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Ipomoea cairica Leaf Extract Counter the Hepatoxic Effect of Diclofenac viz Antioxidant, Anti-inflammatory and Membrane Stabilization.

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Abstract

This study aimed to evaluate the hepatoprotective effect of *I. cairica* leaf extract against the hepatotoxic effect of diclofenac in male Wistar rats. Thirty-five male Wistar rats were randomly divided into five groups of seven rats each. I: normal control; II: orally administered 100 mg/kg of diclofenac (DCF); III: orally administered 100 mg/kg Methanolic extract of *I. cairica* (MEIC) for 14 days before single dose administration of DCF; IV: orally administered 250 mg/kg of MEIC for 14 consecutive days before single dose administration of DCF; V: orally administered 250 mg/kg of MEIC for 14 consecutive days. Animals were sacrificed 24 hours after the last administration. Blood was collected and processed for markers of lipidaemia and hepatic function, the liver was excised and processed for antioxidant (glutathione [GSH], catalase [CAT], superoxide dismutase [SOD], oxidative stress (malondialdehyde [MDA] and protein carbonyl [PC], and pro-inflammatory activities (xanthine oxidase (XO), myeloperoxidase (MPO), and NADP(H) oxidase).The results from the experiment confirmed the pro-oxidative effect of DCF as observed in the increased concentration of MDA and PC, decreased activities of the antioxidant enzymes, and increased activities of the pro-inflammatory enzymes as compared to the control ($P<0.05$). It also showed that DCF induced an increase in AST activity ($P<0.05$) in the serum. Pretreatment with MEIC was able to prevent the oxidative and inflammatory effect of DCF in the liver. Conclusion: The results provide a scientific proof of the potential of *I. cairica* to improve the ability of liver to counter the toxic effect of DCF overdose.

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Introduction

The function of the liver is central to the health and well-being of man. Its role in the detoxification of chemicals and toxic compounds often exposes it to the detrimental effects of toxic compounds and their metabolites (Grzegorzewski *et al*., 2022). Drug abuse or overdose often overburden the liver as it tries to detoxify and eliminate the drugs as fast as possible (Tak and Kim, 2023; Ogunwole *et al*., 2021). Oxidative stress is one of the major mechanisms reported to induce liver damage (Ilesanmi and Odewale, 2020). Oxidative stress is the term that generally describe the condition whereby there is high production of reactive species with concomitant reduction in antioxidant status in the tissues. Oxidative stress can be intensified by drug overdose and the accumulation of their reactive metabolites. Diclofenac, a popular over-the-counter (OTC) pain medication (NSAID) that is readily prescribed for joint pain and aches. Though the drug works by antagonizing inflammatory processes in the body, overdose and discriminate usage of the drug can induce inflammatory processes, causing tissue damage. In addition, DCF metabolites are normally eliminated, however, under high concentration, they

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accumulated in the body, thereby inducing tissue damage, such as liver-related disorders (Heidarian and Nouri, 2021).

The mechanism of DCF-induced hepatotoxicity is linked to oxidative stress and inflammation (Elbaz *et al*., 2022). This discovery has led to the suggestion of antioxidant-rich natural product as an antidote against DCF poisoning. *Ipomoea cairica* is one of the underutilized medicinal plants in Africa. It is of the Convolvulaceae family with more than 500 species identified at different part of the world it is commonly called morning glory. Various part of the plant is used for both medicine and ornamental purpose. The plant can be found in various part of the world, where its different parts are used for various purposes. While the leaves and the roots are edible, the flower is used for beautification purpose. Various parts of the plant are ethnomedicinally used for the treatment of malaria, virus and bacterial infection, in addition, it is also applied therapeutically in the management of disease related to inflammation and oxidative stress (Ilesanmi *et al*., 2023). This research aims to find scientific backing for the protective effect of I. cairica leaf extract against diclofenac hepatotoxicity in male Wistar rats.

Materials and Methods

Plant Collection and Identification

On 8th October 2021, fresh leaves of *Ipomoea cairica* were collected and identified from a community in the state of Nembe in Bayelsa, Nigeria. Parts of the plant were sent to the Department of Botany of the University of Benin for identification and verification by plant taxonomist. A voucher number - UBH-1561 - was allocated to it.

Animal Handling and Experimental Design

A total of thirty-five (35) rats were used for the experiment. They were divided equally into five groups (A, B, C, D and E) of seven rats each.

Group A: Normal control group.

Group B: Orally administered diclofenac (DCF) 100 mg/kg. DCF was dissolved in distilled water and administered

Group C: rats in this group were given 100 mg/kg of methanolic extract from *Ipomoea cairica* leaves (MEIC) for 14 days prior to exposure to DCF. The calculation volume of MEIC in ml was based on the weight of each rat

Group D: rats in this group were administered 250 mg/kg of methanolic extracts of *I. cairica* leaves (MEIC) were administered for 14 consecutive days before single dose administration of DCF. Group E; administered 250 mg/kg of MEIC for 14 consecutive days. The group only administered MEIC during the experiment.

Sacrifice

Animals were observed daily for any physiological changes (such as skin colour, mobility, appetite, aggression, salivation, activeness, etc.). in addition, the amount of food consumed daily and weight were measured every three days. Food was withdrawn overnight on the last day of administration prior to sacrifice. Animals were sacrificed via mild anaesthesia and the blood collected through cardiac puncture. The blood was processed for markers of renal functions and lipid profile. The livers were surgically removed and processed to test for biomarkers indicative of oxidative stress and other biochemical parameters.

These parameters were analysed in the serum: Serum level of alanine aminotransferase (ALT) and aminotransferase (AST) in the rats were analysed following the guidelines from the kits manual, and the results were expressed as units/L. To quantify the amount of albumin in the blood, the established method of Doumas et al., 1971 was followed. The concentration of total cholesterol, triglyceride, high density lipoprotein (HDL), and lower density lipoprotein (LDL) were determined according to the method of Allain *et al*., 1974, Fossati and Prencipe, 1982, and Lee and Nieman, 1996 respectively.

Oxidative stress parameters:

Malondialdehyde (MDA) is an oxidative product of the reaction between lipids and reactive oxygen species, a well-established method by Senthilkumar *et al*., 2021 was employed to measure its concentration. The concentration of glutathione (GS) was determined according to the method of Kalinovic *et al*., 2021. The level of antioxidant enzymes (superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx)) within the hepatocytes was measured according to the method described by Volkov et al., 2021, Kadhum and Hadwan, 2021, and Ahmed et al., 2021 respectively.

Anti-inflammatory enzymes parameters

NAD(P)H oxidase was determined according to the method of Hernández-Espinosa *et al*., 2019. XO was measured according to the method of Della Corte and Stripe, 1972 and described by Battelli *et al*., 1996, and the activity of MPO measured according to the method described by Nishikimi *et al.*, 1972.

Statistical Analysis

All grouped data were statistically performed with Prism (GraphPad Prism, 6.01) software. Differences among groups would be evaluated by one-way analysis of variance followed by Duncan's multiple range tests. All values would be expressed as the mean ± standard deviation of seven animals per group

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Results

Table 1: The serum concentration of albumin, aspartate aminotransferase, and alanine aminotransferase in male Wistar rats after pretreatment with methanolic extract of Ipomoea cairica (MEIC) leaf extract following exposure to diclofenac (DCF) at 100 mg/kg via intraperitoneal administration.

	ALB g/l	AST	ALT
Control	59.2±6.49	0.14±0.02	0.26±0.02
Diclofenac	22.84±5.39***	0.25±0.03***	0.26±0.02
100 mg/kg MEIC+ diclofenac	21.66±7.83	0.32±0.02	0.25±0.04
250 mg/kg MEIC+ diclofenac	22.89±2.89	0.32±0.02	0.24±0.02
250 mg/kg MEIC	59.29±8.41	0.16±0.04	0.30±0.07

The data represents average ± standard deviation (SD), n = 7 rats. *** P<0.0001=Control group vs diclofenac (significantly different)

Biochemical Analysis of Markers of Hepatotoxicity in the Serum

The protective effect of MEIC against diclofenac hepatotoxicity was investigated by evaluating alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities in the serum. In the group administered only diclofenac, there was significant increase in AST, no significant effect on ALT, and significant decrease in bilirubin concentration, when compared to the rats in control group (P<0.05). pretreatment of the rats with 100- and 250 mg/kg of MEIC did not reverse the toxic effect of diclofenac as observed in the insignificant difference in ALT and bilirubin when compared the rats intoxicated with only diclofenac. In addition, there was no significant difference in the activity of AST, ALT, and bilirubin concentration between the rats administered 250 mg/kg of MEIC and the control group.

Table 2: Information about fats in the blood, including total cholesterol (TC), triglycerides (TG), good cholesterol (HDL), and bad cholesterol (LDL) in male Wistar rats after pretreatment with methanolic extract of Ipomoea cairica (MEIC) leaf extract following exposure to diclofenac (DCF) 100 mg/kg via intraperitoneal administration.

	TC	TG	HDL	LDL
Control	1.48±0.10	2.27±0.03	0.73±0.23	0.62±0.23
Diclofenac	5.14±0.47***	1.98±0.77***	0.47±0.36***	3.95±0.04***
100 mg/kg MEIC+ diclofenac	2.45±0.69###	2.66±0.5###	0.34±0.19	1.58±0.19###
250 mg/kg MEIC+ diclofenac	1.92±0.26###	2.79±0.86###	0.31±0.24	1.05±0.24###
250 mg/kg MEIC	1.13±0.04	2.27±0.01	0.87±0.21	0.81±0.2

The data represents average ± standard deviation (SD), n = 7 rats.
*** P<0.0001=Control group vs diclofenac (significantly different); ###P<0.05= diclofenac vs treatment groups (significantly different)

Effect of Diclofenac and Ipomea Cairica Leaf Extract on Lipid Profile

Intoxication of rats with DCF caused a significant elevation in both total cholesterol (TC) and low-density lipoprotein (LDL) in the blood. Conversely, there was a significant decrease in serum levels of triglyceride (TG) and high-density lipoprotein (HDL) when compared to rats in untreated group. The administration of MEIC at 100- and 250 mg/kg was able to significantly (P<0.05) prevent the increased concentration of TC and LDL as compared to the untreated group, in addition to significantly (P<0.05) preventing the decrease of TG and HDL when compared to the untreated group.

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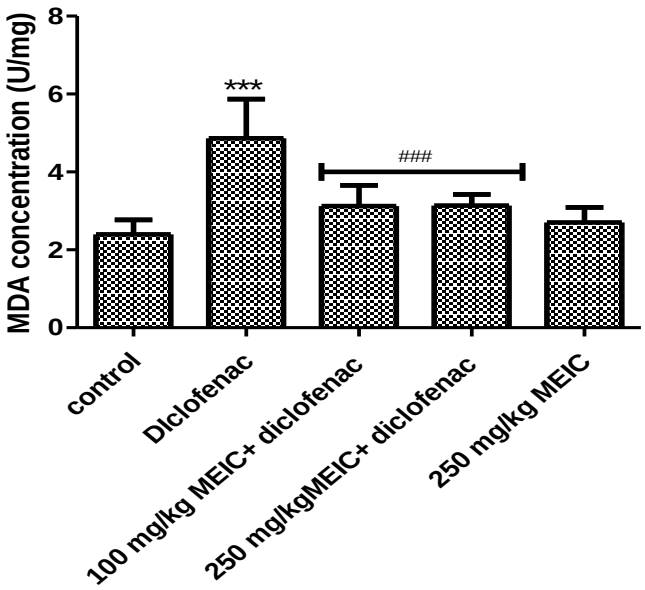

Figure 1: The effect of *I. cairica* leaf extract (MEIC) and diclofenac on the concentration of malondialdehyde in the liver of male Wistar rats. The data represents average ± standard deviation (SD), n = 7 rats. The symbols- *** P<0.0001=Control group vs diclofenac (significantly different) and ### P<0.001= DCF vs treatment (significantly different)

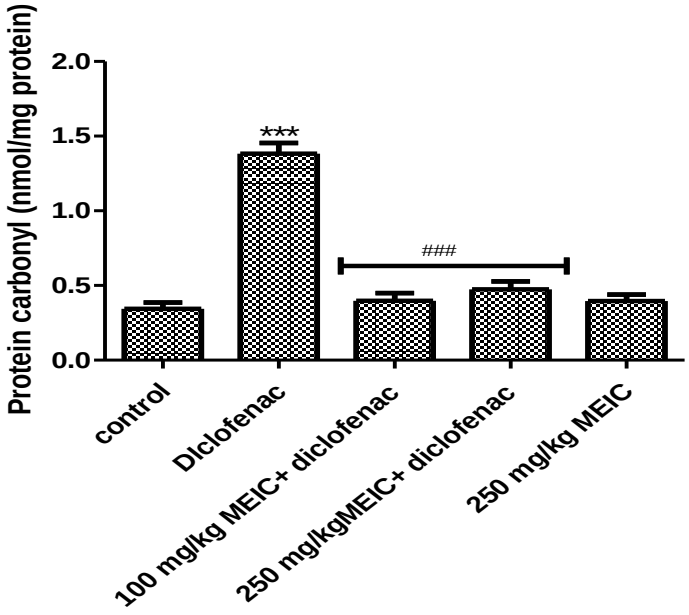

Figure 2: The effect of *I. cairica* leaf extract (MEIC) and diclofenac on the concentration of protein carbonyl in the liver of male Wistar rats. The data represents average ± standard deviation (SD), n = 7 rats. The symbols- *** P<0.0001=Control group vs diclofenac (significantly different) and ### P<0.001= DCF vs treatment (significantly different).

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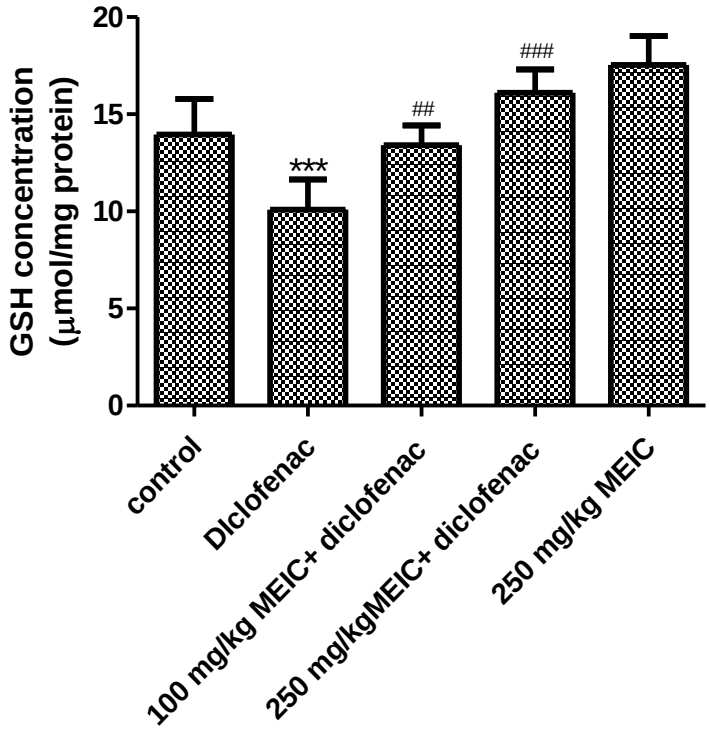

Figure 3: The effect of *I. cairica* leaf extract (MEIC) and diclofenac on the concentration of glutathione in the liver of male Wistar rats. Each bar represents mean value ± standard deviation (SD) (n=5) and the symbols-*** represent $p<0.001$ for control group vs diclofenac (significantly different) and ### represent $p<0.001$ for DCF vs treatment (significantly different).

Measurement of Oxidative Stress in the Liver's Tissue

The oxidative effect of diclofenac and the potential of MEIC to counter it was determined by quantifying the concentration of malondialdehyde (MDA) and protein carbonyl (PC). The results showed that diclofenac at the administered dose significantly increased ($P<0.001$) the concentration of MDA and PC when compared to the rats in the control group. Administration of MEIC at 100- and 250 mg/kg was able to counter the oxidative effect of DCF as observed in the significant decrease ($P<0.001$) in the concentration of MDA and PC when compared to the untreated group.

The pretreatment of rats with MEIC 100 and 250 mg/kg also showed a dose-dependent increase in GSH concentrations compared to untreated rats (DCF). Compared to control rats, the concentration of GSH in rats receiving 250 mg/kg MEIC only increased, but was not significant ($P>0.05$).

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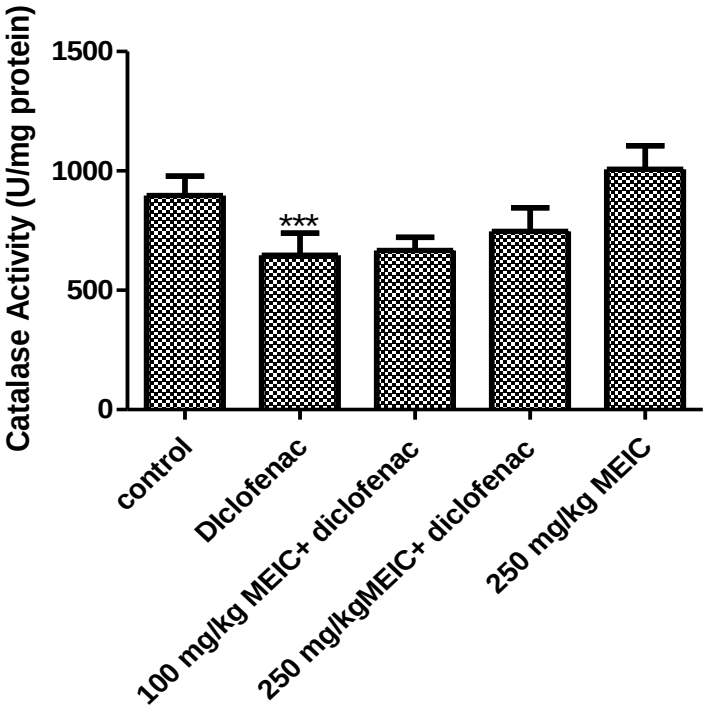

Figure 4: The effect of *I. cairica* leaf extract (MEIC) and diclofenac on the activity of catalase in the liver of male Wistar rats. Each bar represents mean value ± standard deviation (SD) (n=5) and the symbols-*** P<0.001, control group vs diclofenac (significantly different)

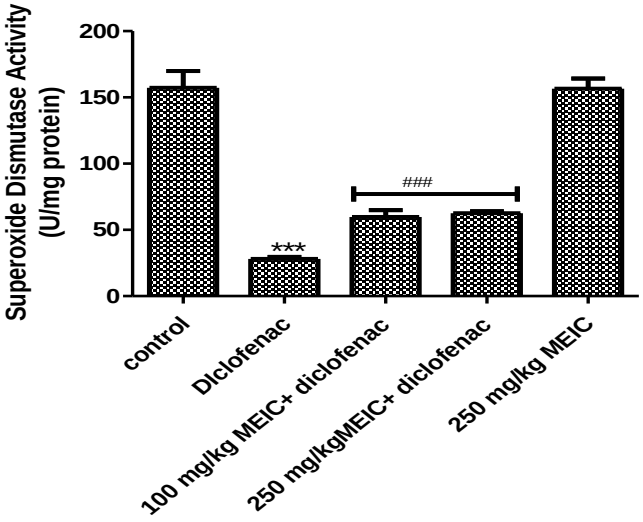

Figure 5: The effect of *I. cairica* leaf extract (MEIC) and diclofenac on the activity of superoxide dismutase in the liver of male Wistar rats. Each bar represents mean value ± standard deviation (SD) (n=5) and the symbols-*** represent p<0.001 for control group vs diclofenac (significantly different) and ### represent p<0.001 for DCF vs treatment (significantly different).

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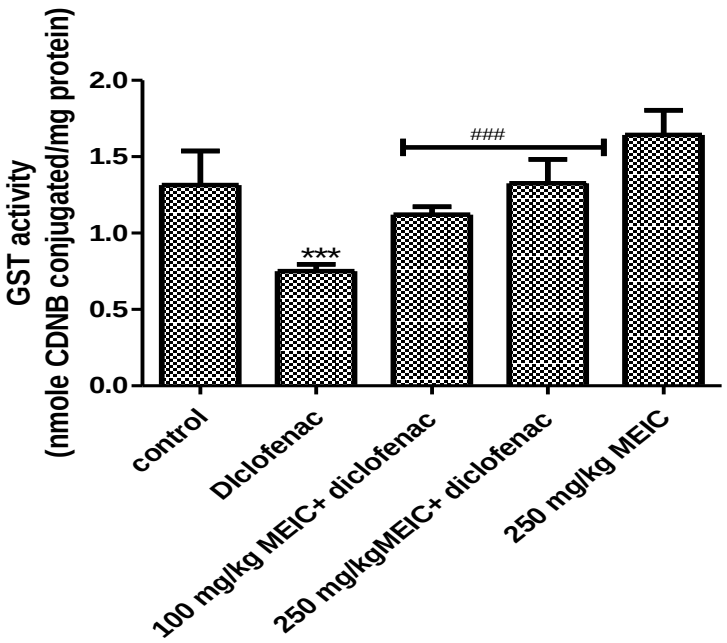

Figure 6: The effect of *I. cairica* leaf extract (MEIC) and diclofenac on the activity of glutathione transferase in the liver of male Wistar rats. Each bar represents mean value ± standard deviation (SD) (n=5) and the symbols-*** and ### indicates significantly different at from control at $p<0.001$ and $p<0.001$ respectively.

The ability of MEIC to counteract oxidative stress was determined by analysing the activity of antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), and glutathione-S-transferase (GST), along with the concentration of the antioxidant molecule glutathione (GSH).

CAT, SOD, and GST activities significantly decreased in the hepatocytes of rats administered diclofenac in comparison to the baseline levels observed in the rats in group I. Pretreatment of rats with MEIC at the dose of 100 mg/kg had no significant effect on catalase activity in comparison to the untreated group (diclofenac), while 250 mg/kg increased the activity of catalase, but it was not significant ($P>0.05$). The administration of 250 mg/kg MEIC had no significant effect on CAT activity compared to rats in the control group. The pretreatment of rats with 100- and 250 mg/kg significantly increased ($P<0.001$) the activity of SOD in comparison to untreated rats, while the administration of 250 mg/kg MEIC did not affect the SOD activity when compared to rats in the control group. The pretreatment of rats with MEIC 100 and 250 mg/kg caused an increase in GST activity in a dose-dependent manner when compared to the rats in the untreated group. As a result, the concentration of GSH in rat's toxic to diclofenac was significantly reduced compared to that of controls.

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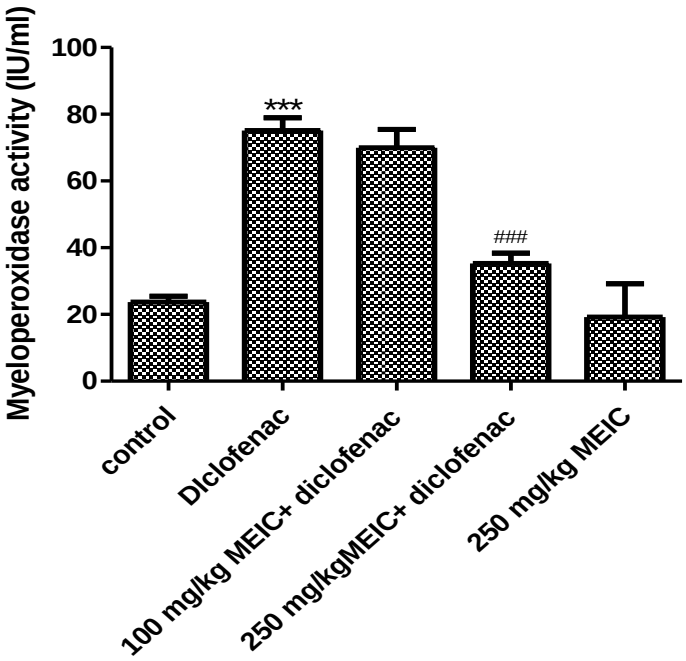

Figure 7: The effect of *I. cairica* leaf extract (MEIC) and diclofenac on the activity of myeloperoxidase in the liver of male Wistar rats. Each bar represents mean value ± standard deviation (SD) (n=5) and the symbols-*** represent $p<0.001$ for control group vs diclofenac (significantly different) and ### represent $p<0.001$ for DCF vs 250 mg MEIC (significantly different).

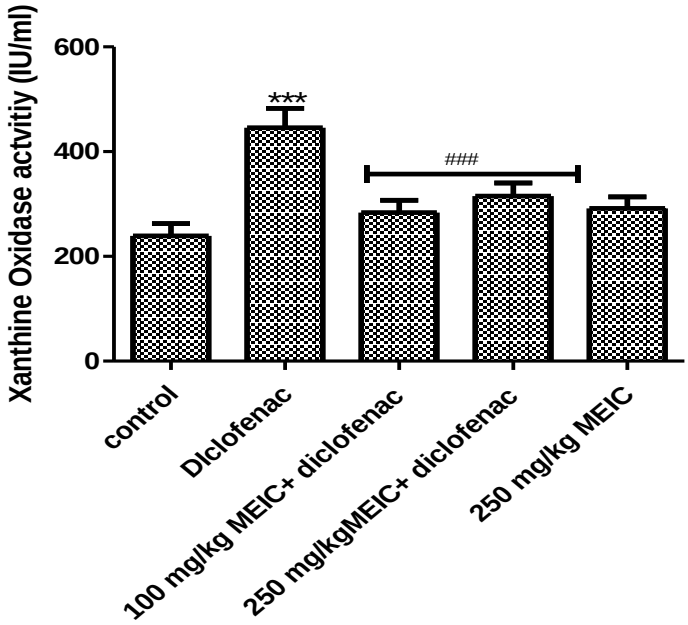

Figure 8: The effect of *I. cairica* leaf extract (MEIC) and diclofenac on the activity of xanthine oxidase in the liver of male Wistar rats. Each bar represents mean value ± standard deviation (SD) (n=5) and the symbols: *** represent $p<0.001$ for control group vs diclofenac (significantly different) and ### represent $p<0.001$ for DCF vs treatment (significantly different).

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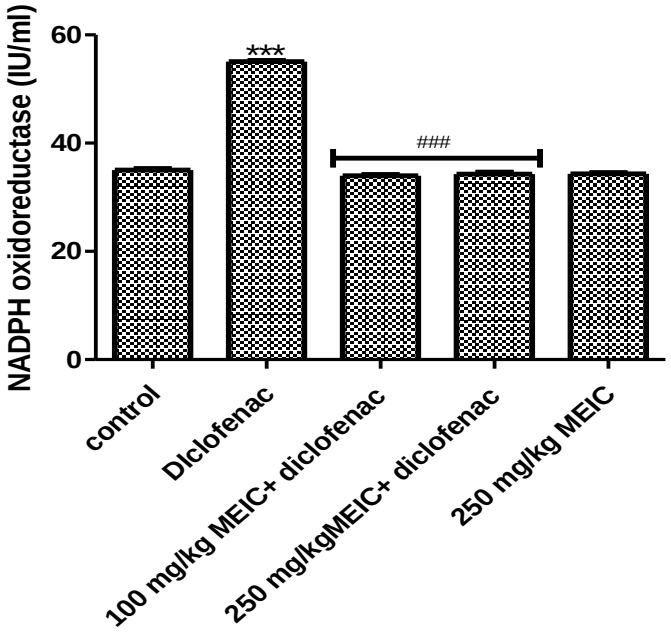

Figure 9: The effect of *I. cairica* leaf extract (MEIC) and diclofenac on the activity of NADPH oxidase in the liver of male Wistar rats. Each bar represents mean value ± standard deviation (SD) (n=5) and the symbols-*** represent $p<0.001$ for control group vs diclofenac (significantly different) and ### represent $p<0.001$ for DCF vs treatment (significantly different).

Assessment of anti-inflammatory enzymes in hepatic tissues after pretreatment with MEIC and diclofenac poisoning.

The results show high inflammation activity in the hepatic cells of rats intoxicated with diclofenac, this was based on the increased activities of myeloperoxidase (MPO), xanthine oxide (XO) and NADPH oxidoreductase (NOR) with significant increase ($P<0.001$) than rats in the control group. The pretreatment of rats with MEIC at a dose of 100 mg/kg did not result in statistically significant effect on MPO activity ($P>0.05$) compared to the untreated group (Diclofenac). However, the group administered 250 mg/kg MEIC had decreased activity of MPO, which was significantly different from MPO activity in the untreated group ($P<0.001$). The administration of 250 mg/kg MEIC did not affect the activity of MPO compared to control rats. For the activities of XO and NOR, the two doses administered significantly reduced their activities in rats' liver ($P>0.001$) compared to those not treated (diclofenac). 250 mg/kg administration does not affect enzyme activity compared to control rats.

Discussion

The liver plays an important role in diclofenac metabolism, it converts the active drug to hydroxylated and sulfated form for easy excretion in humans, while it is excreted in the form of glucuronide in rodents (Ashutosh *et al*., 2021). One of the major indications of liver damage is an elevated level of aspartate aminotransferase and alanine aminotransferase in the serum as well as a decrease in the concentration of albumin in the serum (Heidarian and Nouri, 2019). Findings from the experiment show an increase in AST levels due to DCF exposure indicating liver damage. Pretreatment with MEIC at the two administered doses could not prevent the leakage of the enzyme from the liver. This is contrary to other investigations of MEIC on the hepatotoxicant with similar doses.

Maintaining the homeostatic status of lipid components is vital to prevent cardiovascular diseases. Several reports have shown the role of NSAIDs in the development of CVD. Diclofenac (DCF) is reported to induce toxicity by altering lipid homeostasis (Ashraf *et al*., 2014). This metabolic alteration of lipid profile due to DCF administration was reflected in the experiments as administration of DCF resulted in a significant upregulation of TC and LDL along with a significant downregulation of HDL concentration. No significant changes were noticed in the concentration of triglyceride.

The hyperlipidaemic effect of DCF can be connected to the observed induction of lipolysis by DCF, which is linked to the ability of DCF to inhibit prostaglandin synthesis, which is a critical pathway in the regulation of lipolysis. Increased activity of lipolysis elevates

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plasma free fatty acids (FFA) which might contribute to the hyperlipidaemic effect of DCF. The liver plays a crucial function in maintaining the status of lipid profile in the blood. This might contribute to the hepatotoxic effect of DCF.

Antioxidant-rich plants have been reported to play a therapeutic role in preventing liver toxicity by acting as a cholesterol-lowering agent or improving HDL status. Administration of MEIC for fourteen days before co-administration with DCF was effective in lowering the cholesterol and LDL concentration reflecting its hyperlipidaemic effect.

One of the major biochemical processes involved in hepatotoxicity is oxidative stress. Oxidative stress can cause liver injury via damage to hepatic cells, loss of liver function, and cell death. Since oxidative stress involves an overproduction of oxidants against antioxidants (Villa-Jaimes *et al*., 2023). Most toxic drugs often exert their toxic effect by acting as prooxidants, which involves the production of reactive oxygen species and depletion of the antioxidant system. This are often indicated by increased concentration of malondialdehyde (MDA) and other oxidative species (36. Syed *et al*., 2016; Tang, 2003).

The results from the experiment, confirmed the pro-oxidant effect of DCF as observed in the increased concentration of MDA and protein carbonyl (PC), while it caused depletion of GSH, and activities of CAT, SOD, GST. This corroborated the report of other researchers (Syed *et al*., 2016; Tang, 2003; Boelsterli, 2003. The increased concentration of MDA might be due to increased production of DCF-quinone imine, a reactive metabolite of DCF) which is highly increased due to DCF overload. In addition, the increased concentration of MDA and PC might also be a result of the destruction of the antioxidant defence system, majorly GSH depletion. GSH plays an important role in deradicalizing free radicals involved in oxidative stress. Thus, when the concentration is low, the hepatic cells lose their ability to clear oxidative species. In addition, the decreased activities of CAT and SOD can further decapacitate the cells from deactivating ROS.

Apart from oxidative stress, another mechanism by which drugs induce hepatotoxicity is through inflammatory processes. NAD(P)H oxidases and myeloperoxidase are inflammatory enzymes that are activated when cells/tissues are infected or intoxicated, the two enzymes are also involved in the generation of hydrogen peroxide, further increasing the oxidative effect of toxic chemicals (El-Yazbi *et al*., 2017). Another inflammatory enzyme is xanthine oxidase, which works with inflammatory neutrophils to produce superoxide. the activation and overexpression of these enzymes have been reported to generate deleterious reactive species during the inflammation process. DCF has been reported to damage the liver by disrupting the autophagy via oxidative stress and destroying lysosomal cells (Seung-Hwan *et al*., 2020). Intoxication of the rats with DCF induced an increase in the activities of MPO, NAD(P)H oxidase, and XO in the hepatic cells. The inflammatory activities of DCF have been linked to the activation of macrophages, neutrophils and white blood cells. thereby increasing the inflammatory processes. The findings corroborated the inflammatory activities of DCF.

Pre-treatment with MEIC was able to counter the inflammatory effect of DCF as observed in the decreased activities of MPO, NAD(P)H oxidase, and XO. Previous reports have shown that MEIC has anti-inflammatory activities by testing the extract against egg albumin.

Conclusions

In conclusion, the results of the experiment support the potential usage of *I. cairica* leaves in the maintenance of healthy liver function. The induction of liver damage due to diclofenac assault via oxidative stress and inflammation was reversed by treatment with *I. cairica*. Thus, *I. cairica* protective properties can be linked to phytochemicals present inside it that are antioxidant and anti-inflammatory characteristics.

Funding: None

Institutional Review Board: To ensure animal welfare, the study adhered to the guidelines set forth in the Helsinki Declaration of 1975. All animals used were healthy. The experimental design received approval (code ART2023008) from the Federal University Otuoke's ethical committee on animal research and treatment (ART). The experiments were conducted in the Department of Biochemistry's animal house between May and July 2022.

Conflicts of Interest: None.

Data Availability: It will be made available on request

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