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ANTI-CARDIOTOXIC POTENTIAL OF FERMENTED UNRIPE PAWPAW (CARICA PAPAYA) JUICE ON DICLOFENAC INDUCED CARDIOTOXICITY IN WISTAR RATS

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ABSTRACT

Pawpaw (Carica papaya) is a widely recognized medicinal plant with diverse therapeutic applications. This research focuses on exploring the potential cardioprotective effects of fermented unripe pawpaw juice in mitigating diclofenac-induced cardiotoxicity in Wistar rats.. Twenty healthy adult female Wistar rats were utilized, with weights ranging from 110g to 200g The rats were allocated into four groups using random assignment:. Group 1 Control group: received distilled water and standard feed , Group 2 Diclofenac group: administered 10mg/kg body weight of diclofenac., Group 3 Diclofenac + Pawpaw extract group: received diclofenac and post-treated with 200mg/kg body weight of fermented unripe Carica papaya juice, andGroup 4 Pawpaw extract group: administered 200mg/kg body weight of fermented unripe Carica papaya juice . The plant extract and diclofenac were orally given over a duration of fourteen (14) days. After the treatment was finished, samples of blood and tissue were collected to analyze cardiac biomarkers, oxidative stress markers, and for histological evaluation. Statistical analysis was done using SPSS veriosn 23 and values with $p<0.05$ is considered significant. The result revealed a significant increase in LDH, CK, Total Cholesterol, triglyceride and MDA levels, and reduction in SOD, CAT and GSH activities in diclofenac induced rats. However, post treatment with fermented unripe pawpaw (carica papaya) juice causes significant reduction in LDH, CK, Total Cholesterol, triglyceride and MDA levels, and significant increase in SOD, CAT and GSH activities in diclofenac induced rats. The study highlights the potential of fermented unripe pawpaw juice in alleviating diclofenac-induced cardiotoxicity in male rats by mitigating oxidative stress These findings underscore the therapeutic promise of pawpaw as a natural cardioprotective agent, warranting further exploration in clinical settings for potential human applications In conclusion, the study established the ability of fermented unripe pawpaw (Carica papaya) juice in ameliorating diclofenac-induced cardiotoxicity in male rats by reducing oxidative stress.

KEYWORDS: fermented unripe Carica papaya juice diclofenac Anti-cardiotoxic biomarkers.

INTRODUCTION

Non-steroidal anti-inflammatory medicines (NSAIDs) are frequently prescribed to alleviate pain and reduce inflammation in patients with rheumatoid and osteoarthritis.[16] NSAIDs function by suppressing the activity of cyclooxygenase-1 and cyclooxygenase-2 enzymes, hence diminishing the synthesis of thromboxane and prostaglandin.[14] However, chronic NSAID use can lead to adverse effects like gastrointestinal injury, renal, and cardiovascular damage.[25][12]

Diclofenac, a potent NSAID inhibiting COX-1 and COX-2 enzymes, is used for pain and inflammation management.[29][56] Despite its effectiveness, diclofenac is associated with harmful effects such as gastrointestinal bleeding, nephrotoxicity, hepatotoxicity, and cardiotoxicity.[34] The cardiac damage of diclofenac is associated with an elevation in reactive oxygen species (ROS) production and a decrease in the ability of myocytes to defend against oxidative stress. This impacts the functioning of mitochondria and ultimately leads to programmed cell death, known as apoptosis.[1] Diclofenac, a commonly prescribed nonsteroidal anti-inflammatory drug (NSAID), has been shown to have

detrimental effects on cardiac cells by inhibiting the function of mitochondrial complex III, leading to an excessive generation of reactive oxygen species (ROS) in cardiomyocytes.[36][17] An in vitro study conducted recently discovered that exposure to diclofenac resulted in a reduction in mitochondrial membrane potential and cardiotoxicity in immortalized human cardiomyocytes, ultimately leading to cell death.[60] Furthermore, research has identified diclofenac as a significant contributor to the elevated risk of cardiovascular issues. This is because it disrupts the balance between factors that encourage blood clotting and those that prevent it, which can lead to the occurrence and worsening of cardiovascular disease.[36][43] Long-term diclofenac use can elevate cardiovascular risk by promoting thrombotic events, potentially leading to myocardial infarction and stroke.[66]

Medicinal sources derived from plants have been historically abundant and play a crucial role in managing various diseases.[5] The herbal sources of active ingredients have gained prominence due to their safety and efficacy compared to synthetic drugs, which are often associated with environmental concerns This shift towards herbal remedies is part of a global herbal renaissance, reflecting a growing interest in natural and sustainable healthcare options.[54] Plants contain a myriad of active components with medicinal properties, and the concentration of these components varies across different plant structures. These bioactive compounds are responsible for eliciting physiological changes in the body, leading to therapeutic effects[59] For instance, Carica papaya, commonly known as papaya, has been extensively studied for its pharmacological importance, showcasing anti-inflammatory, anti-fertility, hepatoprotective, wound healing, antihypertensive, and antitumor activities.[63] The utilization of plant materials for medicinal purposes continues to be a significant area of research, with ongoing studies exploring the diverse health benefits and therapeutic potentials of natural remedies. This emphasis on herbal medicine aligns with the increasing recognition of the value of traditional and plant-based treatments in modern healthcare practices.[63]

In contrast to synthetic drugs, herbal sources like Carica papaya (pawpaw) offer natural alternatives with medicinal properties. Pawpaw, rich in nutrients and active compounds, has shown anti-inflammatory, anti-fertility, hepatoprotective, and wound healing properties. Papaya leaves exhibit anti-dengue, anti-cancer, antibacterial, and antioxidant effects, making them valuable in traditional medicine for various conditions. The plant's enzyme papain is used for allergies and sports injuries, while its fruit aids in cardiovascular health, immunity, and cancer prevention.[63] The papaya plant has special cells called laticifers[65] The pharmacological significance of pawpaw lies in its laticifers and active components, contributing to its diverse therapeutic effects. Studies have highlighted the efficacy of pawpaw in treating conditions like kidney failure, low sperm count, dental issues, heart problems, and fibroids in the uterus. In terms of its pharmacological significance, Carica papaya, commonly known as papaya, is a nutrient-rich fruit with various health benefits. Papaya leaves, rich in active components and laticifers, have been studied for their anti-inflammatory, anti-fertility, hepatoprotective, and antitumor properties. The enzyme papain tha found in papaya is utilized in allergy treatment and sports injuries. Papaya's nutrients contribute to cardiovascular health, protection against heart attacks and colon cancer, and boosting immunity. The fruit's beta-carotene content fights free radicals, while its fiber content helps maintain healthy cholesterol levels. Papaya is beneficial for abdominal disorders, wound healing, and immune support against cough. Additionally, Carica papaya's alkaline combination with K2CO3 has shown efficacy in tumor treatment and various skin conditions like warts and sinuses.[65]

Papaya leaves and seeds offer a range of health benefits, including treating dengue fever, preventing malaria, aiding digestion, preventing cancer, boosting immunity, and promoting heart health. Papaya leaves are rich in antioxidants, vitamins, and nutrients like lycopene, phenolic compounds, and alkaloids that enhance immunity and prevent cancer-cell development.[46] Additionally, papaya leaves can help regulate blood sugar levels, lower cholesterol, aid in weight loss, ease menstrual cramps, and support skin and hair health. Consuming papaya daily can contribute to overall well-being.[62][51]

Plants have been used as a source of medicines for centuries, with many herbs and plant-derived compounds having therapeutic properties that can be used in the management of various diseases, including cardiovascular diseases (CVDs).[53][52][11]

These plant-based treatments are derived from natural sources and are biologically compatible. They produce specific components that help defend against attacks from both small and large predators.[53][52][11]

Interestingly, many chemicals, including polyphenolic substances, melatonin, carotenoids, quercetin, resveratrol, vitamin E, vitamin C, L-carnitine, and coenzyme Q10, have been discovered to have therapeutic qualities that can be utilized by humans for the treatment of disorders.[53][52][11]

The utilization of plants for the management of cardiovascular diseases (CVDs) has been extensively recorded in both traditional and contemporary medicine.[53][52][11]

Some herbs, such as Daucus carota, Nerium oleander, Amaranthus Viridis, Ginkgo biloba, Terminalia arjuna, and Citrus medica L., have been found to possess cardioprotective properties due to their potent

antioxidant and free radical scavenging activities).[53][52][11][55]

These herbs have been shown to regulate blood pressure, break down cholesterol and fat deposits in the body, and improve blood and lipid profiles, making them valuable in the management of CVDs).[53][52][11]

Herbs possess not only cardioprotective characteristics, but also exhibit anti-inflammatory, anti-thrombotic, and anti-arrhythmic activities. They are useful in the treatment of a number of cardiovascular diseases, such as cerebral insufficiency, systolic hypertension, angina pectoris, atherosclerosis, and congestive heart failure.[53][52][11] Crataegus monogyna, Terminalia arjuna, and Hawthorn herbs have demonstrated the ability to enhance cardiac function, diminish oxidative stress, and enhance the conversion of LDL or "bad" cholesterol to HDL or "good" cholesterol in the liver.[53][52][11]

However, it is important to note that the use of herbs in the treatment of CVDs is not without risks, and adverse reactions to herbal drugs have been reported.[52][11]

Hence, it is imperative to carry out additional research in order to comprehensively comprehend the mechanisms of action and possible negative consequences of these herbs, in order to guarantee their secure and efficient utilization in the treatment of cardiovascular diseases.[52][11]

Plants are a valuable source of medicines, with many herbs and plant-derived compounds possessing therapeutic properties that can be used in the management of CVDs. These herbs have been shown to possess cardioprotective, anti-inflammatory, anti-thrombotic, and anti-arrhythmic effects, making them useful in the management of various CVDs).[53][52][11]

Previous studies have shown that several diseases and most health-related issues can be taken care of by various forms of the use of the plant Pawpaw. And several preparations and applications of pawpaw results to its various health management therapies. Pawpaw is an important therapeutic drug that can be used for some health issues such as, diabetes, Hepatic disorders, cardiovascular diseases, and Infections.[7]

MATERIALS

Equipment/Apparatus

The equipment/ apparatus used include: Shinlon Grinding mill (model: FFC-15 output 1.1kw); Water bath (electric thermostat water tank; model: HH-W21-Cr4211); Micropipette (Jencons scientific Ltd.); Centrifuge (TDL-4 Bran scientific and instrument company England) ; Spectrophotometer (S23A Techmal and Techmel USA); Weighing balance (Kern 770-15); Scale (Atom electric computer scale, ATOM-A-122); Beaker; Measuring cylinder; hand gloves, conical flask; oral needle; spatula; cheese cloth, dissecting set. Plain bottles, cotton wool; test tubes and racks; mortar and pestle.

Chemicals/Reagents

Diclofenac sodium (DIC) was purchased from Danson Pharmaceutical Company, Opolo Yenagoa Bayelsa State, Nigeria. Chloroform, and Formal Saline was purchased from Angel Medical Store, Onopa, Yenagoa, Bayelsa State.

Animals

Twenty (20) healthy adult female Wister rats weighing between 110g to 200g were obtained from the animal house of Niger Delta University, Amassoma, Bayelsa state and were allowed to acclimatized for two weeks during which they were fed with standard feed (Pellet) and distilled water ad libitum. The rats were housed in plastic cages at a room temperature of about 27°C and photoperiodicity of 12-hour light/12-hour dark. The procedures were conducted in compliance with the National Institutes of Health handbook for the care and treatment of animals used in research (NIH Publications No. 8023, amended 1978).

Collection and Preparation of Extracts

Collection of Plant

The medicinal plant used in this study was the fermented juice of unripe Carica Papaya(pawpaw). The Unripe Carica Papaya fruits were purchased from a Swali market in Yenagoa, Bayelsa State. This was transported to the laboratory in Niger Delta University for use.

Fermented unripe pawpaw juice Extract Preparation

Unripe Carica papaya fruits underwent a washing process to eliminate any dirt and latex. Subsequently, the fruits were peeled and just the flesh was cut into little pieces. The juice was extracted from fresh fruit using a juice extractor and then immediately cooled on ice to prevent the breakdown of biomolecules and antioxidants. The UCP juice underwent filtration using sterile quality paper (Whatman® grade 1 filter paper) and was subsequently stored in closed bottles, away from sunlight, for a duration of 3 days to facilitate proper fermentation.

Experimental Design

The rats were divided into four (4) groups with each group consisting of 5 rats each.

Group 1 (Negative control): Received distilled water and pellet feed for14days.

Group 2 (Test group): Received 10mg/kg per body weight of diclofenac daily by oral route for 14 days.

Group 3 (Test group): Received 10mg/kg per body weight of diclofenac daily by oral route for 14 days and post treated with 200mg/kg body weight of fermented unripe juice of Carica papaya orally for 14 days.

Group 4: Received 200mg/kg body weight of fermented unripe juice of Carica papaya only orally for 14 days.

Sample Collection

After a period of twenty-four hours following the final treatment, the animals were weighed again and then rendered unconscious using chloroform. The blood from each animal was obtained using heart puncture and collected in plain tubes. The tubes were subjected to centrifugation at a speed of 5000 revolutions per minute for a duration of 5 minutes using a swing-bucket centrifuge in order to separate the serum. The serum was collected into several simple tubes for the evaluation of certain indicators of renal and hepatic functioning. A portion of the rat's heart was meticulously removed, measured, and preserved in a 10% formalin solution for histological analysis using hematoxylin and eosin staining.

Ethics

The ethical committee of Niger Delta University Amassoma, Bayelsa State, Nigeria approved all animal protocols, adhering to the National Institutes of Health's Principles of Laboratory Animal Care.[69] The animals were provided with compassionate care in accordance with the standards set out in the "Guide for the Care and Use of Laboratory Animals (1996)" published by the National Academy of Sciences.

Estimations of Biochemical Parameters

Serum parameters

Animals were euthanized on the 15th day of the research. Blood was collected and stored in basic vials for biochemical examination. In order to conduct a histological examination, the heart was extracted and stored in a solution of 10% formalin.

The serum was isolated and the levels of lactate dehydrogenase (LDH) and creatine phosphokinase-MB isoenzyme (CK-MB) were determined using standard kits from Merck Specialities Pvt. Ltd., India. The levels of serum total cholesterol (CHOL) and triglycerides (TG) were measured using enzymatic kits from Accurex Biomedical limited Pvt. Ltd, India, following the instructions provided in the manufacturer's manual.

Determination of Markers of Oxidative stress.

This was by measuring Malondialdehyde (MDA)[70] and superoxide dismutase (SOD)[72] catalase[70] as well as reduced Glutathione GSH.[71]

Statistical Analysis

Data was expressed as Mean ± Standard deviation. The statistical significance was evaluated by ANOVA using SPSS (Statistical Package for Social Sciences). Values wereconsidered statistically significant when <0.05.

3.1: Mean Serum values of lipid profile and Cardiac function Parameters in Adult Albino Rats Exposed to Diclofenac and fermented unripe carica papaya.

Parameters	GP 1	GP 2	GP 3	GP 4	F	P	RMK Statistics value
LDH (UI/L)	13.83±3.10	48.39±6.01a	19.50±4.22ab	13.60±4.7	21.40	0.000	S
CK (UI/L)	14.35±3.56	41.62±5.32a	22.93±4.12ab	14.73±4.12	1.36	0.010	S
Tchol(nmol/L)	139.32±15.20	158.14±15.99a	140.58±14.40ab	139.56±23.02	0.11	0.040	S
TRIG(mmol/L)	3.79±1.00	7.86±2.24a	3.34±1.10ab	3.17±1.25	1.27	0.001	S

Key: Values with superscript alphabet is considered significant (p<0.05). ANOVA was used to compare the different groups. All post hoc testing were done using Bonferroni multiple comparison. GP I = Negative Control, GP 2 = Diclofenac treated, GP 3 = 10mg/kg per body weight of diclofenac + 200mg/kg fermented unripe pawpaw (carica papaya) Juice; GP 4 = Received 200mg/kg body weight of leaves of fermented unripe pawpaw (carica papaya) Juiceonly orally for 14 days.
LDH = Lactate Dehydrogenase
CK= Creatinine Kinase
Tchol= Total cholesterol
TRIG=Triglyceride
NS = Not Significant
S = Significant

All post hoc testing were done using Bonferroni multiple comparison. a Significant difference observed in the LDH levels between GRP 1 and GRP 2, p=.000. ab Significantdifference observed in the LDHlevels between GRP 3 and GRP 2, p =.021.a Significantdifference observed in the CKlevels between GRP 1 and GRP 2, p=.000. ab Significant difference observed in CKlevels between GRP 2 and GRP 3, p =.002. aSignificant difference observed in the Tchollevels between GRP 1 and GRP 2, p=.020. abSignificant difference observed in the Tchol levels between GRP 2 and GRP 3, p=.001.aSignificant difference observed in the TRIG levels between GRP 1 and GRP 2, p=.000. abSignificant difference observed in the TRIG levels between GRP 2 and GRP 3, p=.001.

Table3.1 showed the Effect of Diclofenac and fermented unripe pawpaw (carica papaya) Juice on Cardiac Parameters in Adult Albino Rats Exposed to Diclofenac. The result revealed a statistically significant (p<0.05) increase in serum LDH (48.39±6.01), CK (41.62±5.32),

Total cholesterol (158.14±15.99) and triglyceride (7.86±2.24) levels in the diclofenac alone treated group (group 2) when compared with the group 1 (control group) (13.83±3.10; 14.35±3.56; 139.32±15.20 and 3.79±1.00) respectively. However, post treatment with 200mg/kg body weight of fermented unripe pawpaw (carica papaya) Juice (group 3) causes drastic reduction in the serum LDH (19.50±4.22), CK (22.93±4.12), total cholesterol (140.58±14.40) and TAG (3.34±1.10) levels when compared with the control (group 1). There was no significant difference observed in the serum LDH (13.60±4.72), CK (14.73±4.12), Total cholesterol (139.56±23.02) andTAG (3.17±1.25) levelsof of fermented unripe pawpaw (carica papaya) Juice alone treated rats (group 4) when compared with the group 1 (13.83±3.10; 14.35±3.56; 139.32±15.20 and 3.79±1.00) respectively.

3.2: Mean cardiac tissue values of Oxidative Stress Biomarkers in Adult Albino Rats Exposed to Diclofenac and fermented unripe (carica papaya) pawpaw Juice.

Parameters	GP 1	GP 2	GP 3	GP 4	F	P-RMK Statistics value
SOD (UI/L)	7.08±2.41	2.90±1.02a	5 .62±2.31ab	6.94±2.2	20.730.001	S
CAT (UI/L)	5.97±2.15	2.95±1.12a	6.34±2.03ab	6.01±2.01	1.000.000	S
GSH (UI/L)	6.17±2.70	2.32±1.30a	6.39±2.01ab	6.00±2.21	0.620.034	S
MDA (mmol/L)	1.88±0.74	8.65±2.90a	4.25±1.99ab	2.76±1.10	1.110.010	S

Key: Values with superscript alphabet is considered significant (p<0.05). ANOVA was used to compare the different groups. All post hoc testing were done using Bonferroni multiple comparison. GP I = Negative Control, GP 2 = Diclofenac treated, GP 3 = 10mg/kg per body weight of diclofenac + 200mg/kg carica papaya; GP 4 = Received 200mg/kg body weight of carica papayajuice only orally for 14 days.

SOD = Superoxide dismutase
CAT = Catalase
GSH =Glutathione
MDA = Malondialdehyde
All post hoc testing were done using Bonferroni multiple comparison. a Significant difference observed in the SOD levels between GRP 1 and GRP 2, p=0.000. ab Significantdifference observed in the SODlevels between GRP 3 and GRP 2, p =0.00.a Significantdifference observed in the CATlevels between GRP 1 and GRP 2, p=0.002. ab Significant difference observed in CATlevels between GRP 2 and GRP 3, p =0.000. aSignificant difference observed in the GSH levels between GRP 1 and GRP 2, p=.000. abSignificant difference observed in the GSH concentrations between GRP 2 and GRP 3, p=.001. aSignificant difference observed in the MDA levels between GRP 1 and GRP 2, p=.000.abSignificant difference observed in the MDA concentrations between GRP 2 and GRP 3, p=.000.

Table3.2 showed the effect of diclofenac and carica papaya on some oxidative stress markersin rats exposed to diclofenac. The result revealed that there is statistically significant (p<0.05) reduction in serum SOD (2.90±1.02), CAT (2.95±1.12)and GSH (2.32±1.30) in the diclofenac alone treated group (group 2) when compared with the group 1 (control group) (7.08±2.41; 5.97±2.15; and 6.17±2.70) respectively. While malondialdehyde (8.65±2.90) levels was significantly (p<0.05) higher in the diclofenac alone treated group (group 2) when compared with the group 1(1.88±0.74). However, post treatment with 200mg/kg body weight of carica papayajuice (group 3) causes near to normal significant elevation in the serum SOD (5.62±2.31), CAT (6.34±2.03) and GSH (6.39±2.01) levels when compared with the control (group) (7.08±2.41; 5.97±2.15; and 6.17±2.70) respectively. While malondialdehyde (4.25±1.99) levels causes a drastic reduction of malondialdehyde levels compared with the control (8.65±2.90). There was no significant difference observed in the serum SOD (6.94±2.22), CAT (6.01±2.01), GSH (6.00±2.21) and MDA (2.76±1.10) levelsof carica papayaalone treated rats (group 4) when compared with the group 1 (7.08±2.41; 5.97±2.15; and 6.17±2.70) respectively.

DISCUSSION

The safety of using Non-steroidal anti-inflammatory medicines (NSAIDs) in clinical practice has been questioned. Multiple clinical trials have shown evidence that these medications induce detrimental cardiovascular consequences. Various medicinal plants have specific phytochemical compounds that are utilized in the treatment and control of various ailments, including cardiovascular conditions.

The aim of this study was to investigate the cardioprotective potential of fermented unripe pawpaw (Carica papaya) juice on diclofenac-induced cardiotoxicity in wistar rats.

The present study revealed that there was statistically significant (p<0.05) increase in serum activities of cardiac enzymes (LDH and CK)in the diclofenac alone treated group (group 2) when compared with the group 1 (control group) as shown in (table 3.1). However, post treatment with 200mg/kg body weight of fermented unripe pawpaw (Carica papaya) Juice (group 3) causes drastic reduction in the serum LDH and CK activities when compared with the control as shown in (table 3.1). The research showed that diclofenac alone increased serum LDH and CK activities, indicating cardiac damage, while post-treatment with Carica papaya extract

reduced these enzyme levels, suggesting a protective effect against cardiac injuries. This aligns with previous studies by.[1] on CK activity but contrasts their findings on LDH levels. Additionally, diclofenac induced hyperlipidemia, raising total cholesterol and triglyceride levels, possibly due to enhanced lipolysis. Carica papaya post-treatment significantly reduced these lipid profiles, likely through inhibiting lipogenesis and enhancing lipolysis, supported by.[73] The reduction of LDH and CK activities in the carica papaya post treated rats may be attributed to the antioxidant property of the plants which has ameliorative potential against cardiac injuries.[46]

The present study also demonstrated that oral administration of diclofenac causedhyperlipidemia which represented by marked increase in serum lipid profiles including total cholesesterol (TCh) and triglyceride (TG) compared with control as shown in (table 3.1). This is in agreement with the work of[1], which demonstrated significant increase in serum total cholesterol, triglyceride, LDL-C in diclofenac induced rats. The hyperlipidemia caused by diclofenac can be attributed to the stimulation of lipolysis, resulting in elevated levels of plasma free fatty acids. The stimulating action of diclofenac on lipolysis may be attributed to its strong inhibitory effect on the manufacture of prostaglandins, which play a role in the control of lipolysis. These prostaglandins also regulate the inhibitory influence of lipoprotein lipase activity on lipolysis.[1] The study also revealed that post treatment with Carica papaya causes a drastic reduction in the serum total cholesterol and triglyceride levels in the rats as shown in (table 3.1). This reduction of serum total cholesterol and triglyceride levels in the Carica papayapost treated rats may be attributed to some phytochemical components that inhibit lipogenesis and enhance lipolysis. This is in agreement with the work of[73], which demonstrated that Carica papaya leaves extracts significantly ($p<0.05$) decreased the total cholesterol, triglyceride and LDL-cholesterol levels in rats.

The study on diclofenac-induced cardiac injury in rats highlights the role of oxidative stress in tissue damage. Diclofenac administration led to a significant reduction in antioxidant enzyme activities like SOD, GSH, and CAT, indicating increased oxidative stress in cardiac tissues. This aligns with previous research showing oxidative stress involvement in diclofenac-induced toxicity. Conversely, post-treatment with fermented unripe pawpaw juice demonstrated a significant improvement in antioxidant enzyme activities and a reduction in MDA levels, suggesting a cardioprotective effect.

Oxidative damage occurs in cells or tissues when the production of reactive oxygen species (ROS) exceeds the cell's antioxidant capacity or when the cell's ability to counteract oxidative stress decreases.[1] The current investigation discovered that the application of diclofenac induced oxidative stress in the cardiac tissues of rats. The study revealed a notable reduction in the levels of decreased superoxide dismutase (SOD), glutathione (GSH), and catalase (CAT) in the cardiac tissues of rats exposed to diclofenac, as compared to the control group (group 1), as indicated in Table 3.2. The malondialdehyde levels in the cardiac tissues were markedly elevated ($p<0.05$) in the group that received diclofenac alone (group 2) compared to group 1 (1.88 ± 0.74 vs 8.65 ± 2.90).This finding is consistent with the studies conducted by[8] and[1], which demonstrated that oxidative stress contributes to the tissue damage caused by diclofenac. The cellular antioxidant defense mechanism is mainly controlled by the concentrations of glutathione (GSH) and catalase (CAT).GSH, a non-enzymatic antioxidant, has a vital role in eliminating reactive molecules produced by toxic chemicals and transforming them into less damaging substances.[27]

Catalase is a highly prevalent antioxidant enzyme present in all mammalian tissues. It accelerates the decomposition of hydrogen peroxide and protects the tissue from highly reactive hydroxyl radicals.[21] Thus, the inhibition of metabolizing enzymes of GSH and the reduced activity of CAT in the hearts of diclofenac-intoxicated rats may compromise the tissue's capacity to defend against oxidative damage caused by diclofenac.[1]

Administering fermented unripe pawpaw (Carica papaya) juice to the rats after treatment results in a notable rise in the levels of SOD, CAT, and GSH enzymes, and a substantial decrease in the concentration of MDA in the cardiac tissue of the rats treated with carica papaya. The study conducted by[46] in 2017 indicates that carica papaya juice has the ability to protect the heart and may be beneficial in treating cardiovascular disorders.

The research on diclofenac-induced oxidative stress in cardiac tissues of rats has shown that chronic diclofenac exposure increases mitochondrial oxidative stress, inflammatory mediators, and cardiac dysfunction[60] This aligns with previous research indicating the involvement of oxidative stress in diclofenac-mediated toxicity. However, recent studies provide contrasting perspectives. For instance, research on S-diclofenac, a metabolite of diclofenac, suggests a cardioprotective effect by attenuating doxorubicin-induced cardiac injury through enhanced antioxidant enzyme activities and reduced oxidative stress.[32] This presents a potential counterargument to the detrimental effects of diclofenac on cardiac tissues. The study by[1] on CK activity aligns with the findings of diclofenac-induced oxidative stress in cardiac tissues of rats, but contrasts their findings on LDH levels. Diclofenac has been found to induce hyperlipidemia, raising total cholesterol and triglyceride levels, possibly due to enhanced lipolysis[1] Carica papaya post-treatment significantly reduced these lipid profiles, likely through inhibiting lipogenesis and enhancing lipolysis, supported by.[73] Exploring the

protective effects of S-diclofenac against cardiotoxicity induced by doxorubicin could offer insights into novel therapeutic approaches for managing oxidative tissue damage. Ancient civilizations have long recognized the efficacy of herbs in treating various cardiovascular conditions, including congestive heart failure, systolic hypertension, angina pectoris, atherosclerosis, cerebral insufficiency, and venous insufficiency.[11] Herbs such as Daucus carota, Nerium oleander, Amaranthus Viridis, Ginkgo biloba, Terminalia arjuna, and Citrus medica L. have been discovered to contain therapeutic characteristics that can be utilized by humans in the treatment of ailments.[11] To put it simply, diclofenac has been linked to a higher likelihood of experiencing serious cardiovascular events including heart attack and stroke. Additionally, prolonged exposure to diclofenac can lead to increased oxidative stress in the mitochondria, elevated levels of inflammatory substances, and impaired heart function.[60]

However, recent studies suggest a potential cardioprotective effect of S-diclofenac, a metabolite of diclofenac, against doxorubicin-induced cardiac injury.[32]

CONCLUSIONS

Based on the results from the study, the administration of diclofenac induced oxidative stress in cardiac tissues of rats, leading to a significant reduction in antioxidant enzyme activities like SOD, GSH, and CAT. This reduction in antioxidant defense mechanisms contributed to oxidative tissue damage, as evidenced by the elevated serum concentration of malondialdehyde (MDA), a marker of lipid peroxidation. These findings align with previous research highlighting the involvement of oxidative stress in diclofenac-induced tissue toxicity. On the other hand, post-treatment with fermented unripe pawpaw juice showed a significant improvement in antioxidant enzyme activities and a notable reduction in MDA levels in the treated rats. The findings suggest that carica papaya juice has the ability to protect the heart from cardiovascular illnesses by improving the body's antioxidant defenses and reducing oxidative stress in the heart tissues.Ultimately, the presence of diclofenac can cause oxidative stress in cardiac tissues, resulting in tissue harm caused by the disparity between the production of reactive oxygen species (ROS) and the ability of antioxidants to counteract them.

However, interventions like carica papaya juice post-treatment can mitigate oxidative damage by enhancing antioxidant enzyme activities and reducing lipid peroxidation, suggesting a potential therapeutic approach for cardiovascular health.

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Disclosure of conflict of interest.

The authors confirm that there is no conflict of interest.

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