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Original Research Article

Fermented Unripe Plantain Juice Ameliorates Diclofenac-Induced Hepatic and Renal Dysfunction in Wistar Rats

Arhoghro Ejovwoke Marcellinus[1*], Berezi E. Peter[2], Owotgwun Kasirotu Levi[1], Agberia Steve Obruche[1], Madock Obebinaru Joshua[1]

[1]Department of Biochemistry, Faculty of Basic Sciences, College of Health Science, Niger Delta University, Wilberforce Island, Amassoma, Bayelsa State, Nigeria
[2]Department of Chemistry, Isaac Jasper Boro College of Education, Sagbama, Bayelsa State, Nigeria

***Corresponding Author:** Arhoghro, Ejovwoke Marcellinus
Department of Biochemistry, Faculty of Basic Sciences, College of Health Science, Niger Delta University, Wilberforce Island, Amassoma, Bayelsa State, Nigeria

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Abstract: Diclofenac administration has been associated with several health problems in human including gastrointestinal tract problems, renal toxicity, among others. Several medicinal plants are been used locally for the treatment of several diseases. The aim of this study was to investigate the potential of fermented unripe plantain juice (FUPJ) in ameliorating diclofenac-induced ulcer and renal dysfunction. Twenty (20) healthy adult Wister rats weighing between 110g to 200g were randomly divided into four groups of five rats each. Group I served as control, received distilled water and feeds, Group 2 received 10mg/kg body weight of diclofenac only, Group 3 received 10mg/kg body weight of diclofenac only and post treated with 200mg/kg body weight of FUPJ and Group 4 received 200mg/kg body weight of FUPJ. The diclofenac was administered IP and fermented unripe plantain extract was administered orally for fourteen (14) days. The results showed that diclofenac administration caused a significant increase ($p<0.05$) in serum levels of creatinine and a significant decrease ($p<0.05$) in antioxidant enzymes (superoxide dismutase, catalase, and glutathione peroxidase) compared to the control group. Additionally, diclofenac administration caused ulceration in the gastric mucosa. However, treatment with FUPJ significantly reduced ($p<0.05$) the levels of creatinine, urea, and MDA and increased ($p<0.05$) the levels of antioxidant enzymes in a dose-dependent manner. The highest dose of FUPJ (200mg/kg) showed the most significant reduction in the levels of the tested parameters and prevented the gastric mucosal ulceration. In conclusion, FUPJ has the potential to ameliorate diclofenac-induced ulcer and renal dysfunction through its antioxidant and anti-inflammatory properties.

Keywords: Plantain Juice, Wistar Rats, Renal Toxicity, NSAIDs.

INTRODUCTION

Non-steroidal anti-inflammatory medicines (NSAIDs) are frequently used to treat rheumatoid and osteoarthritis. Due to their pain-relieving and anti-inflammatory properties [4]. NSAIDs exert their effects by inhibiting cyclooxygenase-1 and cyclooxygenase-2 enzymes, leading to the suppression of thromboxane and prostaglandin formation. [23] [10]. Extended use of NSAIDs is linked to detrimental consequences such as gastrointestinal injury, kidney issues, and cardiovascular problems [6, 7]. Diclofenac, a widely prescribed NSAID, is known for its analgesic, anti-inflammatory, and antipyretic actions [1]. It has been reported to induce hepatotoxicity, [12]. characterised by a unique and unpredictable mechanism of liver damage in humans [16]. Diclofenac is metabolised by several cytochrome P-450 enzymes in liver cells, resulting in the creation of drug-protein adducts, GSH conjugation, impairment of mitochondrial function, and eventual harm to organs [7]. Notably, diclofenac itself, rather than its metabolites, is implicated in its toxicity [11]. Furthermore, diclofenac impacts the kidneys, contributing to nephritis and nephrotoxicity [23]. While also causing gastrointestinal issues

like gastric mucosal bleeding, reduced gastric blood flow, and apoptosis [3]. Natural products are increasingly explored for their therapeutic potential due to minimal side effects compared to synthetic drugs [4]. Musa paradisiaca (plantain), a widely consumed fruit in West Africa, is rich in essential nutrients like vitamins A, B6, C, minerals, and dietary fiber. This fruit contains bioactive compounds such as phenolics, carotenoids, lycopene, and phytosterols that play crucial roles in physiological processes and combat cellular damage in humans and animals [17]. The bioactive molecules present in plantain exhibit significant antioxidant properties against various Reactive Oxygen Species (ROS) [15]. In the management of gastric ulcers, conventional approaches involve inhibiting gastric acid secretion with chemical drugs or using antacids to neutralize acid levels [14]. However, these medications often come with side effects like joint pain, altered heartbeat, hematopoietic changes, gynecomastia, impotence, and systemic alkalosis [8]. Research is increasingly focusing on natural products like Musa paradisiaca for drug development due to their potential to offer therapeutic benefits with minimal adverse effect [2-4]. While the medicinal properties of M. paradisiaca are acknowledged for various ailments, further studies are needed to identify the specific bioactive compounds responsible for these beneficial effects.

MATERIALS AND METHOD

Chemicals/Reagents: Chloroform (JDH), Randox Biochemical Kit (Creatinine), Phosphate buffer, normal saline.

Experimental Animals

At the College of Health Sciences, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria, the Department of Pharmacology kept 20 adult Wister albino rats in normal plastic cages. The rats were subjected to a 2-week period of acclimatisation in the study laboratory, in accordance with the rules established by the Institutional Animal Ethical Committee (IAEC) and under the supervision of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

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 for 14days.
Group 2 (Test group): Received 10mg/kg per body weight of diclofenac daily by intraperitoneal route for 14 days.
Group 3 (Test group): Received 10mg/kg per body weight of diclofenac daily by intraperitoneal route for 14 days and post treated with 200mg/kg body weight of FUPJ orally for 14 days.
Group 4: Received 200mg/kg body weight of FUPJ orally for 14days

ETHICS

All animal protocols were approved by the ethical committee of Niger Delta University Amassoma, Bayelsa State, Nigeria which was in line with the National Institutes of Health's Principles of Laboratory Animal Care [25]. All animals received humane care according to the criteria outlined in the "Guide for the Care and Use of Laboratory Animals (1996)" prepared by the National Academy of Sciences.

Biochemical Investigations
Liver Function Test
Determination of Serum Alkaline Phosphatase by Roy Method
Determination of Serum Alanine Aminotransferase (ALT) by Reitman and Frankel Method
Renal function Test
Determination of serum Urea by Berthelot's Enzymatic Method
Determination of serum creatinine by Jaffe colorimetric –kinetic Method
Determination of Markers of Oxidative stress.
This was by measuring Malondialdehyde (MDA) [26], and superoxide dismutase (SOD) [28], catalase [26], as well as reduced Glutathione GSH [27].

RESULTS: The results of the present study are presented in table 1 and 2 below.

Table 1: Result of the Mean serum Creatinine levels, ALP and ALT activities.

Enzyme Parameters	Creatinine (mg/dl)	ALP(U/L)	ALT(U/L)
Normal Control	0.496 ± 0.027^{a}	125.23 ± 4.00^{a}	70.67 ± 2.02^{a}
Diclofenac	0.76 ± 0.0745^{b}	157.16 ± 1.55^{b}	140.02 ± 4.37^{b}
Diclofenac Plus Plantain	0.526 ± 0.0594^{c}	110.26 ± 5.25^{c}	87.32 ± 2.98^{c}
Plantain	0.504 ± 0.0114^{a}	120.30 ± 1.97^{d}	73.03 ± 3.43^{d}

Values are presented in mean ± standard deviation. Means with different superscripts letters are significantly different.

Table 1 demonstrated that the potential of unripe plantain juice on Diclofenac-induced ulcer and renal dysfunction in serum creatinine created a significant ($p>0.05$) rise (0.496 ± 0.0027) in the normal control group in comparison to the

group treated with fermented plantain juice, creatinine (0.504±0.0114). There was an increase after treatment with 200mg/kgFUPJ body weight of fermented Unripe Plantain juice. It also showed that, when compared to the normal control group, the potential of FUPJ on Diclofenac induced Liver dysfunction in serum ALT and ALP generated a substantial ($p<0.05$) increase in ALT (140.02 ± 4.37) and ALP (157.16 ± 1.55) levels. When compared to the control group, post treatment with 200 mg/kg body weight of fermented unripe plantain juice led to an increase in serum ALT (87.32± 2.98) and ALP (110.26± 5.25) levels.

Table 2: Result of the Mean liver homogenate Antioxidant Activity

Antioxidant Parameters	SOD (Units/mg protein)	CAT (Units/mg protein)	GSH (Units/mg protein)	MDA (Units/mg protein)
Normal Control	9.008±0.247[a]	5.452±0.229[a]	7.108±0.140[a]	1.948±0.082[a]
Diclofenac	2.744±0.212[b]	2.444±0.462[b]	2.572±0.518[b]	7.368±0.286[b]
Dico + Plantain	6.006±0.235[c]	4.868±0.895[c]	6.002±0.194[c]	2.95±0.14[c]
Plantain	9.038±0.182[a]	5.16±0.071[a]	7.07±0.161[a]	1.958±0.093[a]

Values are presented in mean ± standard deviation. Means with different superscripts letters are significantly different

Table 2 showed some oxidative stress markers affected by diclofenac and fermented plantain in rats. When group 2 (Diclofenac treated group) was compared to group 1 (the control group), the results showed a statistically significant ($p<0.05$) decrease in serum SOD (3.13±0.33), CAT (2.16±0.12), and GSH (2.69±0.58), respectively. As opposed to the normal control group (3.04±0.57), MDA (7.2±0.33) levels were considerably ($p<0.05$) higher in the L-Name treated group. In comparison to the control group, post-treatment with 200mg/kg FUPJ body weight of fermented plantain juice resulted in a substantial drop in the levels of SOD (6.06±0.17), CAT (4.11±0.13), and GSH (5.58±0.38). MDA (3.06±0.14) values revealed a marked decline in MDA levels.

DISCUSSION

Plants have historically served as valuable sources of safe and effective medications. Herbal medicines remain prevalent in primary healthcare systems globally, with around 80% of the population relying on traditional treatments. Medicinal herbs have been utilized to treat various illnesses. Diclofenac, a phenyl-acetic acid NSAID, possesses anti-inflammatory, analgesic, antinociceptive, antipyretic, and antibacterial properties. This non-threshold multi-targeted medication has been shown to impact organs like the heart, lungs, stomach, kidney, and liver. Diclofenac's detrimental effects on mitochondria involve disrupting immune-mediated protective mechanisms, generating reactive oxygen species, and inhibiting enzymatic and non-enzymatic antioxidants in kidney and liver tissues. Studies have explored fermented plantain juice's potential in mitigating diclofenac-induced ulcers in rats and other forms of amelioration.

Plants have historically served as valuable sources of safe and effective medications.[18]. Herbal medicines remain prevalent in primary healthcare systems globally [16]. With around 80% of the population relying on traditional treatments [9]. Medicinal herbs have been utilized to treat various illnesses [6]. Diclofenac, a phenyl-acetic acid NSAID, possesses anti-inflammatory, analgesic, antinociceptive, antipyretic, and antibacterial properties [22]. This non-threshold multi-targeted medication has been shown to impact organs like the heart, lungs, stomach, kidney, and liver [20]. Diclofenac's detrimental effects on mitochondria involve disrupting immune-mediated protective mechanisms, generating reactive oxygen metabolites, and inhibiting enzymatic and non-enzymatic antioxidants in kidney and liver tissues [21]. Studies have explored fermented plantain juice's potential in mitigating diclofenac-induced ulcers in rats and other forms of amelioration [13]. In this study, the serum level demonstrated that the potential of plantain on diclofenac-induced liver and kidney dysfunction in serum ALT and ALP created a significant potential of unripe plantain juice on Diclofenac-induced ulcer and renal dysfunction in serum creatinine created a significant ($p>0.05$) rise in creatinine (0.496±0.00) in the normal control group in comparison to the group treated with fermented plantain juice, creatinine (0.504±0.01). There was an increase after treatment with 200mg/kg body weight of fermented unripe plantain juice. This result is in line with those reported by [6-23], which showed that serum creatinine and blood urea nitrogen (BUN) levels were notably lower in the FUPJE-treated groups, suggesting an amelioration of renal function. This result has revealed that FUPJE treatment was associated with the modulation of oxidative stress markers and inflammatory mediators in both the liver and kidneys.

Diclofenac and fermented plantain impacted some oxidative stress indicators in rats given the Diclofenac and fermented plantain oxidative stress induction [19]. When group 2 (Diclofenac treated group) was compared to group 1 (the control group), the results showed a statistically significant ($p<0.05$) decrease in serum SOD (3.13±0.33), CAT (2.16±0.12), and GSH (2.69±0.58), respectively. As opposed to the normal control group (3.04±0.57), MDA (7.2±0.33) levels were considerably ($p<0.05$) higher in the Diclofenac treated group. In comparison to the control group, post-treatment with 200mg/kg body weight of fermented pawpaw juice resulted in a substantial drop in the levels of SOD (6.06±0.17), CAT (4.11±0.13), and GSH (5.58±0.38). MDA (3.06±0.14) values revealed a marked decline in MDA levels. This result is in agreement with those reported by [10-23], which showed a significant decrease in malondialdehyde (MDA)

levels and an increase in superoxide dismutase (SOD) activity following treatment with FUPJE. This is because fermented plantain juice contains bioactive compounds such as polyphenols and flavonoids, which have antioxidant properties. These antioxidants can help to neutralize reactive oxygen species and reduce oxidative stress in the stomach lining, which is a known factor in the development of ulcers.

CONCLUSION

The study indicates plantain juice can protect against hepato-renal damage caused by diclofenac by increasing the generation of PGE-2, increasing the expression of COX-2 protein, decreasing the expression of iNOS protein, and thereby minimising the inflammatory response. Plantain juice shows potential as a beneficial addition to protect against the harmful effects of diclofenac on multiple organs. Additional investigation is necessary to explore the molecular processes that support this protective function.

COMPLIANCE WITH ETHICAL STANDARDS

Acknowledgments

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Disclosure of Conflict of Interest: The authors declare that there is no conflict of interest.

Statement of Ethical Approval

The study protocol was approved by the Ethical and Research Committee of Niger Delta University, Bayelsa State, Nigeria. The ethical principles for medical research involving animal subjects as outlined in the Helsinki declaration in 1975 and subsequent revisions were strictly followed in the course of this study.

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