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Original Research Article

Diclofenac Induced Hepatorenal Toxicity on Albino Rats: The Assessment of Mitigative Potential of Fermented *Carica Papaya* Juice

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Abstract: In a study investigating the protective effects of an extract on diclofenac-induced liver and kidney injuries in Wistar rats, twenty healthy adult Wistar rats weighing between 110g to 200g were randomly divided into four groups of five rats each. 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, and Group 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 the liver function parameters (superoxide dismutase, catalase, and glutathione reductase) (Alanine aminotransferase - ALT, and alkaline phosphatase - ALP), renal function parameters (creatinine), oxidative stress markers, and for histological evaluation. The results showed that diclofenac administration caused a significant increase ($p<0.05$) in serum levels of ALT and ALP, creatinine and a significant decrease ($p<0.05$) in antioxidant enzymes (superoxide dismutase, catalase, and glutathione reductase) compared to the control group. However, treatment with FUPJ significantly reduced ($p<0.05$) the levels of ALT and ALP, creatinine, and MDA and increased ($p<0.05$) the levels of antioxidant enzymes. In conclusion, FUPJ has the potential to ameliorate diclofenac-induced hepatic and renal dysfunction through its antioxidant and anti-inflammatory properties.

Keywords: Fermented unripe pawpaw juice diclofenac oxidative stress markers hepatorenal.

1. INTRODUCTION

The kidney and liver play vital roles in the body as they are responsible for regulating electrolytes, acid-base balance, and blood pressure. They also act as natural filters, removing drugs and toxic waste materials from the body system. Additionally, these organs produce hormones and are involved in the production and metabolism of prostaglandins through cyclooxygenase (COX), with the kidney playing a particularly significant role in this process. Furthermore, both the kidney and liver contribute to the functions of the hematopoietic system (Alabi and Akomolafe, 2020). Drug-induced injuries frequently affect the liver and kidneys due to their role in metabolism, detoxification, storage, and elimination of medicines and their byproducts. Drugs can harm these crucial organs. (Alabi and Akomolafe, 2020) Nephrotoxicity and hepatotoxicity are significant medical conditions resulting from the administration of specific medications, such as nonsteroidal anti-inflammatory drugs (NSAIDs) (Alabi and Akomolafe,

2020; Störmer *et al*., 2022). As a nonsteroidal anti-inflammatory drug (NSAID) from the phenylacetic acid class, diclofenac (DF) can reduce swelling and pain, block pain signals, lower body temperature, and kill bacteria. People extensively use methotrexate to manage rheumatoid arthritis and pain; however, it is associated with significant adverse effects such as pulmonary, cardiac, hepatic, and renal tissue destruction, as well as gastrointestinal toxicity (Alabi and Akomolafe, 2020; Störmer *et al*., 2022). Diclofenac, a multitargeted medicine, has demonstrated effects on various body organs, including the lung, stomach, kidney, liver, and heart. This confirmation comes from studies conducted by Alabi and Akomolafe in 2020, Störmer *et al*., in 2022, and Abiola *et al*., in 2019. It's not completely clear how diclofenac hurts the kidneys and liver, but it has something to do with damaging mitochondria, messing up the immune system's defenses, making reactive oxygen species, and stopping enzymes and non-enzyme antioxidants from working in the kidney and liver (Alabi and Akomolafe, 2020; Stormer *et al*., 2022; Abiola *et al*., 2019).

Metabolites of diclofenac, such as 4', 5-hydroxydiclofenac, can also cause the infiltration of neutrophils in hepatic cells. To summarize, diclofenac is a commonly utilized nonsteroidal anti-inflammatory drug (NSAID) that has therapeutic advantages but also presents significant adverse effects, such as nephrotoxicity and hepatotoxicity. The precise mechanism by which diclofenac causes kidney and liver toxicity is not entirely understood. Nonetheless, it is associated with mitochondrial harm, disruption of immune-mediated protective processes, generation of reactive oxygen species, and suppression of enzymatic and non-enzymatic antioxidants in the kidney and liver tissues (Alabi and Akomolafe, 2020; Störmer *et al*., 2022; Abiola *et al*., 2019).Throughout history, there has been a plentiful supply of medicinal resources obtained from plants, which have played a vital role in the treatment and control of many ailments (Anitha *et al*., 2018). Herbal sources of active chemicals have become more prominent since they are considered safer and more effective than synthetic medications, which are sometimes linked to environmental issues. The use of herbal medicines is a manifestation of a worldwide resurgence in herbal medicine, indicating an increasing fascination with natural and environmentally-friendly healthcare alternatives. The citation for this information is from Roshan *et al*. (2014). Plants possess several active constituents that provide therapeutic qualities, and the concentration of these constituents differs among different plant forms. The bioactive chemicals are accountable for inducing physiological alterations in the body, resulting in therapeutic outcomes (Srivastava and Singh, 2016). The pharmacological significance of Carica papaya, also known as papaya, has prompted extensive research. It has demonstrated several beneficial effects, such as anti-inflammatory, anti-fertility, hepatoprotective, wound healing, antihypertensive, and anticancer properties (Ugbogu *et al*., 2023). Research in the field of medicinal plant materials remains an important focus, as continuous investigations investigate the wide range of health advantages and therapeutic potentials offered by natural treatments. The focus on herbal medicine is in accordance with the growing acknowledgment of the importance of traditional and plant-derived therapies in contemporary healthcare methodologies (Ugbogu *et al*., 2023).

The Carica genus belongs to the Caricaceae family and is known for its solitary, somewhat woody, large herbaceous plants that grow rapidly and have indeterminate growth. During its first year of growth, it has the potential to attain a height of 3 meters (Saeed *et al*., 2014; Koul *et al*., 2022).

The Carica papaya is the sole species within the Carica genus. The Caricaceae family is quite prevalent in the neotropics, with six genera and a minimum of 35 species. The transmission of the disease has reached numerous tropical and subtropical locations worldwide (Saeed *et al*., 2014; Koul *et al*., 2022). The plant produces large palmate leaves measuring 0.6 square meters. These leaves possess five to no pinnate lobes, with widths ranging from 40 to 60 centimeters. The growth rate of the papaya plant is rapid, with a duration of 3–8 months from the germination of its seeds to the onset of flowering, and a further 9–15 months until it is ready for harvest (Saeed *et al*., 2014; Koul *et al*., 2022).

Carica papaya, commonly referred to as papaya, is a tropical fruit that offers a multitude of health advantages. The substance contains a high concentration of antioxidants, as well as vitamins A, B, C, and E, and minerals including potassium, magnesium, and calcium (Saeed *et al*., 2014; Koul *et al*., 2022). The fruit is a highly beneficial source of dietary fiber, which promotes efficient digestion and mitigates the risk of constipation (Saeed *et al*., 2014; Koul *et al*., 2022). Herbal therapy utilizes papaya plant leaves for their ability to eradicate intestinal parasites and their antioxidant qualities (Saeed *et al*., 2014; Koul *et al*., 2022). Papaya has demonstrated anti-inflammatory, antibacterial, anticancer, and hepatoprotective properties (Saeed *et al*., 2014; Koul *et al*., 2022). Traditional medicine has utilized papain and chymopapain, two enzymes found in the fruit and its seeds, to treat digestive diseases and inflammation (Saeed *et al*., 2014; Koul *et al*., 2022). Traditional medicine employs the seeds' antiparasitic qualities to treat intestinal parasites and other gastrointestinal illnesses (Saeed *et al*., 2014; Koul *et al*., 2022). In addition to its health benefits, the food industry uses papaya as a natural food preservative and a source of pectin. Jams and jellies use pectin as a material (Saeed *et al*., 2014; Koul *et al*., 2022). The fruit's antioxidant and anti-inflammatory characteristics make it a valuable ingredient in cosmetics and skincare products (Saeed *et al*., 2014; Koul *et al*., 2022).

The Carica papaya plant yields fermented papaya, which offers a variety of health benefits. It is rich in antioxidants, phenolic compounds, carotenoids, and vitamins A, B, C, and E. These components support digestive health and help reduce oxidative stress. The proteolytic enzymes found in papaya, namely papain and bromelain, assist in the digestion process and possess anti-inflammatory properties (Saeed *et al*., 2014). It is important to exercise caution when consuming unripe papaya, as it contains papain, which can be potentially dangerous, particularly for pregnant women. Studies have explored the potential benefits of fermented papaya in battling aging and treating tumors, showcasing promising results in cancer treatment. The process of fermentation amplifies the antioxidant characteristics of papaya, hence augmenting its positive effects on health (Leitão *et al*., 2022).

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MATERİALS AND METHODS

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 kept in plastic cages at a room temperature of about 27°C and photoperiodicity of 12-hour light/12-hour dark. The protocols were executed in compliance with the National Institutes of Health guidelines for the care and utilization of laboratory animals (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 Amassoma Market, Bayelsa State, Nigeria. 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. After the third day of fermentation, the supernatant was filtered and disposed of The fermented unripe pawpaw extract was collected, poured into a container, and kept in the fridge for preservation.

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.

Twenty-four hours after the final treatment, the animals were weighed again and used chloroform to render them unconscious. After the treatment concluded, the rats were sacrificed and blood samples collected via cardiac puncture to measure liver function parameters such as Alanine aminotransferase (ALT), and alkaline phosphatases (ALP), renal function parameters like creatinine, and oxidative stress biomarkers like malondialdehyde (MDA), catalase, and superoxide dismutase (SOD).

Sample Collection

Sample Preparation and Collection

Blood samples were collected by cardiac puncture into standard bottles. The blood samples were allowed to clot for 3 hours to enable coagulation. Afterward, the samples were centrifuged at 2000rpm for 10 minutes, and the supernatant (serum) was collected and stored in the refrigerator. The other part was mixed with Phosphate buffer and homogenized.

Ethics

The ethical committee at Niger Delta University in Amassoma, Bayelsa State, Nigeria, authorized all animal protocols, following the Principles of Laboratory

Animal Care set forth by the National Institutes of Health [NRC]. The animals received compassionate care in line with the guidelines outlined in the "Guide for the Care and Use of Laboratory Animals (1996)" published by the National Academy of Sciences.

Biochemical Parameters

The following biochemical parameters were determined spectrophotometrically using the respective kits and instructions provided in the biochemical kit manual.

Estimations of Biochemical Parameters

Serum Parameters

On the 15th day of the research, we euthanized the animals. We collected the blood and stored it in simple vials for biochemical analysis. The serum was isolated for the determination of liver function parameters: aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatases (ALP). Enzymatic kits from Accurex Biomedical Limited Pvt. Ltd., India, Renal function parameters: creatinine and urea were determined using standard kits from Merck Specialities Pvt. Ltd., India, following the instructions provided in the manufacturer's manual.

Determination of Markers of Oxidative stress. This was by measuring Malondialdehyde (MDA) [Aebi,1984] and superoxide dismutase (SOD)[Butler,1963] catalase [Aebi, 1984] as well as reduced Glutathione GSH [Sun *et al*.,1988]

Statistical Analysis

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

RESULTS

The results of the present study are presented in the Table and bar charts below. Table 3.1 shows the mean serum levels of ALT, ALP and CREATININE. Figure 3.1 shows the mean tissue homogenate antioxidant activities.

Table 3.1: Result of the Mean serum level of Alanine aminotransferase and Alanine phosphatase and Creatine

ENZYME PARAMETERS	ALT(U/L)	ALP(U/L)	CREATINE(U/L)
NORMAL CONTROL	71.07±2.24[a]	128.53±4.21[a]	0.5±0.03[a]
DICLOFENAC	141.23±4.51[b]	160.36±1.79[b]	0.81±0.02[b]
DICO + PAWPAW	90.73±3.11[c]	120.58±7.95[d]	0.52±0.01[c]
PAWPAW	72.03±3.10[a]	124.33±1.27[d]	0.5±0.02[c]

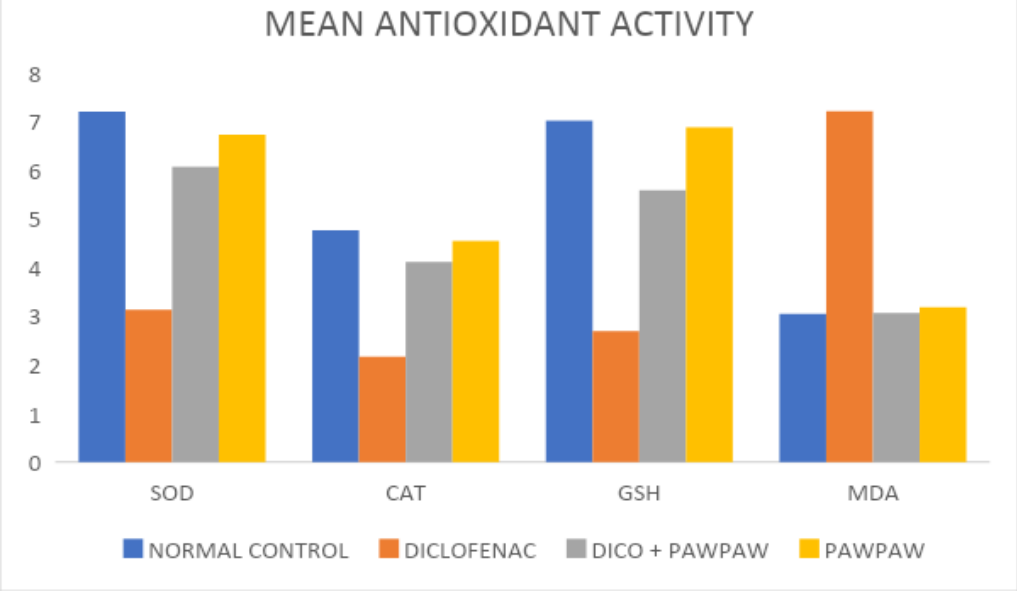

Figure 3.1: The study demonstrated the impact of Diclofenac and fermented Pawpaw on oxidative stress markers in rats subjected to oxidative stress induction. Group 2, which received Diclofenac, exhibited a statistically significant decrease ($p<0.05$) in serum SOD (3.13±0.33), CAT (2.16±0.12), and GSH (2.69±0.58) levels compared to the control group (group 1). Conversely, MDA levels were significantly higher ($p<0.05$) in the L-Name treated group (7.2±0.33) compared to the control group (3.04±0.57)

The data is presented as the mean ± standard deviation (SD). Values with a different superscript from the control group are statistically significant at $p < 0.05$.

Table 3.1 demonstrated that the potential of Pawpaw on Diclofenac-induced Liver and kidney dysfunction. Our study found a significant increase in serum levels of ALT (alanine transaminase), ALP

(alkaline phosphatase), and creatinine in the experimental group treated with Diclofenac, compared to the normal control group. Specifically, ALT levels rose to 141.23±4.51, ALP levels to 160.36±1.79, and creatinine levels to 0.81±0.02.

However, after administering fermented Pawpaw juice at a dosage of 200 mg/kg body weight, we observed a notable decrease in these levels compared to the control group. ALT levels decreased to 90.73±3.11, ALP levels decreased to 120.58±7.95, and creatinine levels decreased to 0.52±0.01.

Post-treatment with 200 mg/kg body weight of fermented pawpaw juice resulted in a notable reduction in SOD (6.06±0.17), CAT (4.11±0.13), and GSH (5.58±0.38) levels compared to the control group. Furthermore, MDA levels showed a significant decrease (3.06±0.14) post-treatment with fermented pawpaw juice. All values are presented as mean ± standard deviation.

DISCUSSION

Diclofenac (DF), an NSAID derived from phenyl-acetic acid, possesses anti-inflammatory, analgesic, antinociceptive, antipyretic, and antibacterial effects. It is commonly used to alleviate symptoms of rheumatoid arthritis and discomfort. Although DF offers therapeutic benefits, it also has significant and severe adverse effects. These encompass harm to the pulmonary, cardiac, hepatic, and renal tissues, along with gastrointestinal toxicity (Alabi and Akomolafe, 2020). Diclofenac is a non-threshold multi-targeted medicine that has been demonstrated to affect various organs in the body, such as the heart, lungs, stomach, kidney, and liver (Alabi and Akomolafe, 2020).

Diclofenac can hurt mitochondria by messing up the immune system's defenses, creating reactive oxygen species, and decreasing the activity of enzyme- and non-enzyme-based antioxidants in the liver and kidneys (Alabi and Akomolafe, 2020).

A study by Owumi and Dim (2015) found that drug-induced liver injury is estimated to have an annual incidence of 10 to 15 per 10,000 to 100,000 persons exposed to the drug, highlighting the importance of understanding potential drug interactions and their effects on liver function (Ogbe *et al*., 2022). Another study by MedlinePlus Drug Information (n.d.) notes that NSAIDs, including diclofenac, may cause ulcers, bleeding, or holes in the stomach or intestine, emphasizing the potential side effects on the gastrointestinal tract [2]. Additionally, a report by the Food and Drug Administration (FDA) tracks the side effects of approved drugs, including diclofenac, and encourages patients to report any side effects experienced.

In this study, the serum levels showed that Pawpaw has the potential to mitigate diclofenac-induced liver and kidney dysfunction. There was a significant increase in ALT (141.23±4.51) and ALP (160.36±1.79) levels in the group treated with diclofenac compared to the normal control group. Previous research has linked diclofenac to damage in liver and kidney cells, including mitochondrial damage, impairment of immune-mediated protective systems, and inhibition of antioxidants in these cells (Kadhim *et al*., 2022).

The concentrations of ALT (90.73±3.11), ALP (120.58±7.95), and creatinine (0.52±0.01) in the blood dropped compared to the control group after 200 mg/kg body weight of fermented Pawpaw juice was given. Alabi and Akomolafe's 2020 study found that Kolaviron effectively avoids or minimizes the harmful effects of diclofenac in the plasma, liver, and kidney of rats. This implies that Kolaviron can shield the liver and kidneys from the damaging effects of diclofenac-induced hepatorenal toxicity. Additionally, studies have demonstrated that Artemisia possesses hepatorenal protective and preventative properties against diclofenac-induced liver and kidney damage in rats. The citation "Kadhim *et al.*, 2022" refers to a publication by Kadhim and colleagues in the year 2022. Studies have demonstrated that fermented papaya extract possesses antioxidant characteristics and has the potential to safeguard against liver and kidney harm (Leitão *et al*., 2022).

Diclofenac and fermented Pawpaw impacted some oxidative stress indicators in rats, given the diclofenac and fermented Pawpaw oxidative stress induction. Also, when group 2 (the diclofenac-treated group) was compared to group 1 (the control group), serum SOD (3.13±0.33), CAT (2.16±0.12), and GSH (2.69±0.58) decreased statistically significantly ($p<0.05$). MDA levels in the diclofenac-treated group were significantly ($p<0.05$) higher than in the normal control group (3.04±0.57). Increased kidney MDA and H2O2 levels demonstrate that diclofenac induces kidney injury in rats through oxidative damage. This damage is due to the oxidative deterioration of polyunsaturated fatty acids in kidney membranes (Abiola *et al*., 2019).

As compared to the control group, post-treatment with 200 mg/kg body weight fermented pawpaw juice resulted in a significant increase in SOD (6.06±0.17), CAT (4.11±0.13), and GSH (5.58±0.38) levels. MDA (3.06±0.14) data revealed a significant decrease in MDA.

The findings suggest that fermented Pawpaw juice may offer defense against oxidative stress in the liver and kidney caused by diclofenac.

Stormer *et al*., (2022) found that a solitary oral administration of diclofenac resulted in long-term kidney damage when there was already a pre-existing

subclinical acute kidney injury (AKI). According to Stormer *et al*., (2022), the research discovered that taking one dose of diclofenac by mouth led to a shift from an experimental, asymptomatic form of acute kidney injury (AKI) to chronic kidney disease. This underscores the possible enduring impacts of diclofenac on renal function. Diclofenac also increased the concentrations of MDA and H_2O_2 in the kidneys. MDA is a consequence of lipid peroxidation, while H_2O_2 is a byproduct of aerobic metabolism (Abiola *et al*., 2019). These findings suggest that diclofenac has the potential to cause oxidative stress in the kidney, resulting in kidney injury. To summarize, diclofenac has therapeutic advantages but also presents significant adverse effects, such as pulmonary, cardiac, hepatic, and renal tissue damage, as well as gastrointestinal toxicity (Alabi and Akomolafe, 2020). Alabi and Akomolafe's (2020) study suggests that consuming fermented Pawpaw juice could shield the liver and kidney from diclofenac's oxidative stress. Diclofenac has the potential to cause long-term damage to the kidneys in cases where there is already a pre-existing subclinical acute kidney injury (AKI) (Stormer *et al*., 2022). Additionally, it can trigger oxidative stress in the kidneys, resulting in kidney damage.

CONCLUSION

Diclofenac causes kidney and liver damage through oxidative damage and hinders immune-mediated defensive mechanisms. Artemisia and Kolaviron are potential protective drugs that have demonstrated hepatoprotective and preventative actions against diclofenac-induced damage. Fermented papaya leaf extract has the potential to contain antioxidant properties that may help protect against hepatorenal impairment.

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