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Hepatoprotective Effect of *Ipomoea cairica* Leaf Extract against Acetaminophen Hepatotoxic Effect in Rats viz antioxidant and anti-inflammatory activities

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ABSTRACT

Background: Acetaminophen is one of the over-the-counter drugs commonly used by humans as a pain reliever. While it is generally safe at a prescribed dose, misuse and overdose of the drug make it one of the substances linked to liver damage. *Ipomoea cairica* is one of the medicinal plants reported to be an alternative source of treatment and prevention of toxic effects of chemicals against organ function. This study evaluated the hepatoprotective effect of *I. cairica* leaf extract against the hepatotoxic effect of acetaminophen in rats.

Methods: Thirty-five male Wistar rats were randomly divided into five groups of seven rats each. I: Normal control; II: orally administered 2000 mg/kg of acetaminophen (ACET); III: orally administered 100 mg/kg Methanolic extract of *I. cairica* (MEIC) for 14 days before single dose administration of ACET; IV: orally administered 250 mg/kg of MEIC for 14 consecutive days before single dose administration of ACET 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, oxidative stress, and pro-inflammatory activities

Results: The results showed that ACET (group II) caused a significant increase in the concentration of serum total cholesterol (TC), triglyceride (TG), and low-density lipoprotein (LDL). In contrast, the concentration of HDL was significantly reduced when compared to the rats in the control group. Pretreatment with MEIC at a higher dose was able to prevent the production of malondialdehyde (MDA) and phosphatidylserine (PC) as well as glutathione (GSH) levels. MEIC also prevented the depletion of SOD and CAT activities by ACET. In addition, ACE overdose elevated the activities of NADPH oxidoreductases, xanthine oxidases, and MPO. However, administration of the two doses of MEIC was able to reverse all the toxic effects of acetaminophen on hepatic tissues.

Conclusion: The results provided scientific proof of the potential of *I. cairica* to protect against hepatotoxicity that can be linked to the antioxidant and anti-inflammatory activity of the plant.

1. INTRODUCTION

Acetaminophen overdose can damage the liver (hepatotoxicity) through several mechanisms, including oxidative stress, apoptosis, inflammation, and disruption of cell membranes[1]. This process starts with the conversion of acetaminophen to its toxic metabolite, N-acetyl-P-benzoquinoneimine (NAPQI). Reportedly, 90% of acetaminophen ingested undergoes glucuronidation or sulfation for biliary excretion, 8% is oxidized to the reactive metabolite N-acetyl-P-benzoquinoneimine (NAPQI), and the remaining 2% is excreted unmetabolized[2].

At a safe dose, the body has sufficient glutathione, a natural antioxidant, to neutralize NAPQ1, the harmful byproduct of acetaminophen, thereby preventing the toxic effect of the drug. At high doses, however, NAPQI overwhelms the endogenous glutathione (GSH), leading the remaining NAPQI to react with other biological molecules, causing organ damage and loss of function[3]. Exceeding the recommended dose can overwhelm the body's ability to detoxify NAPQ1. This can compromise liver function and, in severe cases, lead to liver failure, a serious global health concern[4]. Natural products have been one of the solutions the drug industry is looking at to provide succor and prevent the development of liver damage[5]. They are rich in antioxidant and anti-inflammatory phytochemicals, and their abundance and safety have contributed to their appeal.

They are readily available in both developed and developing countries. *Ipomoea cairica*, commonly known as morning glory, is one of the underutilized medicinal plants in Africa. *Ipomoea cairica* belongs to the Convolvulaceae family, which has more than 500 identified species found in different parts of the world. It is commonly called morning glory. Various parts of the plant are used for both medicinal and ornamental purposes[6]. The plant can be found in various parts of the world, where its different parts are used for various purposes. While the leaves and roots are edible, the flower is used for beautification purposes. Various parts of the plant are ethnomedicinally used for the treatment of malaria, viral and bacterial infections. In addition, they are also applied therapeutically in the management of diseases related to inflammation and oxidative stress[6]. In order to evaluate the hepatoprotective activity of *Ipomoea cairica* leaf extract against acetaminophen-induced hepatotoxicity, the levels of several markers were determined, including liver function tests (alanine aminotransferase, aspartate aminotransferase, and albumin), oxidative stress markers (lipid peroxidation and protein carbonyl), and anti-inflammatory enzymes (myeloperoxidase, xanthine oxidase, and NAD(P)H oxidase).

Materials and Methods

Collection and identification of Plant

Fresh leaves of *Ipomoea cairica* were harvested from a community in Nembe Local Government, Bayelsa State, Nigeria, on the 8th of October, 2021. A section of the plant was taken to the Department of Botany at the University of Benin for identification by Prof. Emmanuel I. Aigbokhan and a voucher number- UBH-1561 was allotted to it.

Animal handling and experimental design

Thirty-five male Wistar rats were randomly divided into five groups of seven rats each. The rats were allowed to acclimatize for two weeks in clean cages under laboratory conditions. They were fed with commercially formulated pelletized feed and given clean water*, ad libitum*.

Group I (Control): Orally administered distilled water.

Group II (Acetaminophen): Orally administered 2000 mg/kg of acetaminophen (ACET). ACET was dissolved in distilled water, and the volume administered was adjusted according to the weight of each rat[7].

Group III (Lower Dose Treatment Group): Administered 100 mg/kg of methanolic extract of *Ipomoea cairica* leaf (MEIC) orally for 14 consecutive days before exposure to ACET[8].

Group IV (Higher Dose Treatment): Administered 250 mg/kg of methanolic extract of *Ipomoea cairica* leaf (MEIC) orally for 14 consecutive days before exposure to ACET.

Group V (Extract): Administered 250 mg/kg of methanolic extract of *Ipomoea cairica* leaf (MEIC) orally for 14 consecutive days. This group was administered only MEIC for the duration of the experiment.

Animals were sacrificed via mild aesthesia and the blood collected through cardiac puncture. The blood was processed for markers of liver functions and lipid profile. The liver was excised and processed for markers of oxidative stress and other biochemical parameters. Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were analysed according to the guidelines made available in the kit manual and the results were expressed as units/L. Serum albumin concentration was measured using the method described by Doumas et al.[9]. Similarly, total cholesterol (TC), triglyceride (TG), high-density lipoprotein (HDL), and low-density lipoprotein (LDL) concentrations were determined following the standard methods established for TC[10], TG[11], and HDL and LDL[12].

Oxidative stress parameters

As a marker of oxidative stress, the concentration of malondialdehyde (MDA) was measured using the method established[13]. Additionally, we determined glutathione (GSH) concentration, a key cellular antioxidant, following the standard method[14]. The activities of superoxide dismutase, catalase, and glutathione peroxidase were also determined[15,16,17].

Determination of Pro-inflammatory enzymes parameters

NAD(P)H oxidase, Xanthine oxidase (XO), and the activity of myeloperoxidase (MPO) were determined using the standard methods[18,19,20,21].

RESULTS

TABLE 3.1: Effect of Methanolic extract of *Ipomoea cairica* (MEIC) leaf on the serum concentration of total protein (TP), albumin (ALB), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities in male Wistar rats exposed to acetaminophen.

	TP (g/l)	ALB (g/l)	AST (IU)	ALT (IU)
Control	62.39 ± 6.80	59.25 ± 6.40	0.14 ± 0.02	0.26 ± 0.04
Acetaminophen (2000 mg/kg)	67.75 ± 17.70	15.03 ± 2.80**	0.39 ± 0.03**	0.33 ± 0.06*
100 mg/kg MEIC+ Acetaminophen	62.93 ± 6.20	29.66 ± 2.60##	0.36 ± 0.05	0.30 ± 0.07
250 mg/kg MEIC+ Acetaminophen +	60.18 ± 8.50	52.07 ± 2.30##	0.32 ± 0.03#	0.29 ± 0.05#
250 mg/kg MEIC	64.64 ± 8.90	59.29 ± 8.40	0.16 ± 0.04	0.24 ± 0.07

Values are expressed as mean ± SD (n=7). Statistically significant differences: *P<0.05= control vs acetaminophen; **P<0.0001=Control group vs acetaminophen; ##P<0.001= acetaminophen vs treatment groups. **TP= total protein; ALB= albumin; AST= aspartate aminotransferase; and ALT= alanine aminotransferase**

TABLE 3.2: Effect of Methanolic extract of *Ipomoea cairica* (MEIC) leaf on the serum level of high-density lipoprotein (HDL), total cholesterol (TC), total glyceride (TG), and low-density lipoprotein (LDL) in male Wistar rats exposed to acetaminophen.

	TC	TG	LDL	HDL
Control	1.81 ± 0.10	2.27 ± 0.03	0.73 ± 0.23	2.17 ± 0.21
Acetaminophen (2000 mg/kg)	5.14 ± 0.19**	2.57 ± 0.59	2.83 ± 0.05**	0.62 ± 0.23**
100 mg/kg MEIC + acetaminophen	3.88 ± 0.32##	1.79 ± 0.46	1.51 ± 0.04##	1.53 ± 0.03##
250 mg/kg MEIC + Acetaminophen	1.95 ± 0.35##	2.74 ± 0.27	1.25 ± 0.03##	2.02 ± 0.44##
250 mg/kg MEIC	1.49 ± 0.04	2.27 ± 0.01	0.87 ± 0.12	3.15 ± 0.27

Values are expressed as mean±SD (n=7). Statistically significant differences: **P<0.0001=Control group vs acetaminophen; ##P<0.001= acetaminophen vs treatment groups. **HDL= high-density lipoprotein; TC= total cholesterol; TG= total glyceride; and LDL= low-density lipoprotein**

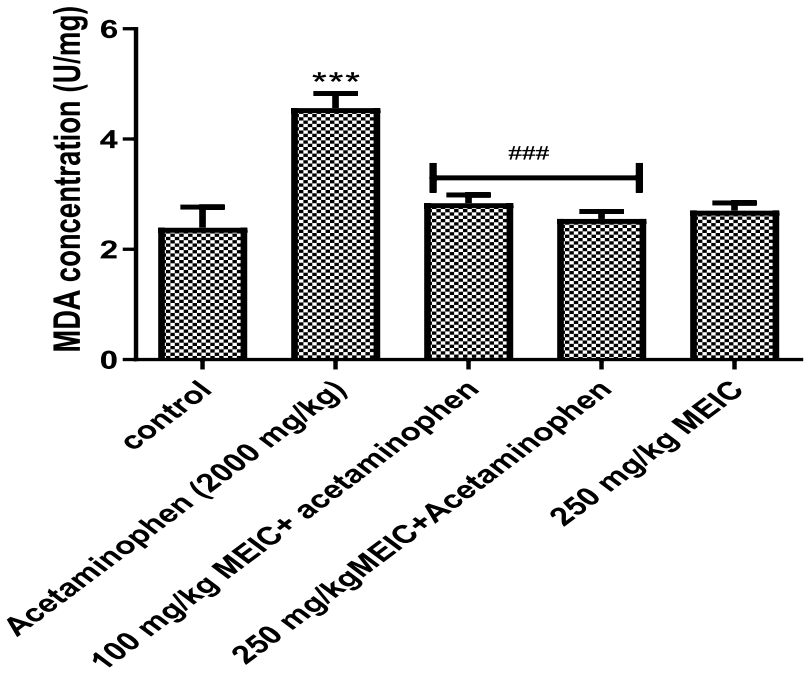

Figure 3.1: The effect of *I. cairica* leaf extract (MEIC) and acetaminophen on the concentration of malondialdehyde (MDA) in the liver of male Wistar rats. Each bar represents mean value ± standard deviation (SD) (n=7) and the symbols-*** P<0.001 = Control group vs acetaminophen and $^{\#\#\#}$P<0.001 acetaminophen vs treatment groups. MEIC: Methanolic extract of *I. cairica*: MDA= Malondialdehyde

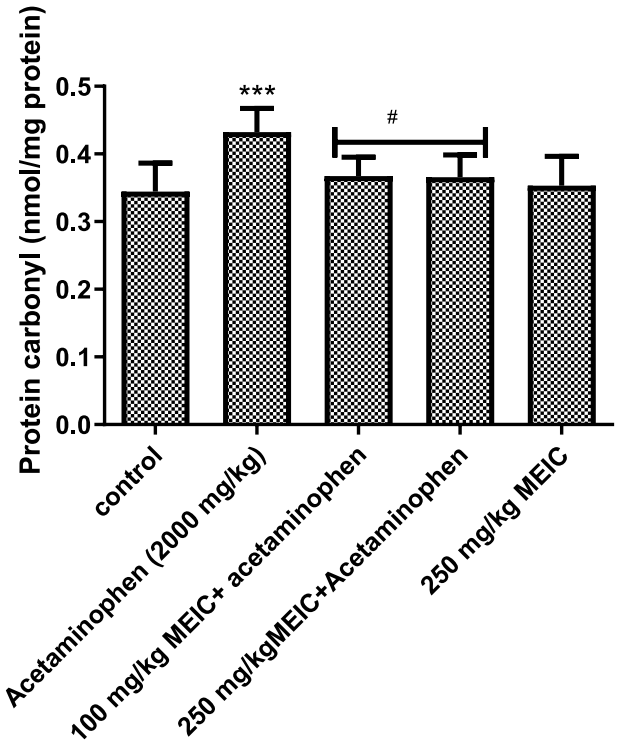

Figure 3.2: The effect of *I. cairica* leaf extract (MEIC) and acetaminophen on the concentration of protein carbonyl in the liver of male Wistar rats. Each bar represents mean value ± standard deviation (SD) (n=7) and the symbols-***P<0.001= Control group vs acetaminophen and $^{\#}$P<0.05= acetaminophen vs treatment groups. MEIC: Methanolic extract of *I. cairica*

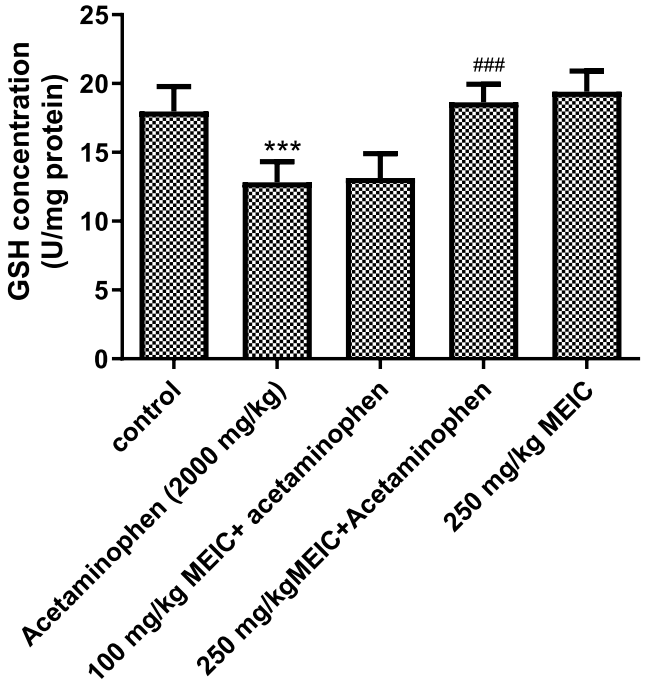

Figure 3.3: The effect of *I. cairica* leaf extract (MEIC) and acetaminophen on the concentration of glutathione in the liver of male Wistar rats. Each bar represents mean value ± standard deviation (SD) (n=7) and the symbols-***P<0.001= Control group vs acetaminophen and #P<0.05= acetaminophen vs treatment groups. MEIC: Methanolic extract of *I. cairica.* GSH: glutathione.

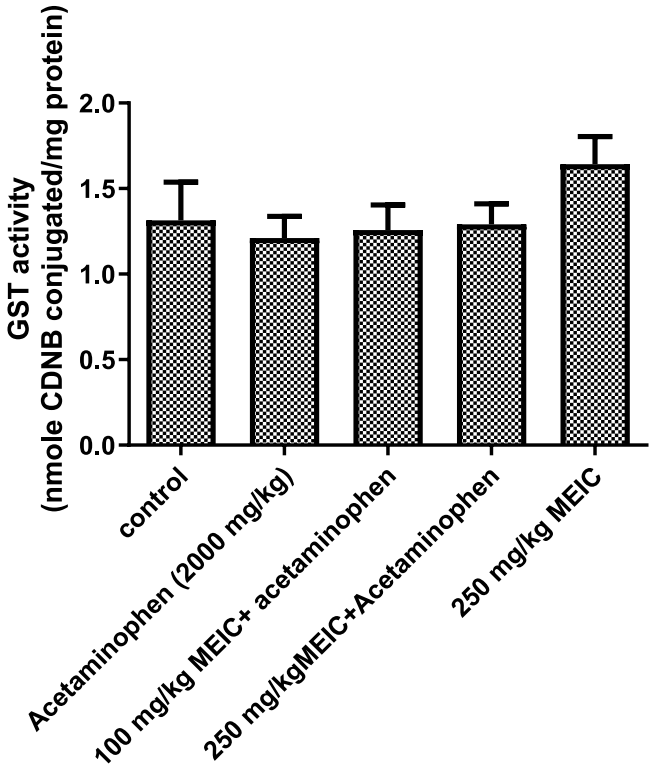

Figure 3.4: The effect of *I. cairica* leaf extract (MEIC) and acetaminophen on the activity of glutathione transferase in the liver of male Wistar rats. Each bar represents mean value ± standard deviation (SD) (n=7). MEIC: Methanolic extract of *I. cairica;* GST: Glutathione-S-transferase

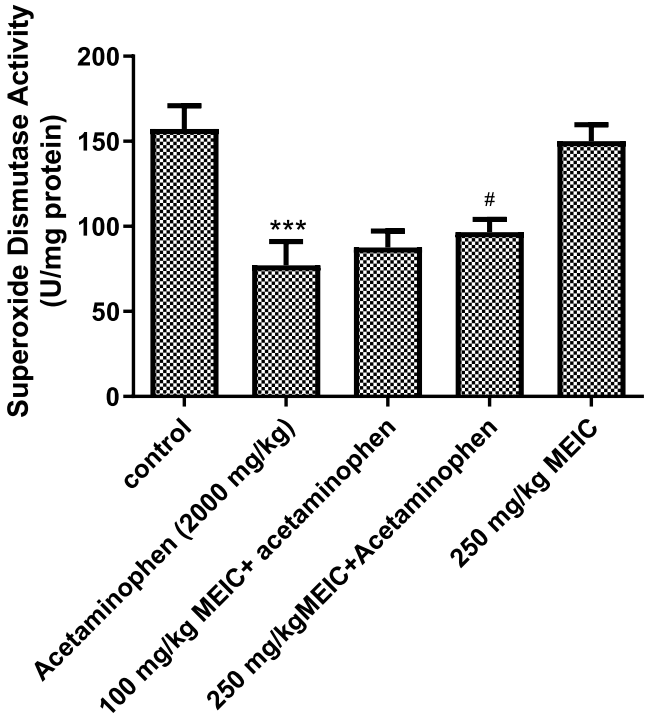

Figure 3.5: The effect of *I. cairica* leaf extract (MEIC) and acetaminophen on the activity of superoxide dismutase in the liver of male Wistar rats. Each bar represents mean value ± standard deviation (SD) (n=7) and the symbols-***P<0.001= Control group vs acetaminophen and $^{\#}$P<0.05= acetaminophen vs treatment groups. MEIC: Methanolic extract of *I. cairica*.

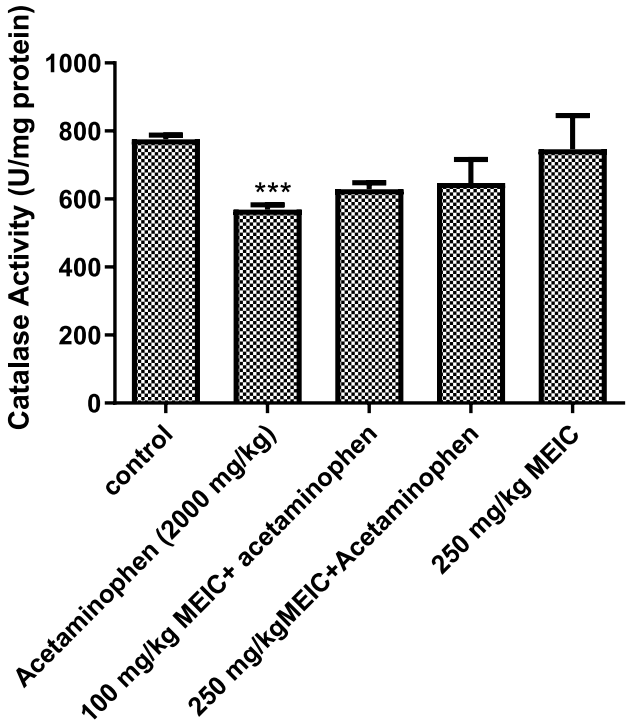

Figure 3.6: The effect of *I. cairica* leaf extract (MEIC) and acetaminophen on the activity of catalase in the liver of male Wistar rats. Each bar represents mean value ± standard deviation (SD) (n=7) and the symbols-***P<0.001= Control group vs acetaminophen. MEIC: Methanolic extract of *I. cairica*.

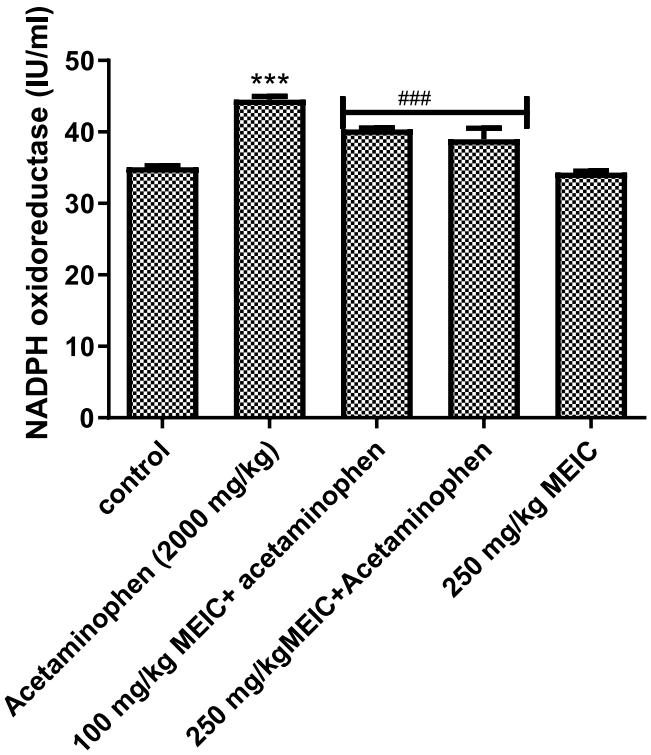

Figure 3.7: The effect of *I. cairica* leaf extract (MEIC) and acetaminophen on the activity of NADPH oxidase in the liver of male Wistar rats. Each bar represents mean value ± standard deviation (SD) (n=7) and the symbols-***P<0.001= Control group vs acetaminophen and $^{###}$P<0.001= acetaminophen vs treatment groups. MEIC: Methanolic extract of *I. cairica*.

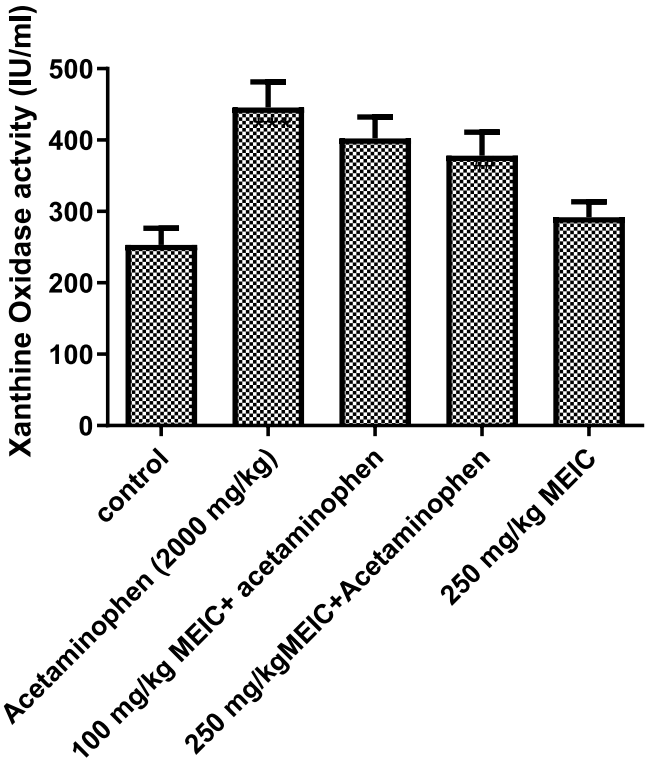

Figure 3.8: The effect of *I. cairica* leaf extract (MEIC) and acetaminophen on the activity of xanthine oxidase in the liver of male Wistar rats. Each bar represents mean value ± standard deviation (SD) (n=7) and the symbols-***P<0.001= Control group vs acetaminophen and $^{##}$P<0.001= acetaminophen vs treatment groups. MEIC: Methanolic extract of *I. cairica*

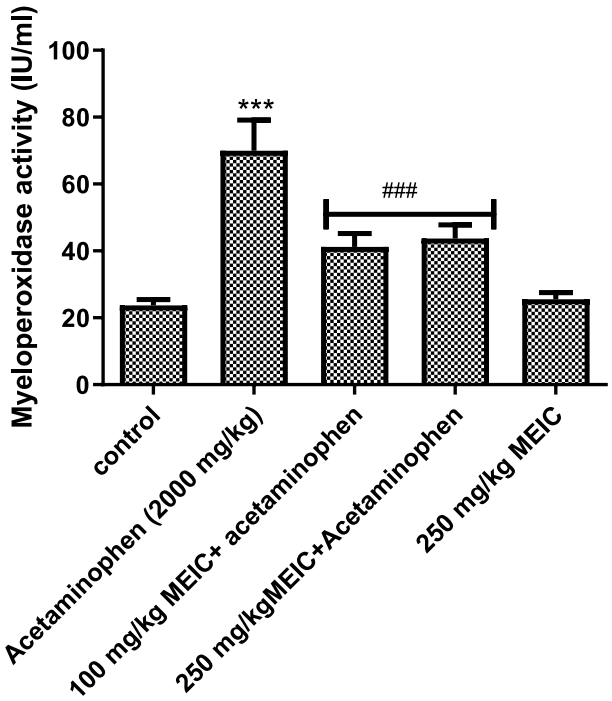

Figure 3.9: The effect of *I. cairica* leaf extract (MEIC) and acetaminophen on the activity of myeloperoxidase in the liver of male Wistar rats. Each bar represents mean value ± standard deviation (SD) (n=7) and the symbols-***P<0.001= Control group vs acetaminophen and $^{###}$P<0.001= acetaminophen vs treatment groups. MEIC: Methanolic extract of *I. cairica*

RESULTS

Effects of MEIC on lipid profile.

The results showed that the intoxication of rats with acetaminophen caused a significant alteration in the lipid profile. Acetaminophen caused a significant increase in total cholesterol (TC), triglyceride (TG), and low-density lipoprotein (LDL) compared to the control group, while the concentration of high-density lipoprotein (HDL) was significantly ($p<0.05$) reduced. Pretreatment of rats with both 100 and 250 mg/kg of MEIC significantly decreased TC, TG, and LDL levels compared to rats administered only acetaminophen. In addition, the higher dose of MEIC (250 mg/kg) significantly decreased the levels of TC, TG, and LDL compared to rats administered 100 mg/kg of MEIC. Administration of 250 mg/kg of MEIC alone showed no significant effect on TC, TG, and LDL compared to 100 mg/kg of MEIC. However, it significantly increased ($p<0.05$) the concentration of HDL compared to rats administered 100 mg/kg.

Effect of MEIC on markers of oxidative stress (malondialdehyde and protein carbonyl)

The results showd that there was a significant ($p<0.001$) increase in the concentration of malondialdehyde (MDA) and protein carbonyl (PC) compared to the control group. Pretreatment of rats with both 100 and 250 mg/kg of MEIC caused a significant decrease in the concentration of MDA ($p<0.001$) and PC ($p<0.05$) compared to the acetaminophen-treated group. In addition, there was no significant difference between the two doses administered. Administration of 250 mg/kg of MEIC had no significant difference in the concentration of MDA and PC compared to the control group.

Effect of MEIC on the antioxidant status (glutathione, glutathione transferase, superoxide dismutase, and catalase).

Intoxication of rats with acetaminophen caused a significant ($p<0.001$) decrease in the concentration of glutathione (GSH) compared to the control group. Pretreatment with 100 mg/kg of MEIC showed no significant effect on GSH concentration compared to the acetaminophen-only group. However, pretreatment with 250 mg/kg of MEIC significantly ($p<0.001$) increased the GSH concentration compared to the acetaminophen-only group. Administration of 250 mg/kg of MEIC alone had no significant effect on GSH concentration compared to the control group. The results also show that acetaminophen

intoxication caused a significant ($p<0.001$) decrease in superoxide dismutase (SOD) activity compared to the control group. Pretreatment with 100 mg/kg showed no significant difference in SOD activity compared to the acetaminophen-only group, while 250 mg/kg of MEIC significantly ($p<0.05$) increased SOD activity compared to the acetaminophen-only group. Administration of 250 mg/kg of MEIC had no significant effect on SOD activity compared to the control group. For catalase (CAT), acetaminophen intoxication caused a significant ($p<0.001$) decrease in activity compared to the control group. Pretreatment with both 100 and 250 mg/kg of MEIC increased CAT activity, however, this increase was not statistically significant. In addition, administration of 250 mg/kg of MEIC showed no significant difference in CAT activity compared to the control group.

Effects of MEIC on anti-inflammatory enzymes (NADPH oxidoreductase, xanthine oxidase, and myeloperoxidase)

The results showed that acetaminophen intoxication caused a significant ($p<0.05$) increase in the activity of NADPH oxidoreductase (NOR), xanthine oxidase (XO), and myeloperoxidase (MPO) compared to the control group. Pretreatment of rats with both 100 and 250 mg/kg of MEIC significantly ($p<0.001$) decreased the activity of NOR compared to the acetaminophen-treated group. Similarly, pretreatment with both doses of MEIC (100 and 250 mg/kg) significantly decreased ($P<0.001$) the activity of MPO compared to the acetaminophen-treated group. However, pretreatment with 100 mg/kg of MEIC did not significantly decrease XO activity, while pretreatment with 250 mg/kg significantly ($p<0.01$) decreased XO activity compared to the acetaminophen-treated group. Administration of 250 mg/kg of MEIC alone had no significant effect on NOR, XO, and MPO compared to the control group.

DISCUSSION

The study was designed to establish the hepatoprotective effect of *I. cairica* leaf extract against acetaminophen-induced liver damage. The findings showed that pre-treatment of rats with MEIC caused a significant ($p<0.05$) decrease in the activity of AST and ALT, as well as an increase in the concentration of albumin. The hepatoprotective effects of MEIC against lead and cadmium toxicity have been reported previously[22]. The ability of acetaminophen overdose to disrupt the homeostasis of AST and ALT is a proven indicator of its hepatotoxicity effect[23]. Thus, MEIC's ability to prevent acetaminophen-induced alterations in AST and ALT levels supports its hepatoprotective potential. It has also been reported that hepatotoxicity can alter lipid metabolism. The findings from the experiment also supported the hepatotoxicity effect of acetaminophen as it was observed in this study increase in the concentration of triglyceride (TG), low-density lipoprotein (LDL), and total cholesterol (TC). This toxic effect was reversed by pre-treatment with MEIC. It has earlier been reported that liver injury caused lipid metabolism[24,25] that was also confirmed in this study.

Endogenous antioxidants are a natural defence against free radicals that initiate oxidative stress[26]. Antioxidant enzymes catalyse the conversion of free radicals to non-radicals, while antioxidant molecules provide electrons that stabilize free radicals, preventing them from reacting with functional biomolecules[27]. MDA and PC are the end product of the oxidative process involving lipids and proteins. An increase in the level of MDA and PC is an indicator of oxidative damage. This condition has been reported concerning several investigations on the toxic effects of various chemicals. In this study, acetaminophen increased the concentration of MDA and PC, while pretreatment with MEIC was able to prevent the production of MDA and PC due to acetaminophen overdose. The study further showed that pre-treatment with MEIC was able to prevent the depletion of GSH by acetaminophen. Catalase and superoxide dismutase are two important enzymes that catalyse the conversion of highly reactive radicals to non-radicals. SOD catalyses the reduction of superoxide to hydrogen peroxide, which is further reduced to oxygen and water by the activity of catalase. Depletion of these enzymes causes oxidative stress[28, 29]. Acetaminophen has been reported to decrease the activities of SOD and CAT[5]. Pretreatment with MEIC at a higher dose was able to protect the liver from complete reduction of SOD and CAT activities by acetaminophen. This finding is similar to previous results obtained on the antioxidant-boosting effect of MEIC[8].

NAD(P)H oxidoreductase, an enzyme involved in the generation of superoxide anion, is physiologically active in various liver cells (Kupffer cells, hepatocytes, and stellate cells)[18]. Myeloperoxidase (MPO) is an oxidative enzyme in neutrophils that catalyses the production of hypochlorous acid, a potent oxidant[18] is an oxidative enzyme that catalyses the production of hypochlorous acid, a very strong oxidant in the neutrophil. Xanthine oxidase (XO) is another oxidative enzyme sometimes used as a marker for liver disease[20]. The activities of these enzymes are implicated in various models of hepatotoxicity.

Physiologically, these enzymes participate in the inflammatory response to eliminate infected cells; however, their overexpression leads to the overproduction of reactive species that can damage healthy cells. High MPO activity is a known indicator of inflammation[30]. The results showed that acetaminophen overdose elevated the activities of MPO and XO[31,32]. An increase in NAD(P)H oxidoreductase activity was also observed with acetaminophen administration. However, pretreatment with MEIC was able to protect the hepatocyte against acetaminophen-induced inflammatory enzymes.

Conclusion

In conclusion, the findings showed that methanol extract of *I. cairica* leaf can prevent or reverse the hepatotoxic effect of acetaminophen that might have been mediated through the plant antioxidant and anti-inflammatory.

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