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Research Article

Iodoacetamide, A Disinfection By-Product, Induces Hepatic Oxidative Stress and Inflammation in Rats: Ameliorative Role of *Cyperus Esculentus*

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ABSTRACT

Iodoacetamide (IAA), an emerging nitrogenous disinfection by-product (N- DBPs) formed during water treatment, poses an emerging concern due to its potential toxicity. *Cyperus esculentus* (CE) possesses antioxidants and anti-inflammatory properties. Thus, this study investigated the protective effect of CE on IAA-induced hepatotoxicity in Wistar rats. Thirty-five healthy rats (180 - 190 g) were randomly divided into five groups (n = 7): control, IAA only (0.1% IAA), CE only (1.0 ml/100g), IAA + CE (0.5 ml/100g), and IAA + CE (1.0 ml/100g). Following a 14-day administration period, hepatocellular integrity and liver function were assessed. IAA exposure significantly ($p < 0.05$) elevated serum levels of liver enzymes (AST, ALT, ALP, and GGT) indicating hepatocellular damage. It also reduced hepatic antioxidant enzyme activities and increased malondialdehyde (MDA) levels. Co-administration of CE significantly reversed these changes, improving liver enzyme profiles, restoring antioxidant enzyme activity, and reducing MDA levels. Moreover, CE also attenuated hepatic inflammation induced by IAA. Histological evaluation showed that CE mitigated IAA-induced necrosis, congestion, vacuolar degeneration, and other structural alterations in hepatocytes. CE ameliorates IAA-induced hepatotoxicity via mechanisms involving the mitigation of oxidative stress and inflammation in rats.

Keywords: *Iodoacetamide; Cyperus esculentus; Oxidative stress; Inflammation; Hepatotoxicity*

INTRODUCTION

Water pollution remains a significant environmental challenge worldwide, posing threats to aquatic ecosystems, public health, and the accessibility of safe drinking water (Srivastav *et al*., 2020). To minimize the risk of water-borne diseases and maintain drinking water quality, disinfectants like chlorine, chloramines, and ozone are routinely employed during the treatment process. Nevertheless, these agents can interact with naturally occurring organic substances in water, leading to the formation of disinfection by-products (DBPs) (Krasner *et al*., 2006; Zhou *et al*., 2019; Xu *et al*., 2022), which are associated with carcinogenic, teratogenic and mutagenic risks (Richardson *et al*., 2007; Li *et al*., 2018; WHO, 2000). Currently, more than 600 DBPs have been identified, leading to extensive human exposure across the globe via ingestion of drinking water, inhalation of vapors, and skin contact (Villanueva *et al*., 2015). Among these, iodoacetamide (IAA), a nitrogenous and iodinated DBP, which is widely used as a sulfhydryl alkylating chemical, has drawn increasing attention due to its toxicity

and occurrence in treated water systems. This highly reactive alkylating agent exerts its toxicity primarily by depleting cellular sulfhydryl (-SH) groups, including glutathione (GSH), resulting in reactive oxygen species production, oxidative tissue damage and cell death. Previous studies have demonstrated that IAA induces cytotoxic and genotoxic effects in various cell types. For example, IAA has been reported to induce cytotoxic and genotoxic effects in Chinese hamster ovary cells (Plewa et al., 2008) and promotes both apoptotic and necrotic cell death in porcine kidney cells (van De Water et al., 1999). In vivo, Deng et al. (2014) reported that 30-day exposure to IAA in mice led to a significant decline in the activities of antioxidant enzymes, namely superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px). Recently, Dai et al. (2022) demonstrated that IAA induces oxidative stress in human hepatocellular carcinoma cells. Moreover, Xiao et al. (2025) also reported that IAA triggers ovarian dysfunction in mice through Transforming Growth Factor Beta (TGF-β) signaling pathway activation and apoptosis induction, further highlighting the systemic toxicity associated with these compounds.

Medicinal plants and their derivatives have been used for the treatment of various diseases. Multiple studies have provided experimental evidence highlighting the protective potential of phytonutrients against environmental contaminants-induced liver toxicity (Ige et al., 2012; Ige and Akhigbe, 2013; Aja et al., 2020; Etemadi et al., 2020; Handan et al., 2020). In fact, antioxidant supplementation via phytonutrients has been highlighted as a promising therapeutic strategy for mitigating hepatic injury (Medina and Moreno-Otero, 2005). Thus, there is growing scientific interest in medicinal plants rich in bioactive antioxidants capable of protecting the liver against oxidative damage.

Cyperus esculentus L. (CE), also referred to as tiger nut, is a member of the Cyperaceae family and has a widespread global distribution (Sanchez-Zapata et al., 2012). This energy-rich tuber is composed of substantial amounts of starch, fats, proteins, sugars, and essential dietary minerals (Zhang et al., 1996). CE has been utilized for alleviating ailments such as diarrhea, dysentery, indigestion, and reproductive dysfunction (Al Essawe and Almashhadani, 2010; Abano and Amoah, 2011; Al-Shaikh et al., 2013; Ekaluo et al., 2015; Saheed et al., 2016; Atoigwe-Ogeyemhe et al., 2018). In fact, it is a known source of antioxidants like vitamins C and E, quercetin, as well as minerals including zinc, potassium, and phosphorus (Belewu and Belewu, 2007; Allouh et al., 2015). Moreover, several studies have demonstrated its neuro-hepatic effect via its antioxidative and anti-inflammatory properties (Jing et al., 2020; Umukoro et al., 2020; Ma et al., 2022; Ojakovo et al., 2025). For instance, Adelakun et al. (2022) reported that CE reduces hepato-renal oxidative damage, inflammatory responses, and caspase-3 activation after chronic exposure to sodium fluoride. Similarly, Oyedepo and Odoje (2014) demonstrated hepatoprotective effects when tiger nut flour was incorporated into rat diets prior to CCl_4 exposure. Also, Onuoha et al. (2017) have reported that pre-treatment with tigernut-based nutri-milk effectively reduced acetaminophen-induced hepatic damage in rats.

Considering the well reported antioxidative and anti-inflammatory properties of CE, and the paucity of information from literature on the effect of CE on IAA-induced hepatotoxicity, we reasoned that CE could be a promising hepatoprotective agent in IAA-induced hepatotoxicity.

MATERIALS AND METHODS

Chemicals and reagent

Iodoacetamide was purchased from Fluka, Germany. All other reagents used were of analytical grade.

Collection and preparation of tiger nut milk

Fresh Tiger nuts (*Cyperus esculentus*) were purchased from local vendor in Sabo market, Oyo, Oyo State, Nigeria. The method of Naeem and Youssef (2022) was used with slight modifications. Briefly, fresh tiger nuts (250 g) were soaked overnight in potable water at room temperature using a 1:3 (w/v) ratio. The soaked nuts were then blended until a smooth homogenate was obtained, filtered through cheesecloth to remove coarse particles, and portioned into 100-mL aliquots, which were stored at -20 °C until use.

Animal care

We randomly divided 35 Wistar rats (weighing between 180 and 190 grams) into five groups containing seven animals each. The experimental animals were acquired from the Central Animal House, University of Ibadan, Ibadan, and housed for two weeks at the Department of Chemical Sciences, Ajayi Crowther University, Oyo for acclimatization before the experiments began. They were housed under standard laboratory conditions (24 ± 2 °C, 43 % humidity, and 12-h light/12-h dark cycle) in polycarbonate plastic cages located in a well-ventilated vivarium with free access to standard laboratory chow and water *ad libitum*. All animal handling and treatment procedures adhered to standard protocols in strict accordance with the "Principle of Laboratory Animal Care" (NIH Publication No. 85-23).

Experimental design

Rats were randomly allotted into five groups (n = 7): Group 1 served as control and received distilled water orally at 2 ml/kg/day for 14 days; Group 2 (IAA Only) received 0.1 % iodoacetamide (IAA) for 14 days; Group 3 (CE Only) received 1.0 ml/100g of tigernut milk for 14 days; Group 4 (IAA + CE (0.5)) received 0.1 % IAA and 0.5 ml/100g of tigernut milk for 14 days; Group 5 (IAA + CE (1.0)) received 0.1 % IAA and 1.0 ml/100g of tigernut milk for 14 days. CE was administered orally by gavage. The selection of CE doses (0.5 mL/100 g and 1.0 mL/100 g body weight) was guided by the work of Ezekwesili et al. (2016), in which equivalent doses of tigernut milk were administered to rats under comparable experimental conditions. Similarly, the IAA dose and administration protocol were modelled on the established approach of Elseweidy et al. (2008). IAA-containing drinking

water was prepared freshly every day by dissolving 0.2 g IAA in 200 mL drinking water (0.1% concentration) and administered via oral consumption for 14 days.

Biochemical analysis

Twenty-four hours post-final dose, animals received intraperitoneal anesthesia consisting of ketamine (75 mg/kg) and xylazine (10 mg/kg). Serum was obtained by collecting blood from the jugular vein and centrifuging it at 3000 rpm for 10 minutes, then stored at -20 °C until analysis. The animals were subsequently euthanized by cervical dislocation, and liver tissues were excised. The isolated livers were rinsed with normal saline and individually homogenized in 50 mM Tris-HCl buffer (pH 7.4). Centrifugation of the homogenates was performed at 12,000 × g for 10 min at 4 °C using a refrigerated centrifuge, and the supernatants obtained were portioned and stored at -20 °C until biochemical assays were conducted. For histological evaluation, liver tissues were fixed in 4% phosphate-buffered formalin.

Assay of liver function indices

Serum activities of AST, ALT, ALP, and GGT were determined using commercial assay kits obtained from Quimica Clinica Aplicada (QCA), Spain.

Assessment of oxidative stress biomarkers

Catalase (CAT) activity was measured following the method of Goth (1991), while superoxide dismutase (SOD) activity was evaluated using the protocol described by Misra and Fridovich (1972). Malondialdehyde (MDA) levels were quantified using the TBA reagent to assess lipid peroxidation, as outlined by Esterbauer and Cheeseman (1990). The method of Habig et al. (1974) was used to assess glutathione-S-transferase (GST) activity while glutathione (GSH) levels were measured following the method of Jollow et al. (1974).

Determination of biomarkers of inflammation

IL-1β and TNF-α concentrations were evaluated using ELISA Kits (Biolegend, San Diego, CA, USA), while iNOS and COX-2 concentrations were determined with ELISA kits supplied by Elabscience Biotechnology, following the manufacturers' protocols.

Histological examination

Ketamine (75 mg/kg) and xylazine (10 mg/kg) were administered intraperitoneally to anesthetize rats, after which transcardial perfusion was performed using sodium chloride solution followed by 4% formalin in 0.1 M sodium phosphate buffer (pH 7.4). Subsequently, the liver was carefully harvested and immersed in 4% formalin for fixation. Tissues were embedded in paraffin, and serial sections of 5 µm thickness were obtained using a rotary microtome (Leica RM2125 RTS). These sections were stained with hematoxylin and eosin (H&E) to evaluate general liver histomorphology. Photomicrographs of the stained sections were obtained with a Leica digital camera mounted on a light microscope.

Statistical analysis

All data were expressed as mean ± SEM and subjected to statistical analysis using one-way analysis of variance (ANOVA) via GraphPad Prism® software version 10.01 (GraphPad Software, Inc.) to evaluate intergroup differences. Where significant main effects were identified, Bonferroni post hoc test was employed for multiple comparisons. Statistical significance was defined at $p < 0.05$.

RESULTS

Biomarkers of liver function

Treatment with IAA alone resulted in a significant ($p < 0.0001$) rise in serum ALT, AST, ALP, and GGT activities in rats (Figure 1). Conversely, co-administration of IAA and CE (IAA + CE (0.5) and IAA + CE (1.0)) significantly decreased the activities of ALT, AST, ALP and GGT when compared to the group treated with IAA alone ($p < 0.0001$). However, the decrease in ALP and GGT activities in the group treated with IAA + CE (0.5) was not significant ($p = 0.4513$ and $p = 0.0864$, respectively) when compared with group exposed to IAA alone. Rats treated with CE alone showed no treatment-related effects on liver function enzymes, as there were no significant ($p > 0.05$) differences in ALP, ALT, and GGT levels compared to the control group. However, a statistically significant ($p = 0.0129$) decrease was observed in AST activity relative to controls (Figure 1).

Figure 1

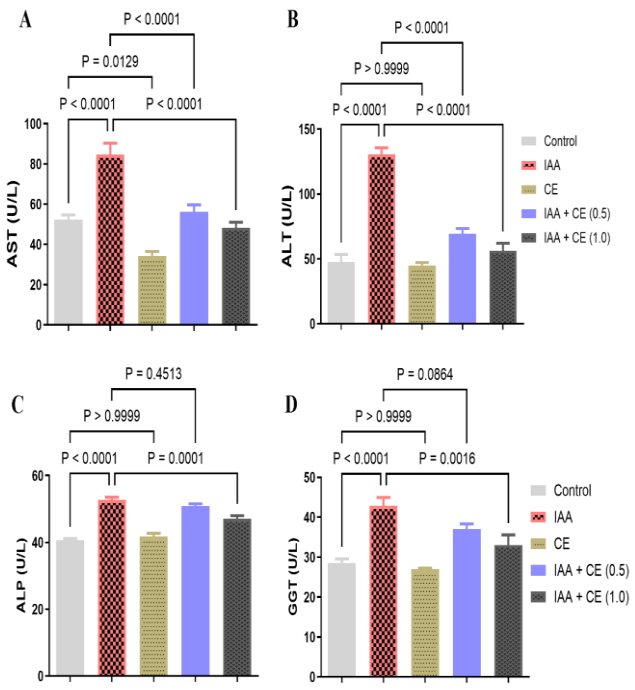

Figure 1. Effect of Iodoacetamide on hepatic function markers and the ameliorative impact of Cyperus esculentus. (A) aspartate aminotransferase, (B) alanine aminotransferase, (C) alkaline phosphatase, and (D) Gamma-glutamyltranferase. Data are expressed as mean ± SEM; n = 7 rats, error bars represent SEM.

Hepatic oxidative and antioxidant concentration

Rats treated exclusively with IAA showed significantly lower ($p < 0.05$) hepatic antioxidant enzyme activities (SOD, CAT, and GST) and GSH levels compared to untreated controls (Figure 2). Co-treatment with IAA + CE (0.5) and IAA + CE (1.0) significantly ($p < 0.05$) increased the levels of these antioxidant markers compared to the IAA-alone group (Figure 2). However, the rise in SOD activity detected in the IAA + CE (0.5) group was not statistically significant ($p = 0.1154$) when compared with the IAA-alone group. On the other hand, rats treated with CE alone showed a significant increase in CAT activity ($p < 0.0161$) and GSH levels ($p = 0.274$) compared to the control group, while SOD and GST activities remained statistically unchanged ($p = 0.3550$ and $p > 0.05$). Moreover, rats treated with IAA alone showed significant ($p < 0.0001$) increase in the concentration of liver MDA compared to control. However, simultaneous administration of CE (IAA + CE (0.5) and IAA + CE (1.0)) significantly ($p < 0.0001$) decreased liver MDA levels relative to rats treated exclusively with IAA.

Figure 2

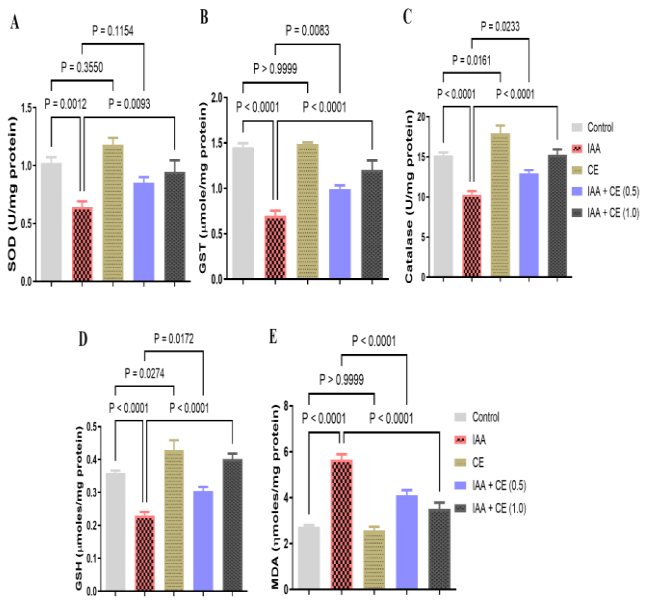

Figure 2. Effect of *Cyperus esculentus* on hepatic oxidative stress indices in Iodoacetamide-treated rats. Data are expressed as mean ± SEM; n = 7 rats, error bars represent SEM.

Effects of IAA and C. esculentus on selected markers of inflammation in the liver

Administration of IAA alone significantly ($p < 0.0001$) increased hepatic levels of IL-1β, TNF-α, COX-2, and iNOS, compared to the control group (Figure 3). Co-treatment with CE (IAA + CE (0.5) and IAA + CE (1.0)) significantly ($p < 0.05$) reduced the levels of these inflammatory markers when compared to the IAA-alone group. However, the reduction in iNOS level observed in the IAA + CE (0.5) group was not statistically significant ($p = 0.1102$) relative to the IAA-alone group. Rats treated with CE alone showed no treatment related effects relative to controls.

Figure 3

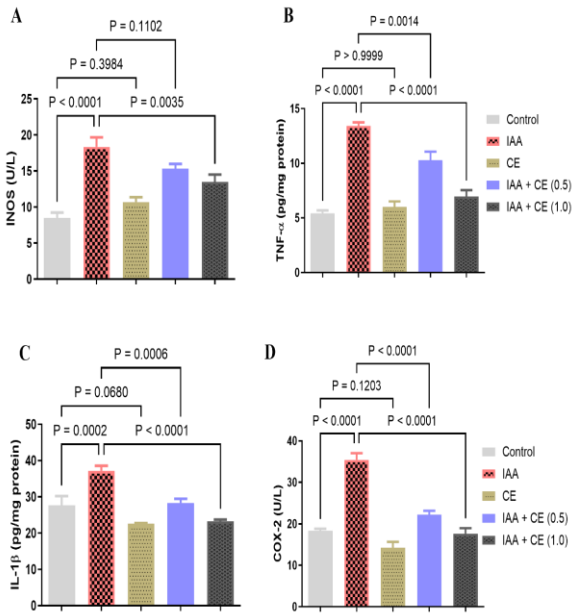

Figure 3. Influence of *Cyperus esculentus* on hepatic inflammatory markers in Iodoacetamide-treated rats. Data are expressed as mean ± SEM; n = 7 rats, error bars represent SEM.

Histological observation of liver following exposure to IAA and/or CE

The photomicrographs illustrating liver tissue morphology in rats exposed to IAA and/or CE (IAA + CE (0.5) and IAA + CE (1.0)) are displayed in Fig. 4. Both the control and CE-treated groups exhibited well-preserved hepatic histo-architecture with a normally organized monolayer of hepatocytes. Rats exposed to IAA alone show pronounced histopathological alterations, such as acinar hepatocyte necrosis, vascular congestion, sinusoidal dilation, vacuolar degeneration, marked hemorrhage, and hydropic degeneration. However, rats exposed to IAA and CE (IAA + CE (0.5) and IAA + CE (1.0)) show normal and organized layer of hepatic tissue with mild periportal cellular infiltration.

Figure 4

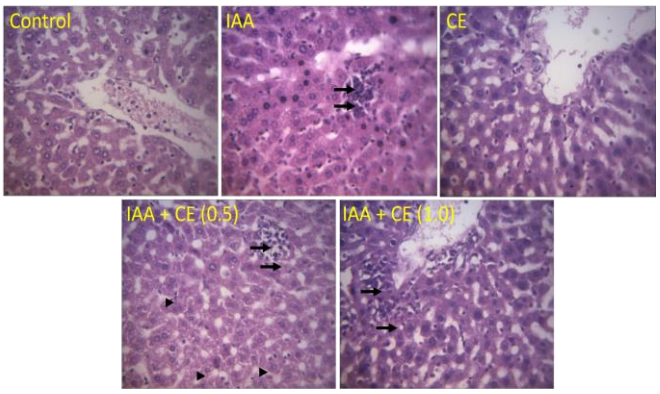

Figure 4. Microscopic images of hepatic tissue from experimental rats. Control and CE rats showing normal liver histology. IAA alone treated rats showing necrosis of acinar hepatocytes, congestion, and tissue degeneration. IAA and CE (IAA + CE (0.5) and IAA + CE (1.0)) treated rats showing normal and organized layer of hepatic tissue with mild periportal cellular infiltration. H & E x 400.

DISCUSSION

The present study investigated the impact of IAA, a disinfection by-product, on hepatic function, oxidative stress, and inflammatory signaling, and evaluated the hepatoprotective potential of CE against these effects in Wistar rats. To the best of our knowledge, this is the first in vivo study to specifically examine the protective role of CE against IAA-induced hepatotoxicity. While IAA toxicity has been demonstrated in cell-based systems and other tissues (Plewa *et al*., 2008; van De Water *et al*., 1999; Dai *et al*., 2022), and CE hepatoprotection has been reported against hepatotoxicants such as CCl_4, sodium fluoride, and acetaminophen (Oyedepo and Odoje, 2014; Adelakun *et al*., 2022; Onuoha *et al*., 2017), the interaction between IAA and CE in the hepatic context had not previously been characterised in vivo. Crucially, beyond demonstrating that IAA is hepatotoxic, the present study provides mechanistic insight into the inflammatory pathways involved, specifically implicating the NF-κB/COX-2/iNOS inflammatory axis in IAA-driven hepatic injury, and suggesting that CE-mediated protection operates through coordinated suppression of oxidative stress and pro-inflammatory signalling, plausibly via Nrf2/ARE pathway activation. Elevated hepatic oxidative stress and inflammation were observed following IAA treatment, and co-administration of CE significantly mitigated these effects in a dose-dependent manner.

ALT, AST, ALP, and GGT are key serum biochemical markers commonly used to assess liver injury (Asejeje *et al*., 2023; Zhou *et al*., 2023). These enzymes are primarily located in the cytoplasm and are released into the bloodstream following cellular damage. In fact, several studies have demonstrated that elevated serum levels of these enzymes are characteristic indicators of liver injury (Asejeje *et al*., 2023; Zhou *et al*., 2023; Zheng *et al*., 2024). Thus, they are used clinically to evaluate the extent of acute hepatocellular injury and to detect early signs of liver dysfunction (Chen *et al*., 2021). In this study, we observed that the activities of serum AST, ALT, and ALP in the IAA exposed group were significantly higher which suggested that IAA caused liver damage in rats. However, we observed that treatment with CE independently, as well as in combination with IAA, led to a reduction in the activities of these enzymes. The hepatoprotective effect of CE observed in this study aligns with previous findings by Adelakun *et al*. (2022), and Adepoju *et al*. (2025), who reported significant reductions in ALT and AST levels in rats treated with extracts of CE tubers and tannin fractionate of CE respectively. Similarly, Oyedepo and Odoje (2014) documented notable decreases in ALT, AST, and ALP levels in rats pretreated with various concentrations of CE flour incorporated into their diet for 21 days prior to CCl_4 exposure.

Hepatotoxicity development involves oxidative stress as one of the key pathogenic mechanisms. It occurs when reactive oxygen species (ROS) generation surpasses the antioxidant defence system's neutralizing capacity. Thus, an effective protective strategy for preventing and treating liver damage involves decreasing ROS generation through enhancement of antioxidant enzyme levels, including SOD and glutathione peroxidases (GPx) (Liu *et al*., 2010; Kubrak et al., 2012; Zheng *et al*., 2014). In this study, we found that exposure of rats to IAA resulted in a decrease in the activities of hepatic enzymatic antioxidants (SOD, CAT, GST) as well as GSH level coupled with an increase in hepatic MDA (lipid peroxidation product) level. This is mechanistically consistent with the known alkylating action of IAA: as a potent electrophile, IAA covalently modifies and depletes cellular sulfhydryl (-SH) groups, including GSH, thereby both directly consuming a critical non-enzymatic antioxidant and compromising the GSH-dependent activity of GST (van De Water *et al*., 1999; Dai *et al*., 2022). The resultant GSH depletion impairs the cell's capacity to neutralize ROS, leading to accumulation of cytotoxic radicals and the downstream lipid peroxidation reflected in elevated MDA levels, consistent with findings by Dai *et al*. (2022). Mechanistically, this pattern of antioxidant suppression is characteristic of Nrf2 pathway inhibition: under electrophilic stress, Nrf2 is normally released from its inhibitor Keap1 and translocates to the nucleus to drive transcription of antioxidant response element (ARE)-regulated genes, including those encoding SOD, CAT, GST, and enzymes of GSH biosynthesis. The concurrent decline in all four antioxidant parameters observed in the IAA-exposed group suggests that IAA-induced electrophilic burden may overwhelm or suppress this Nrf2/ARE axis in hepatic tissue. Interestingly, simultaneous administration of CE led to a significant reduction in MDA levels and a corresponding increase in SOD, CAT, and GST activities, as well as GSH levels, in rat liver tissues compared to the IAA alone group. The antioxidative effect of CE observed in this study was dose-dependent. This restoration of antioxidant defenses by CE is consistent with Nrf2/ARE pathway activation by its phytoactive constituents. CE is a rich source of polyphenols, quercetin, and vitamins C and E (Belewu and Belewu, 2007; Innih *et al*., 2021; Hasan *et al*., 2013), many of which are well-characterized Nrf2 inducers. Quercetin in particular has been shown to promote Nrf2 nuclear translocation, upregulate ARE-driven antioxidant gene expression, and restore GSH levels in models of hepatic oxidative injury (Liu *et al*., 2010). While direct measurement of Nrf2 protein expression or nuclear localization was not performed in the present study, the observed biochemical profile strongly supports this mechanistic interpretation and warrants confirmation in future work using western blotting or immunohistochemistry for Nrf2, HO-1, and NQO1.

Oxidative stress and inflammation are intimately linked in hepatic injury: ROS generated during oxidative insult can directly activate NF-κB, the master transcriptional regulator of inflammatory gene expression (Morgan and Liu, 2011). Activation of NF-κB promotes the transcription of pro-inflammatory cytokines (TNF-α, IL-1β) and inflammatory enzymes (COX-2, iNOS), all of which were elevated in the IAA-exposed group in the present study. Specifically, IAA-induced GSH depletion and ROS accumulation likely facilitates IκB kinase (IKK) activation, IκBα phosphorylation

and degradation, and the subsequent nuclear translocation of NF-κB p65, which drives hepatic inflammatory gene expression and amplifies tissue injury. TNF-α and IL-1β, as upstream cytokines, further potentiate NF-κB activity in a feed-forward loop that perpetuates hepatic inflammation (Popa *et al*., 2007). Additionally, iNOS-derived nitric oxide (NO) generated under these conditions can react with superoxide to form peroxynitrite, a highly reactive nitrogen species that causes nitrosative damage to hepatocytes, contributing to the histopathological alterations observed. COX-2, a downstream effector of NF-κB, produces prostaglandins that amplify the local inflammatory response, further promoting hepatocellular damage (Somade *et al*., 2023). Our finding that IAA simultaneously elevated TNF-α, IL-1β, COX-2, and iNOS in a coherent pattern is consistent with unified upstream NF-κB activation. This is further supported by the study of Xiao *et al*. (2025), which demonstrated that IAA triggers systemic inflammatory responses in a dose-dependent fashion, and aligns with the established capacity of electrophilic DBPs to activate innate immune signalling in multiple tissues. Importantly, co-administration of CE with IAA significantly attenuated all four inflammatory markers in a dose-dependent manner. The anti-inflammatory effect of CE is mechanistically plausible through two converging pathways. First, by restoring GSH levels and reducing ROS (as evidenced by decreased MDA), CE reduces the oxidative trigger of NF-κB activation, thereby indirectly limiting pro-inflammatory gene transcription. Second, quercetin and other polyphenolic constituents of CE have been independently shown to directly inhibit NF-κB nuclear translocation and suppress IKK activity, reducing downstream COX-2 and iNOS expression (Aja *et al*., 2020; Somade *et al*., 2023). The dose-dependent suppression of all four inflammatory mediators observed here further supports a coherent CE-mediated inhibition of the NF-κB/COX-2/iNOS inflammatory axis, rather than a non-specific effect.

The liver plays a central role in detoxification and xenobiotic metabolism. Its histological integrity is an essential indicator for assessing chemical-induced toxicity. Following exposure to IAA, we observed histopathological alterations such as necrosis of acinar hepatocytes, vascular congestion, tissue degeneration, vacuolar changes, pronounced haemorrhage, and hydropic degeneration in the liver of rats when compared with the control. These structural lesions are the morphological correlates of the biochemical mechanisms described above: GSH depletion and ROS-driven lipid peroxidation compromise membrane integrity, leading to cellular swelling and vacuolar degeneration; sustained NF-κB-driven inflammation with elevated TNF-α promotes hepatocyte apoptosis and necrosis; and iNOS-derived peroxynitrite contributes to vascular endothelial injury and haemorrhage (Popa *et al*., 2007; Somade *et al*., 2023). The convergence of oxidative and inflammatory injury thus explains the severity of the histopathological damage observed in the IAA-alone group. Co-treatment with CE markedly reversed these structural changes, with treated livers displaying near-normal hepatic architecture with only mild periportal cellular infiltration.

This histological recovery is consistent with the biochemical restoration of antioxidant defenses and suppression of inflammatory mediators documented in this study and collectively supports the conclusion that CE acts at multiple mechanistic nodes to protect against IAA-induced hepatic injury. The histoarchitecture of the control and CE-only groups appeared entirely normal, with hepatocytes arranged in an organized monolayer, confirming the intrinsic safety of CE at the doses examined.

CONCLUSION

Taken together, the present study demonstrates that IAA-induced hepatotoxicity is mediated through coordinated oxidative stress and hepatic inflammation, and that CE exerts a significant hepatoprotective effect against these insults in a dose-dependent manner. Notwithstanding, the study has limitations that should be considered when interpreting these findings. First, no standard hepatoprotective agent was included as a positive control. The absence of a reference comparator means that the absolute efficacy of CE cannot be directly benchmarked against an established clinical standard. Consequently, while our data clearly demonstrates that CE significantly attenuates IAA-induced hepatotoxicity relative to the untreated toxic group, quantitative statements about how CE compares in potency to conventional hepatoprotective agents cannot be made from the present dataset alone. Future studies should incorporate a positive control to contextualize the magnitude of CE's protective effect. Second, the mechanistic pathways proposed in this study, specifically Nrf2/ARE pathway activation and NF-κB inhibition, are inferred from biochemical endpoints rather than directly demonstrated through molecular analyses. Measurement of Nrf2 nuclear translocation, HO-1 and NQO1 expression, IκBα phosphorylation, and NF-κB p65 nuclear localization by western blotting or immunohistochemistry would confirm these mechanistic interpretations and is recommended for future work. Third, the CE preparation used in this study was an unfractionated tigernut milk, making it impossible to attribute the observed protective effects to any single bioactive constituent. Fractionation studies and in vitro mechanistic work with isolated phytochemicals (e.g., quercetin, vitamin E, polyphenolic fractions) are needed to identify the principal active component(s) responsible.

AUTHORS' CONTRIBUTIONS

Conceptualization, FOA; Methodology, FOA, AAA, MAA, and BOA; Investigation, FOA, AAA, STA, and BOA; Data curation and analysis, FOA, MAA, and STA; Writing-original draft, FOA, MAA, and BOA; Writing - review & editing. FOA, MAA, and BOA. All authors have read and agreed to the published version of the manuscript.

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CONFLICT OF INTEREST
The authors declare that there is no conflict of interest

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