hadwan's (2018) method.

Histological studies

The harvested organs (heart) were preserved in buffered 10% formalin saline solution for histopathological processing following the Emelike et al. (2020), Aloke et al. (2021), Badi et al. (2022) and Rahmati et al. (2022) methods. Next, the heart weight/body weight ratio was calculated (Emelike et al., 2020).

Statistical analysis

Data were analyzed by 1-way analysis of variance using GraphPad Prism (GraphPad® Software, San Diego, CA, USA) and presented as Mean±SEM. The Tukey post hoc test was also used for the differences between the groups. $P<0.05$ was considered to indicate a significant difference.

3. Results

There existed a significant ($P<0.05$) increase in absolute organ weight of the heart in groups 3, 4, and 5 compared to group 1 (normal control) and group 2 (feed-restricted control). While group 2 had no significant ($P<0.05$) increase compared to group 1. There was no significant ($P<0.05$) increase in the relative organ weight of the heart in groups 3, 4, and 5 compared to group 1 (normal control) and group 2 (feed-restricted control). Group 2 had no significant ($P<0.05$) increase, similar to group 1. While group 5 had a significant ($P<0.05$) increase in the relative organ weight of the heart compared to groups 3 and 4. There was a significant ($P<0.05$) increase in body weight and length in groups 3, 4, and 5 compared to group 1 (normal control) and group 2 (feed-restricted control). Group 2 had a significant ($P<0.05$) increase compared to group 1 (Table 1).

There was a significant ($P<0.05$) increase in serum levels of CK in groups 3, 4, and 5 compared to group 1 (normal control). Groups 4 and 5 only had a significant ($P<0.05$) increase compared to group 2. There was also a significant ($P<0.05$) increase in serum levels of MDA in groups 3, 4, and 5, similar to group 1 (normal control) and group 2 (feed-restricted control). Group 2 also had a significant ($P<0.05$) increase compared to group 1. There was also an increase in serum levels of H_2O_2 in group 3, group 4, and group 5 compared to group 1 (nor-

Table 1. Effects of perinatal administration of flavonoid-rich methanol extract of hibiscus sabdariffa to food-restricted rats on offspring organ (heart), weight, and body parameters at the onset of puberty

Variables	Mean±SEM				
	Group 1	Group 2	Group 3	Group 4	Group 5
Absolute heart weight (g)	0.21±0.00	0.23±0.01	0.27±0.00**	0.30±0.01**	0.31±0.01**
Relative heart weight (g)	0.0048±0.0001	0.0048±0.0001	0.0056±0.0001	0.0050±0.0002	0.0044±0.0001
Body weight (g)	44.83±0.35	47.80±0.58*	53.73±0.58**	58.95±0.95**	71.13±0.83**
Body length (cm)	13.28±0.06	13.60±0.04*	14.38±0.06**	14.60±0.04**	14.73±0.05**

* $P<0.05$ versus normal control (group 1); ** $P<0.05$ versus feed-restricted control (group 2)

mal control) and group 2, though not significant (Table 2).

The photomicrograph revealed degenerative changes in aggregate myocardial inflammation, moderate intra-myocardial hemorrhage, focal area necrosis, and dilated cardiac fibers compared to the control group (Figures 1-5).

4. Discussion

The increase in body weight and length recorded in offspring of dams suggest that the possible corticosterone-enhanced lactation in the *H. sabdariffa* dams and the growth-promoting constituents (flavonoids) in the *H. sabdariffa* extract may be responsible for the accelerated rate of weight and length gain in the offspring of the *H. sabdariffa* dams during the period of lactation (Clemente et al., 2016; Briffa et al., 2019). Flavonoids in *H. sabdariffa* extract impede the action of 11β-hydroxysteroid dehydrogenase type-2 (11βHSD-2). It decreases the transformation of the active glucocorticoid to the inactive form causing increased glucocorticoid levels that affect the fetus (Janssen et al., 2015; Beck et al., 2017). The postpartum decline in 11βHSD-2 activity and increased active glucocorticoids is a prerequisite for the endocrine induction of lactation. Glucocorticoids induce the expression of milk proteins such as casein and lactalbumin (Zheng et al., 2016). Also, earlier researchers have suggested that flavonoids impede the action of 11βHSD-2 (Clayton et al., 2015; Beck et al., 2017). The consumption of the extract of *H. sabdariffa* during lactation is rich in flavonoids (Zheng et al., 2016), further increasing the concentration of active glucocorticoids. This event explains the raised corticosterone level, as observed in the study by Iyare and Adegoke (2008), and may contribute to the lactation process and thus enhance nutrient delivery to the suckling neonates.

The elevation in absolute and relative organ weights of the heart in offspring is presented in Table 1, which might be due to increased metabolic activities of the heart. This event results in the blood-pumping action of the heart following the pathophysiology of oxidative

Table 2. Effects of perinatal administration of flavo noid-rich methanol extract of hibiscus sabdariffa to food-restricted rats on offspring serum levels of biochemical parameters

Groups	Mean±SEM		
	Creatine kinase (U/L)	Malondialdehyde (mg/mL)	H_2O_2 (µmole)
1	20.39±0.19	4.73±0.13	0.46±0.01
2	21.69±1.60	5.15±0.08*	0.48±0.01
3	24.67±0.96*	5.68±0.07**	0.49±0.02
4	27.05±0.69**	5.91±0.05**	0.51±0.01
5	28.47±0.31**	6.12±0.06**	0.53±0.02

* $P<0.05$ versus normal control (group 1); ** $P<0.05$ versus feed-restricted control (group 2)

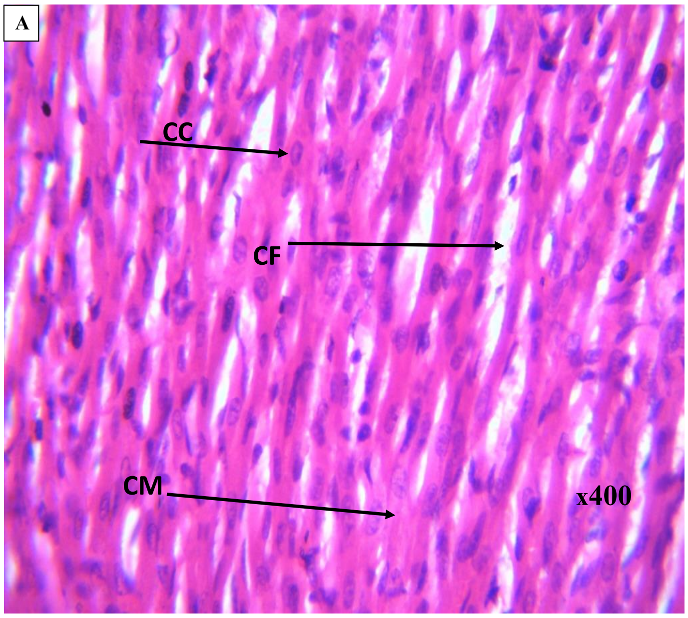

Figure 1. Photomicrograph of group 1 section of heart

(x400) (H/E) showing normal cardiac tissue with normal Cardiac Fiber (CF), cardiac cell (CC) and cardiac muscles (CM)

myocardial impairment, slaying of cells by the death of the cell body, or programmed cell death, which may have resulted from free radicals, such as reactive oxygen species. In pathophysiological conditions, excess production of reactive oxygen species (ROS) prevails over the antioxidant defenses leading to oxidative stress is deleterious as the excess ROS is responsible for causing the injury of cellular components (Carta et al., 2017). The increased heart weight could be attributed to the observed myocardial degenerative changes that might result in poor oxygen carriage. The heart needs to pump more blood leading to increased metabolic activities, resulting in enlargement and an increase in heart weight.

The increase in serum levels of H_2O_2 and MDA in offspring of dams suggests that oxidative stress may play a critical role in these observed degenerative changes that may cause the pathogenesis of hypertension and other cardiovascular diseases. MDA (as an indicator of lipid peroxidation in vitro and in vivo) and H_2O_2 (as ROS)

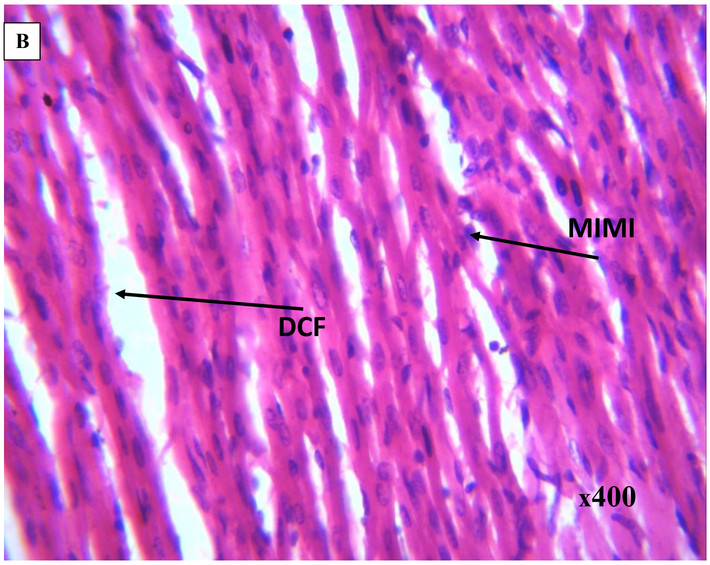

Figure 2. Photomicrograph of group 2 section of heart

(x400) (H/E) showing mild to moderate effect on the cardiac tissue with Mild Infiltration Myocardial Inflammation (MIMI) and Dilated Cardiac Fibers (DCF)

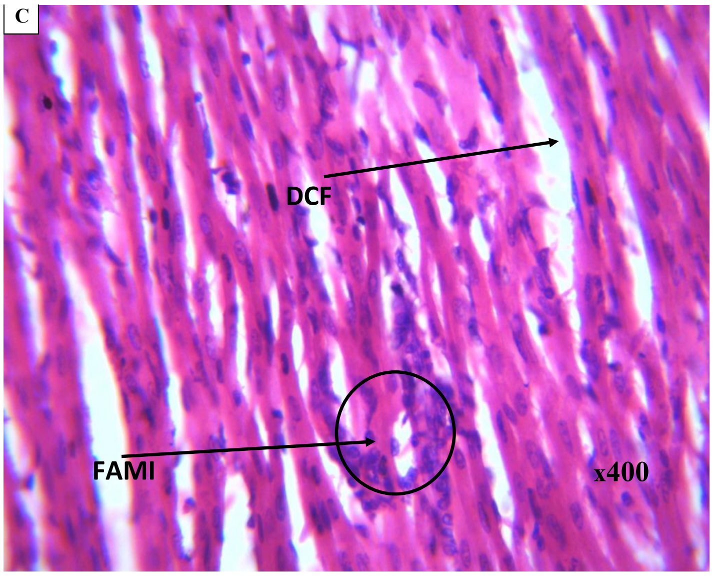

Figure 3. Photomicrograph of group 3 section of heart

(x400) (H/E) showing mild to moderate effect on the cardiac tissue with focal aggregate myocardial inflammation (FAMI) and dilated cardiac fibers (DCF)

in blood serum or tissue homogenates are methods to predict the levels of oxidative stress. It contributes to vascular impairment via stimulating cell maturation, extracellular matrix protein precipitate, endothelial dysfunction, and raised vascular fashion, distinct components of the vascular phenotype in hypertension (Tangvarasittichai, 2015). Substantial proof has been stockpiled in recent decades that oxidative stress supports the outcome of heart disorders and advances to heart failure (Vejrup et al., 2021). Human studies have shown that higher serum levels of MDA are seen in hypertensive patients compared to normotensive control (Armas-Padilla et al., 2007). Also, increased levels of MDA and decreased levels of catalase in hypertensive pregnant women (Bosch et al., 2015).

There is a significant ($P<0.05$) increase in serum levels of MDA in the progeny of dams in group 2 compared to group 1. This study result agrees with earlier research on oxidative tension generated by food limitations. It suggests that fare constraint alters the operation of the glutathione biological process and

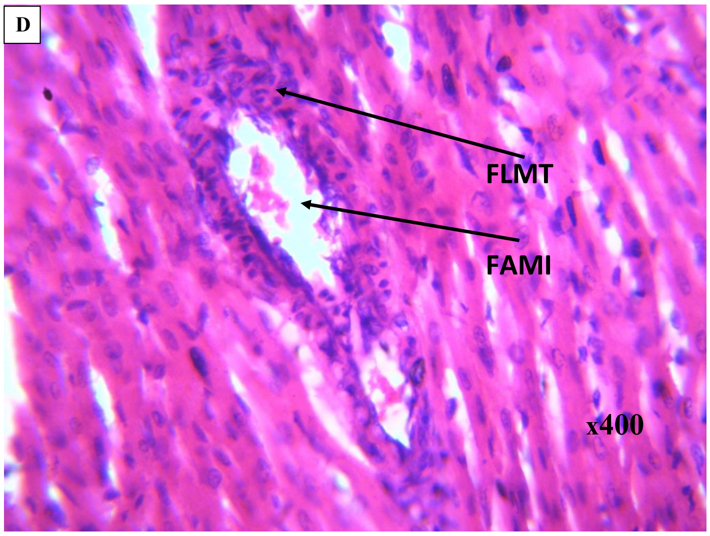

Figure 4. Photomicrograph of group 4 section of heart

(x400) (H/E) showing moderate effect on the cardiac tissue with moderate focal loss of myocardial tissue (FLMT) and moderate focal aggregate myocardial inflammation (FAMI)

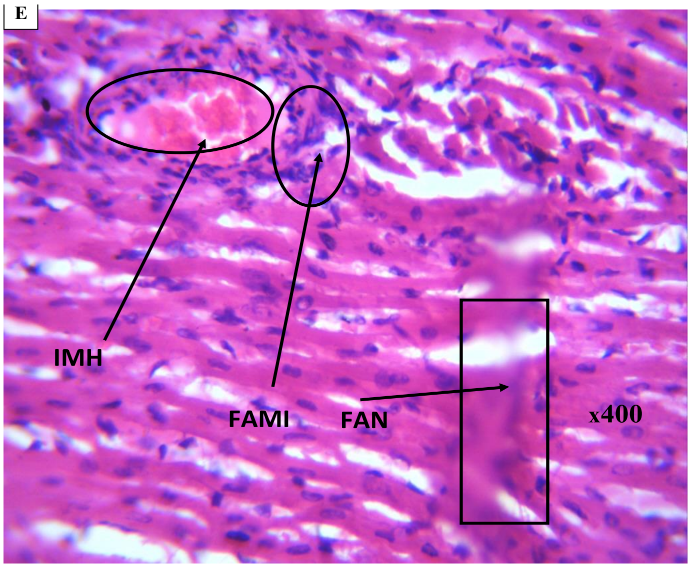

Figure 5. Photomicrograph of group 5 section of heart

(x400)(H/E) showing moderate to severe effect on the cardiac tissue with moderate focal aggregate myocardial inflammation (FAMI), moderate intra myocardial hemorrhage (IMH), and focal area necrosis (FAN)

changes the antioxidant defense mechanism (Bosch et al., 2015; Emelike et al., 2021). The ROS activities developed by dietary limitation ushering to oxidative tension have been reported by researchers (Bosch et al., 2015). The increased production of superoxide anion and hydrogen peroxide reduced nitric oxide synthesis. The decreased bioavailability of antioxidants has been documented in experimental and human hypertension (Camara et al., 2017).

The increase in serum levels of CK in offspring of dams can be attributed to degenerative changes since creatine kinase is an intracellular enzyme in striated and smooth muscles and the brain. Creatine kinase is an enzyme regulator of high-energy phosphate production and utilization in contractile tissues. Rat serum or plasma CK activity is widely used for the quantitative evaluation of myocardial infarction (Camara et al., 2017).

Other markers include myopathic processes, muscle damage following intramuscular injections, and testing the myotoxicity of different drugs (Oerbeck et al., 2017). Although not all myopathies produce a rise in CK activity, it is increased in muscle fiber destruction after mechanical trauma, toxic injury, or alteration of enzymatic or structural proteins. CK elevation also varies within disorders. The increased CK concentration in pups of dams that consumed flavonoids from *H. sabdariffa* may indicate an acute myocardial injury and or myocarditis (Abel et al., 2017). These findings suggest the accumulation of free radicals in cardiac tissue, causing injuries to intracellular components of the myocardium and its membranes. Injuries to the myocardial membranes result in the release of CK in serum or plasma. CK remains the standard biomarker for cardiac injuries. The cardiac tissues are particularly vulnerable to free radical injuries as they contain low levels of antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase.

This condition may have resulted from free radicals and reactive oxygen species and played a function in the pathophysiology of oxidative myocardial impairment and destroying cells by the death of the cell body or programmed cell death (Cioffi et al., 2017). Maternal malnutrition may have reduced cardiomyocytes in developing pups. It can boost age-associated vascular and structural modifications and improve excess deposition of extracellular matrix in the cardiac muscle, formation, and development of a network of capillaries at maturity (Corstius et al., 2005; Skilton et al., 2005). A study by Cheema et al. (2005) reported altered heart morphology and reduced heart cell mass during development because of the adverse effects of maternal malnutrition. These changes in the heart morphology of pups of rats that consumed flavonoid-rich methanol extract from *H. sabdariffa* may also accrue to increased norepinephrine levels (Tricò et al., 2020).

5. Conclusion

We can say that since the offspring of *H. sabdariffa* dams were at no period during their growth administrated, the flavonoid-rich methanol extract from

H. sabdariffa, the myocardial degenerative changes suggest that oxidative stress and reactive oxygen species may have played a role in the pathophysiology of myocardial deterioration and in destroying cells by the death of the cell body or programmed cell death, ushering to myocardial injury.

Ethical Considerations

Compliance with ethical guidelines

There were no ethical considerations to be considered in this research.

Funding

This research did not receive any grant from funding agencies in the public, commercial, or non-profit sectors.

Authors' contributions

All authors equally contributed to preparing this article.

Conflict of interest

The authors declared no conflict of interest.

Acknowledgments

The authors thank the Department of Physiology, Faculty of Basic Medical Sciences, College of Medical Sciences, AE-FUNAI, Ebonyi State, for supporting this study.

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