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Ferulic acid interventions ameliorate NDEA-CCl_4-induced hepatocellular carcinoma via Nrf2 and p53 upregulation and Akt/PKB-NF-κB-TNF-α pathway downregulation in male Wistar rats

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ABSTRACT

Hepatocellular carcinoma is a prevalent form of liver cancer that is life threatening. Many chemically synthesized anti-cancer drugs have various degrees of side effects. Hence, this study investigated the effect of FEAC interventions on NDEA-CCl_4-induced HCAR in male Wistar rats. HCAR was induced by intraperitoneal administration of 200 mg/kg of NDEA and 0.5 mL/kg CCl_4 (as a promoter of HCAR). Following the induction of HCAR, rats were treated differently with two different doses (25 and 50 mg/kg) of FEAC. HCAR induction was confirmed by the significant elevation of serum levels of ALT, AST, and α-FP. Also elevated significantly were liver levels of Akt/PKB, NF-κB, TNF-α, MDA, GSH, and activities of GST, SOD, and CAT, while levels of liver p53 and Nrf2 were significantly lowered compared with normal rats. Treatment interventions with both 25 and 50 mg/kg of FEAC against the DEN-CCl_4-induced HCAR gave comparable effects, marked by a significant reduction in the levels of serum ALT, AST and α-FP, as well as liver levels of MDA, GSH, Akt/PKB, NF-κB, TNF-α, GST, SOD, and CAT, while levels of liver p53 and Nrf2 were significantly elevated compared with normal rats. Put together and judging by the outcomes of this study, FEAC being a potent antioxidant may also be potent against chemical-induced HCAR via upregulation of p53 and Nrf2, as well as downregulation of the Akt/PKB-NF-κB pathway in rats.

Abbreviations: HCAR, hepatocellular carcinoma; VCM, vinyl chloride monomer; NDEA, N-nitrosodiethylamine; CCl_4, carbon tetrachloride; FEAC, ferulic acid; ALT, alanine aminotransferase; α-FP, alpha-fetoprotein; CAT, catalase; Akt/PKB, protein kinase B; AST, aspartate aminotransferase; SOD, superoxide dismutase; p53, tumor suppressor protein; GST, glutathione S-transferase; NF-κB, nuclear factor kappa B; GSH, reduced glutathione; Nrf2, nuclear erythroid 2-related factor 2; MDA, malondialdehyde; IκB, inhibitor of NF-κB; BaP, benzo(a)pyrene; CLD, chronic liver disease; NAACCR, North American Association of Central Cancer Registries; PCBs, polychlorinated biphenyls; PDK1, 3-phosphoinositide-dependent kinase 1; ELISA, enzyme linked immunosorbent assay; IACUC, Institutional Animal Care and Use Committee; ANOVA, One-way analysis of variance; PFOA, perfluorooctanoic acid; PFCs, perfluorinated chemicals; HIV-1, human immunodeficiency virus 1; TACE, transarterial chemoembolization; TSGs, tumor suppressor genes; PIP3, phosphatidylinositol 3,4,5-trisphosphate.

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1. Introduction

HCAR is the most prevalent type of primary hepatic cancer. HCAR, occurring as a result of cirrhosis, CLD and cirrhosis, is responsible for the recent emerging cases totaling 906,000 and about 830,000 cases of mortality in the year 2020, making it the 6th most rampant diagnosed cancer and ranked the 3rd most death causing cancer, a trend that is rapidly rising [1]. NAACCR, in 2023 estimated recently diagnosed cases of HCAR in the United States were 41,210, which has tripled compared with what was recorded 40 years ago, with a stabilized increase since 2015 [2]. HCAR is highly heterogeneous, with various associated etiologies simultaneously occurring during its development. Therefore, HCAR requires urgent attention to understand and acquire more knowledge in terms of its pathogenesis, diagnosis, prevention, and treatment.

The liver is continually exposed to hepatotoxins that can lead to alterations in hepatic homeostasis and ultimately lead to the induction of cancer [3]. NDEA is a genotoxin and carcinogen that is extensively utilized for the induction of HCAR in mice and rats [4]. NDEA alkylation of DNA forms adducts that can initiate hepatocarcinogenesis [5]. Induction of HCAR can be promoted by the combined administration of a single dose of NDEA and cancer promoters like CCl_4 [6]. CCl_4 induces cell destruction via free radical-mediated hepatocyte attack. Following hepatocyte exposure to CCl_4, prompt cell destruction results, which can be partially reversed and further result in cellular damage through lipid peroxidation [7]. The development of HCAR generally involves the stepwise stages of hepatic fibrosis, cirrhosis, and cancer. The combined use of NDEA and CCl_4 has not only helped against the prolonged period that is usually taken by most traditional chemical carcinogens but has also helped to improve the simulation of liver cancer and the process of developing hepatic cancer in humans.

FEAC (4-hydroxy-3-methoxycinnamic acid) is a phenolic acid rampantly seen in medicinal plants. FEAC is primarily present in cell walls of plants and adds to the structural rigidity via direct covalent linkage to structural polysaccharides like arabinoxylans, a precursor of a complex polymer called lignin that confers mechanical resistance against plant tissue biodegradation [8]. FEAC and FEAC-derived compounds have been mostly shown to possess various pharmacological properties, which are majorly anti-fibrotic, anti-cancer, anti-allergic, anti-inflammatory, and anti-oxidative [9,10]. FEAC has effectively demonstrated different mechanisms of amelioration against various models of diseases [11,12]. One of such is the NF-κB and Nrf2-HO1 signaling that are controlled by FEAC to restrain or prevent inflammatory responses and oxidative stress, respectively, thereby eliciting cardio-protection [13].

In this study, we looked at the mechanism through which FEAC exerts its therapeutic effect in rat model of NDEA-CCl_4-induced HCAR.

2. Materials and methods

2.1. HCAR-inducing chemicals, kits, and other reagents

NDEA ($C_4H_{10}N_2O$; 102.137 $gmol^{-}1$) and tetrachloromethane (CCl_4; 153.82 g/mol) are products of Sigma Chemical Co., USA; FEAC (95% purity; $C_{10}H_{10}O_4$) was obtained from AK Scientific, United State. NF-κB, Nrf2, p53, Akt/PKB, AFP, and TNF-α ELISA kits were produced by Elabscience Biotechnology, USA.

2.2. Animals and treatments

A total of 20 male Wistar rats (average weight of 150 g) were obtained and used for the study. They were given free access to standard rat food and potable drinking water. Certification to proceed with the study was issued by the IACUC of our Department, with a certification number FUNAABBCHREC 998,863 IDRD2023/091. After 7 days of acclimatization, rats were grouped into 4. Control rats were placed in group I and were intraperitoneally administered normal saline (the vehicle for NDEA) and sunflower oil (the vehicle for CCl_4). HCAR-induced rats were placed in group II and were intraperitoneally injected with 200 mg/kg of NDEA and 0.5 mL/kg of CCl_4 (a tumor promoter) simultaneously once a week for 3 weeks [14,15]. Rats placed in groups III and IV were treated equally like rats in group II but were orally treated with 25 and 50 mg/kg of FEAC [16] for another 3 weeks.

2.3. Collection of samples

After the subjection of rats to ketamine (50 mg/kg) euthanasia, they were dissected, and blood samples were drawn from the abdominal artery into plain 5 mL tubes and tubes were allowed to stand for at least an hour. The blood samples were processed in a centrifuge at 3000 rpm for 10 min and the serum was separated for the estimation of liver function indices (ALT and AST) and a liver cancer marker (α-FP). Also collected was the liver of each rat. The homogenized liver sections (in 0.1 M phosphate buffer; pH 7.4) were also processed in a centrifuge at 5000 rpm for 10 min, and the supernatants obtained were used for the quantification of p53, Akt/PKB, Nrf2, NF-κB, TNF-α, and some oxidative stress indices. Histopathological evaluations were also conducted on other sections of the liver samples.

2.4. Assays

2.4.1. Serum quantification of AST, ALT, and α-FP

The levels of AST and ALT in the serum of rats were quantified by following the protocols embedded in their respective kit (produced by Randox Laboratory Ltd, UK). The level of α-FP in the serum of rats was determined by using the ELISA method as described in the inserted leaflet of the ELISA kit (produced by Elabscience Biotechnology, USA).

2.4.2. Liver quantification of MDA, GSH, GST, SOD, and CAT

Hepatic MDA was assayed using the Bobadoye et al. [17] method, and hepatic GSH was assayed using the Begum et al. [18] method, and hepatic GST was assayed using the Abdulmalek et al. [19] method, while the hepatic SOD and CAT activities were assayed using the Somade et al. [20] method.

2.4.3. Liver quantification of TNF-α, p53, Nrf2, Akt/PKB, and NF-κB

The levels of TNF-α, p53, Nrf2, Akt/PKB, and NF-κB in the liver were quantified by following the protocols that come with each ELISA kit (Elabscience Biotechnology, USA), using the Sandwich-ELISA principle.

2.4.4. Liver histopathology

The 10% formalin-embedded liver sections were washed in ice-cold phosphate buffer (pH 7.4) for half a day. At the end of dehydration, the liver specimens were paraffin-embedded, sectioned, and stained with eosin-hematoxylin dye. Slides were examined under the microscope at a magnification of x100.

2.5. Data analysis using statistical methods

Results were represented by the mean ± standard error of the mean. ANOVA followed by Tukey's test packages in GraphPad Prism version 8.0.0 were employed to statistically differentiate the various experimental groups in terms of significance. *P*-values lower than 0.05 were considered significant.

3. Results

3.1. Effect of FEAC treatments on serum levels of α-FP, AST, ALT, and AST:ALT ratio in NDEA-CCl_4-induced HCAR

α-FP (Fig. 1A), AST (Fig. 1B) and ALT (Fig. 1C) levels in the HCAR-induced rats were significantly elevated by 51.97%, 37.48%, and

180.64%, respectively compared with the control rats. Treatment interventions by 25 and 50 mg/kg of FEAC ameliorated this increase in α-FP level (Fig. 1A) in the HCAR-induced rats by lowering the level by 58.01% and 53.67%, respectively and AST level (Fig. 1B) by 37.88% and 40.43%, respectively, while ALT level (Fig. 1C) was lowered by 29.33% and 28.54%, respectively compared with HCAR only. Similarly, the serum AST:ALT ratio (Fig. 1C) in the HCAR-induced rats was significantly elevated by 13.87% compared with the control rats. Treatments with 25 and 50 mg/kg of FEAC significantly reduced this increase in the AST:ALT ratio in the HCAR-induced rats by lowering the ratio by 14.41% and 18.90%, respectively compared with HCAR only.

3.2. Effect of FEAC treatments on liver MDA and GSH levels in NDEA-CCl_4-induced HCAR

In Fig. 2, the induction of HCAR led to a significant increase in the hepatic concentrations of MDA (Fig. 2A) and GSH (Fig. 2B) by 139.60% and 106.18%, respectively compared with control. FEAC treatments significantly lowered the concentration of MDA (Fig. 2A) by 48.74% (for 25 mg/kg) and 38.89% (for 50 mg/kg), while GSH (Fig. 2B) concentration was significantly lowered by 51.13% (for 25 mg/kg) and 43.76% (for 50 mg/kg), compared with HCAR only.

3.3. Effect of FEAC treatments on liver GST, SOD, and CAT activities in NDEA-CCl_4-induced HCAR

GST (Fig. 3A), SOD (Fig. 3B), and CAT (Fig. 3C) activities in the liver of HCAR-induced rats were significantly raised by 155.57%, 107.78%, and 107.90%, respectively compared with the control rats. Following FEAC treatments, the elevated hepatic activity of GST was lowered by 56.02% (by 25 mg/kg) and 54.50% (by 50 mg/kg) in rats (Fig. 3A) compared with HCAR only. For SOD, FEAC treatment interventions at 25 and 50 mg/kg significantly reduced the elevated hepatic activity of SOD by 93.62% and 70.96%, respectively (Fig. 3B), while for CAT, FEAC treatment interventions at 25 and 50 mg/kg significantly reduced the elevated activity of CAT by 51.13% and 43.60%, respectively (Fig. 3C) compared with HCAR only.

3.4. Effect of FEAC treatments on liver TNF-α, Akt/PKB, NF-κB, p53 and Nrf2 levels in NDEA-CCl_4-induced HCAR

The TNF-α (Fig. 4A), Akt/PKB (Fig. 4B), and NF-κB (Fig. 4C) levels in

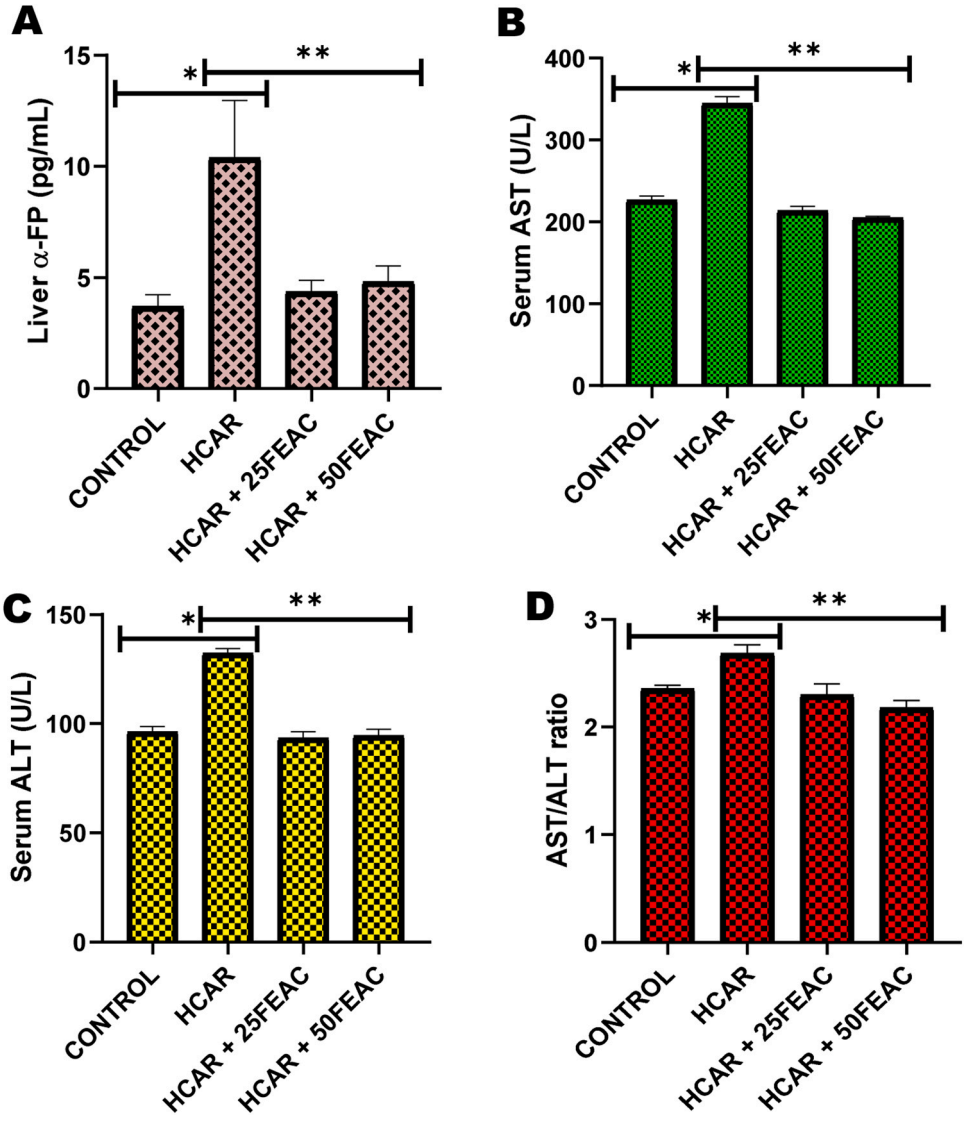

Fig. 1. Effect of FEAC treatments on serum levels of α-FP (A), AST (B), ALT (C), and AST:ALT ratio (D) in NDEA-CCl_4-induced HCAR. Each bar depicts the mean ± SEM of 5 samples in the group. * ($p < 0.05$) means significantly different compare with control; * *($p < 0.05$) means significantly different compared with HCAR. HCAR = hepatocellular carcinoma; FEAC = ferulic acid.

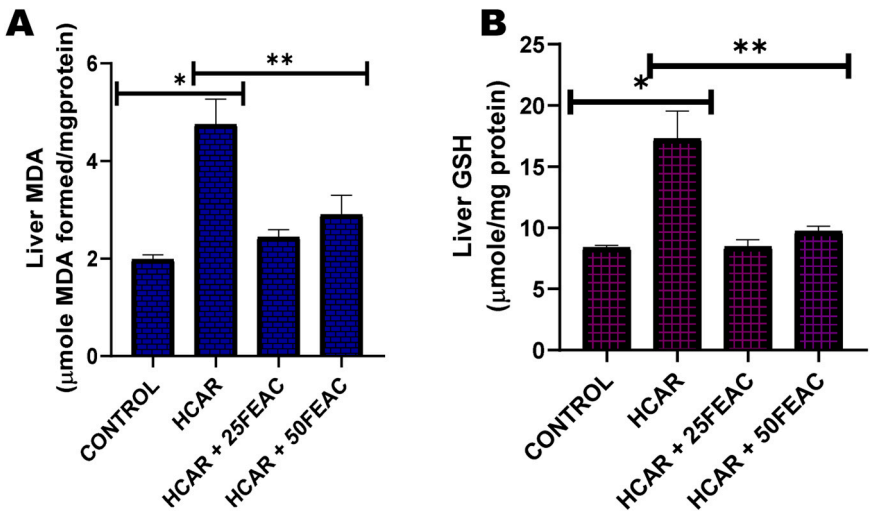

Fig. 2. Effect of FEAC treatments on liver MDA (A) and GSH (B) levels in NDEA-CCl_4-induced HCAR. Each bar depicts the mean ± SEM of 5 samples in the group. * ($p < 0.05$) means significantly different compare with control; * *($p < 0.05$) means significantly different compared with HCAR. HCAR = hepatocellular carcinoma; FEAC = ferulic acid.

the HCAR-induced rats were significantly elevated by 69.78%, 65.05%, and 24.69%, respectively compared with the control rats. Treatment interventions at 25 and 50 mg/kg of FEAC ameliorated this increase in TNF-α level in the HCAR-induced rats by lowering the level by 30.84% and 35.09%, respectively (Fig. 4A); for Akt/PKB by 21.65% and 34.17%, respectively (Fig. 4B), while for NF-κB by 10.87% and 13.12%, respectively (Fig. 4C) compared with HCAR only. On the other hand, p53 (Fig. 4D) and Nrf2 (Fig. 4E) levels in the HCAR-induced rats were significantly lowered by 55.78% and 36.14%, respectively compared with the control rats. Treatments with 25 and 50 mg/kg of FEAC also ameliorated this decrease in p53 level in the HCAR-induced rats by raising the level by 76.60% and 96.45% (Fig. 4D), respectively as well as Nrf2 (Fig, 4E) by 35.99% and 31.51%, respectively compared with HCAR only.

3.5. *Effect of FEAC treatments on hepatocyte architecture*

Histopathological examination of the hepatocyte of control rats (Fig. 5) revealed normal architecture compared with the HCAR only exposed rats that revealed micro-trabecular well differentiated hepatocellular carcinoma with large cytoplasmic eosinophilic inclusion and very large nucleoli. Following treatments with 25 and 50 mg/kg of FEAC, the hepatocyte architecture revealed neoplastic cells with large cytoplasmic eosinophilic inclusion and large nucleoli and neoplastic cells with very large nucleoli, respectively.

4. Discussion

Numerous cytotoxic and chemicals that can destroy DNA have been proposed to be among the root causes of HCAR. Examples of these agents are PFOA, PFCs, acrylamide, PCBs, BaP, VCM, as well as food contaminants such as ochratoxins and aflatoxins. Agents also documented include abused substances like alcohol and viruses such as hepatitis B, HIV-1, and hepatitis C. As a result, this study investigated the effect of FEAC treatments and its probable mechanism of action in a rat model of NDEA-CCl_4-induced HCAR.

AST and ALT are common and regular laboratory indices for ascertaining hepatic function. Bezan et al. [21] revealed that the De Ritis ratio (an elevated preoperative AST:ALT ratio) is a predicting factor for kidney cancer that is non-metastatic [21]. After that, other research has investigated the predicting value of this ratio in different cancers, including prostate, oropharyngeal, HCAR, and colorectal cancers [22–24]. Elevated AST:ALT value was reported to significantly correlate with poor overall survival in primary HCAR patients [25]. To further support this, Liu et al. [26] reported that an elevated AST:ALT ratio is an indication of poor overall survival in HCAR patients subjected to TACE. The elevated serum levels of AST, ALT, and in particular the elevated ratio of AST/ALT observed in this study in HCAR-induced rats is an indication of liver destruction, which led to the elevated levels of the two transaminases in the serum. This finding is corroborated by the published results of Yakubu et al. [14] and Zhang et al. [15], which reported elevated levels of AST and ALT in rat model of NDEA-induced HCAR. FEAC interventions ameliorated the NDEA-CCl_4-induced HCAR by lowering the levels of the 2 transaminases and their ratio (AST:ALT ratio), which can be attributed to its hepatoprotective, antioxidative, and anticancer prowess as previously documented by Adeyi et al. [16] and Zhai et al. [27].

α-FP is the most recurrently and persistently used bio-index for HCAR [28]. Studies have shown that constantly elevated serum α-FP concentration correlates with belligerent tissue morphology of HCAR, like vascular invasion and satellitosis [29,30]. In this study, the elevated level of serum α-FP that was seen is a corroboration of the NDEA-CCl_4-induced HCAR in the rats [14,31,32]. FEAC's ability to lower the serum level of α-FP following its interventions against the NDEA-CCl_4-induced HCAR is also pointing to its anticancer effect as documented by Zhai et al. [27] in a review article.

Free radicals and oxidative stress play a major role in NDEA-induced liver toxicity [33,34] and HCAR [14,35]. This is not in any way different from the outcomes of this study, where a significant increase in the hepatic levels of MDA, GSH, and activities of GST, SOD, and CAT were recorded following the induction of HCAR. The elevation in the liver level of MDA may be the effect of free radical attack on the liver cell membrane components that are electron rich, while the elevation in the liver level and activities of the endogenous antioxidants may be attributed to a cellular response to NDEA-CCl_4-induced oxidative stress in rats. The ability of FEAC to ameliorate the NDEA-CCl_4-induced HCAR can be attributed to its antioxidant property as previously reported in studies by Yakubu et al. [14], Adeyi et al. [16], Hilary et al. [36], and Ghasemi-Dehnoo et al. [37].

HCAR is a type of cancer that is typically inflammation driven. This tissue inflammation is a progressive type, which is chronic and non-resolving [38]. In this study, NDEA-CCl_4-induced hepatic inflammation characterized by an elevated level of TNF-α was observed. This is in line with the recent report by Sarkar et al. [39], where elevated levels of liver TNF-α was recorded in a mouse model of NDEA-CCl_4-induced HCAR. FEAC interventions at both doses investigated demonstrated an

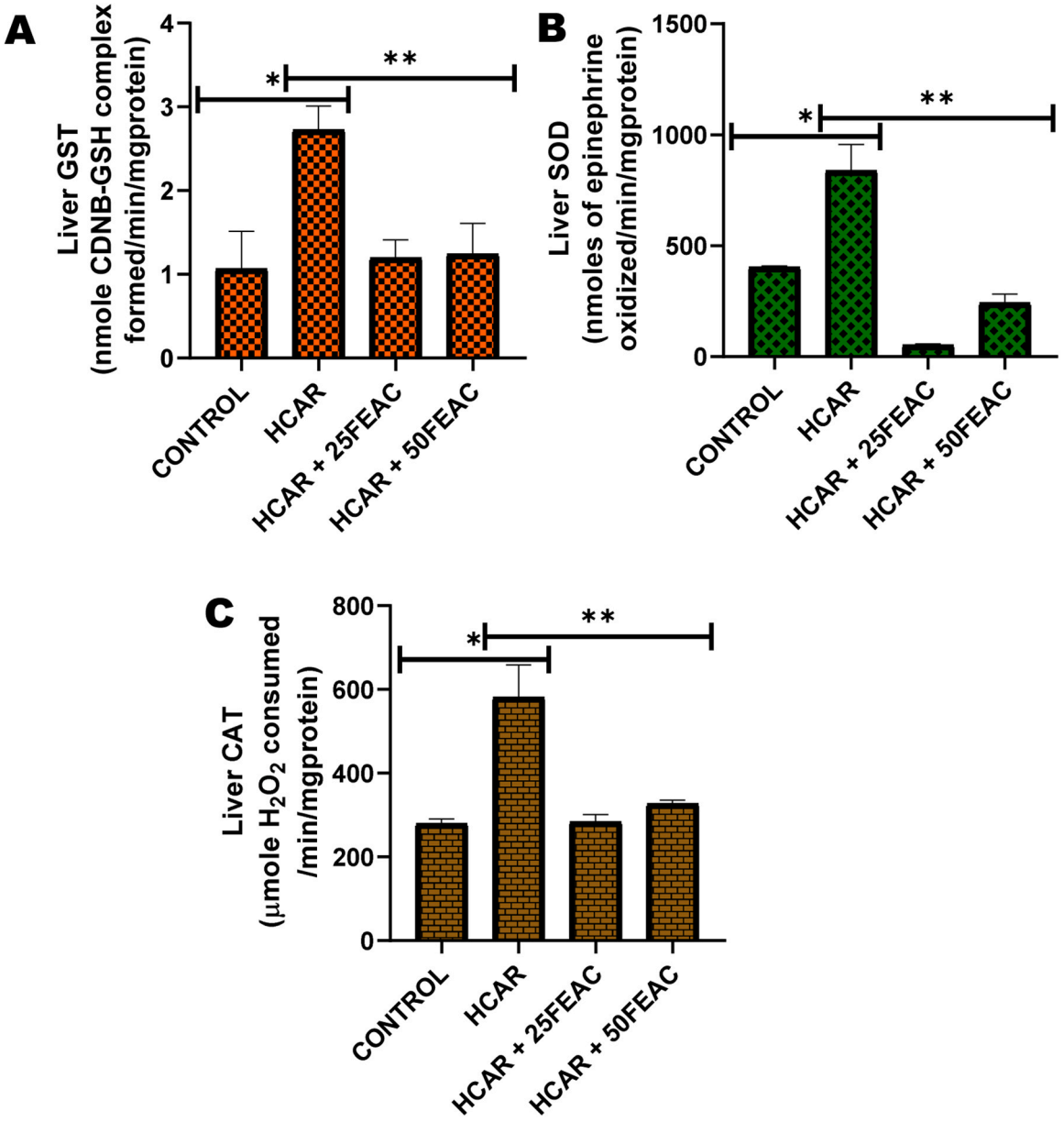

Fig. 3. Effect of FEAC treatments on liver GST (A), SOD (B), and CAT (C) activities in NDEA-CCl_4-induced HCAR. Each bar depicts the mean ± SEM of 5 samples in the group. * ($p < 0.05$) means significantly different compare with control; * *($p < 0.05$) means significantly different compared with HCAR. HCAR = hepatocellular carcinoma; FEAC = ferulic acid.

anti-inflammatory effect, which was marked by the decrease in liver TNF-α level, as earlier reported by Zhai et al. [27].

Cancer development involves two genes that are grouped into TSGs and oncogenes [40]. Being a key TSG that participates in the control of cell cycle and apoptosis induction due to damaged DNA and activation of oncogenes, p53 activation, stabilization, and accumulation ensue through cellular post-translational modifications, leading to a reduced risk of tumorigenesis [41]. p53 induces G1 arrest, apoptosis, and cell senescence [42]. It promotes apoptosis, but cyclin D1 overexpression can lead to reduced G1 phase activity, leading to an accelerated progression of cancer [43]. The p53 concentration following the induction of HCAR in this study was significantly reduced, which we think may be due to the NDEA-CCl_4-induced inhibition of its synthesis, its degradation as a result of prolong exposure or severe damage, excessive oxidative stress that can also lead to p53 degradation, or phosphorylation and inactivation by the PI3K/Akt pathway that can cause its degradation. FEAC interventions restored the hepatic p53 level, suggesting its antioxidant, gene-protective, cell repairing, and apoptotic potentials against the NDEA-CCl_4-induced HCAR.

Nrf2 is a major coordinating factor in the cellular response to oxidative stress. It plays a cytoprotective role in various organs, like the colon [44], liver [45], kidneys [46], and lungs [47]. Like p53, Nrf2 in this study was significantly reduced in the NDEA-CCl_4-administered rats. This may be attributed to the NDEA-CCl_4-induced generation of free radicals that led to hepatic stress and a concomitant decrease and depletion of the liver Nrf2, a situation that occurred as a result of its response to oxidative stress. Also, the decreased Nrf2 level in the cancer-induced rats may also be due to the NDEA-CCl_4-induced interference and activation of the PI3K-Akt/PKB pathway, which Nrf2 is downstream, thereby enhancing the degradation of Nrf2. FEAC exerted an antioxidant effect against NDEA-CCl_4-induced oxidative stress by maintaining and conserving the hepatic endogenous antioxidative markers, thereby relieving Nrf2 of the burden of nuclear translocation and Nrf2-induced nuclear transcription of anti-stress genes that were

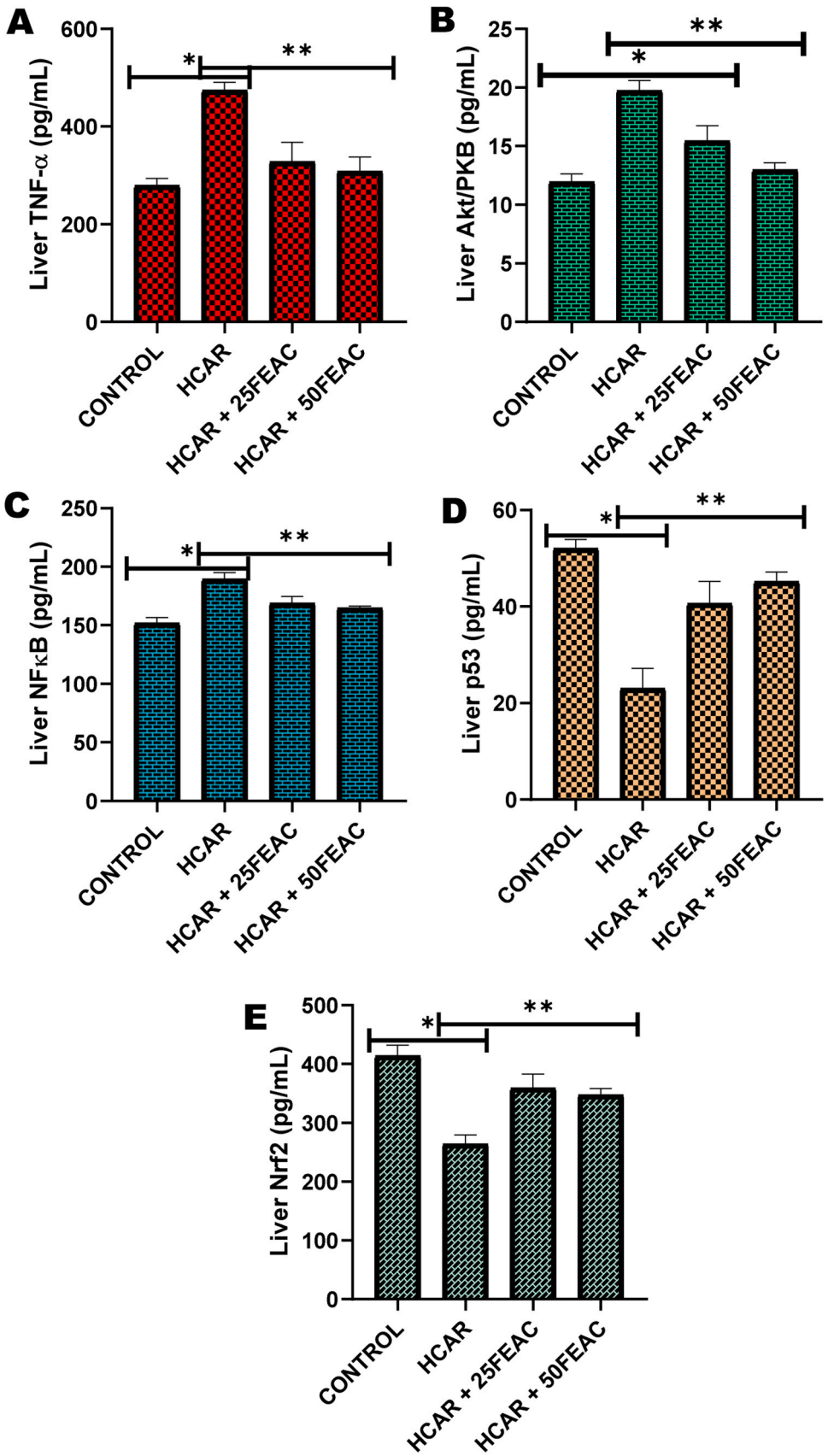

Fig. 4. Effect of FEAC treatments on liver TNF-α (A), Akt/PKB (B), NF-κB (C), p53 (D), and Nrf2 (E) levels in NDEA-CCl_4-induced HCAR. Each bar depicts the mean ± SEM of 5 samples in the group. * ($p < 0.05$) means significantly different compare with control; * *($p < 0.05$) means significantly different compared with HCAR. HCAR = hepatocellular carcinoma; FEAC = ferulic acid.

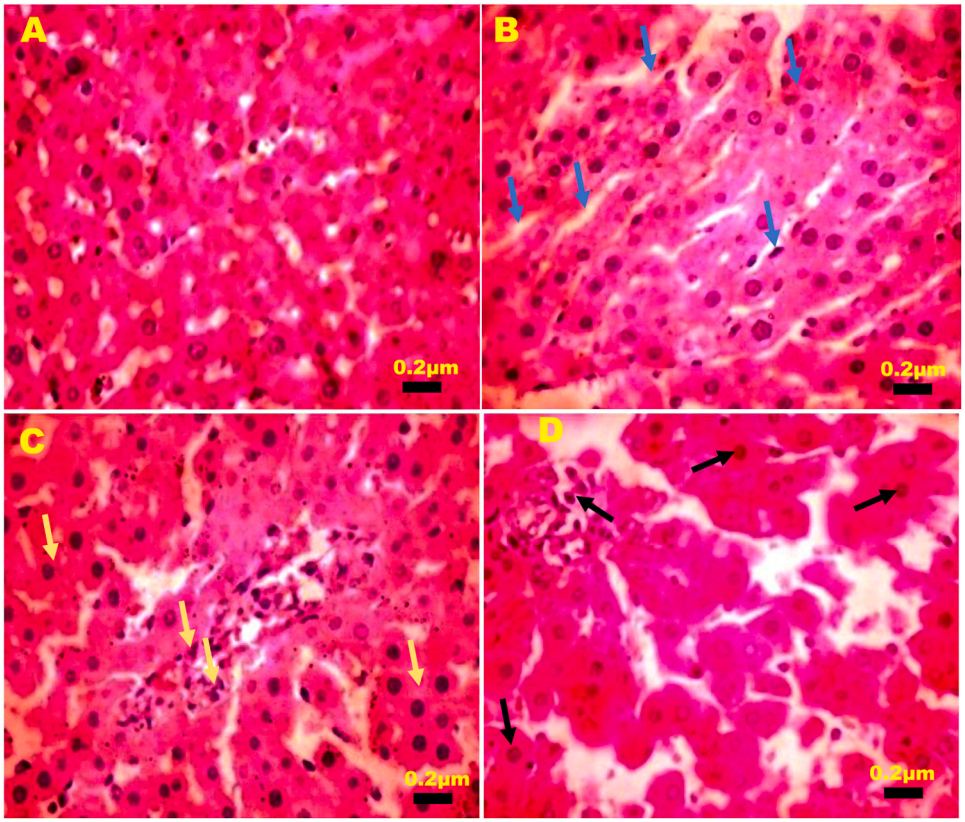

Fig. 5. Effect of ferulic acid (FEAC) treatments on hepatocyte architecture (x 100). Control (A) showing normal hepatocyte; HCAR only (B) showing micro-trabecular well differentiated hepatocellular carcinoma with large cytoplasmic eosinophilic inclusion and very large nucleoli (blue arrows); HCAR + 25FEAC (C) showing neoplastic cells with large cytoplasmic eosinophilic inclusion and large nucleoli (yellow arrows); HCAR + 50FEAC (D) showing neoplastic cells with very large nucleoli (black arrows).

also reported in this study and discussed above.

Akt/PKB controls different cellular or biological activities including apoptotic inhibition, genome stabilization, synthesis of proteins, metabolism of glucose, cell survival, and proliferation in response to receptor binding by growth factors and other extracellular stimuli. One corporate molecular feature of malignancies in humans is the excessive Akt/PKB activation, which leads to tumor aggression and resistance to drugs [48]. In this study, NDEA-CCl_4-induced HCAR resulted to hepatic activation of Akt/PKB. The activation may be a way of ensuring and maintaining liver cell survival in response to NDEA-CCl_4-induced oxidative stress and DNA damage. It may also be due to the activation of a signaling molecule (PI3K) that is upstream, which may have catalyzed the formation of PIP3 and recruited Akt to the membrane and then its activation (by phosphorylation) by PDK1. This hepatic Akt activation may be responsible for the hepatic p53 and Nrf2 inhibitions discussed above are a mechanism that allows cellular growth and survival and a key characteristic of cancer cells. FEAC demonstrated an Akt-inhibitory role following its interventions, pointing to its anti-cancer, apoptotic, and antioxidative properties.

NF-κB is a transcription factor that is made up of 5 members, out of which p65 happens to be the most studied member [49]. NF-κB is another downstream target of Akt, the former being cellularly sequestered with IκB. Following cellular stress, the phosphorylation of IκB by IκB kinase encourages the degradation IκB and the released NF-κB translocates into the nucleus to promote pro-inflammatory gene transcriptions [50]. The hepatic NF-κB activation/upregulation recorded in this study may be attributed to the NDEA-CCl_4-induced excessive generation of free radicals that causes liver cell stress and the eventual activation of NF-κB. The hepatic activation of this transcription factor may also be due to the elevated level of hepatic TNF-α that was seen in this study, being a potent activator of NF-κB, as previously documented by Somade et al. [51]. FEAC exerts its antioxidant and anti-inflammatory effects by inhibiting the NDEA-CCl_4-induced activation of NF-κB. The mechanism through which it may have done this could be through the inhibition of IκB de-sequestration from NF-κB and degradation, inhibition of p65 phosphorylation [27] and inhibition of NF-κB nuclear translocation [52].

Finally, the hepatocyte architecture confirmed the induction of HCAR, while FEAC treatments to some extent demonstrated anti-cancer effect by lowering the degree of the NDEA -CCl_4-induced HCAR in rats.

Gathering the findings from this study, it is concluded that FEAC's mechanisms of action against NDEA-CCl_4-induced HCAR may be through the inhibition of oxidative stress via upregulation of Nrf2, promotion of apoptosis via upregulation of p53, and inhibition of inflammation via downregulation of the Akt/PKB-NF-κB-TNF-α pathway in male Wistar rats.

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CRediT authorship contribution statement

Shonoiki Olaoluwawunmi M.: Resources, Project administration, Methodology, Investigation. **Adewole Ezekiel:** Resources, Conceptualization. **Ajiboye Opeyemi I.:** Resources, Project administration, Methodology, Investigation. **Oyinloye Babatunji E.:** Resources. **Inalu David C.:** Resources, Project administration, Methodology, Investigation. **Akamo Adio J.:** Resources. **Ayofe Mukodaz A.:** Resources, Project administration, Methodology, Investigation. **Adegoke Ayodeji M.:**

Resources. **Dada Temitope A.:** Resources, Project administration, Methodology, Investigation. **Olaniyan Tunde O.:** Resources. **Adeyi Olubisi E.:** Resources, Investigation. **James Adewale S.:** Resources. **Ajayi Babajide O.:** Resources, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation. **Omotoso Opeyemi:** Resources. **Somade Oluwatobi T.:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Isiaka Ismaila D.:** Resources. **Onikola Rasaq T.:** Resources. **Adelabu Aminat O.:** Resources, Project administration, Methodology, Investigation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

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