Ferulic acid attenuated diethylnitrosamine-provoked hepato-renal damage and malfunction by suppressing oxidative stress, abating inflammation and upregulating nuclear factor erythroid related factor-2 signaling

Hussein G. Sawie[a], Osama M. Ahmed[b], Marwa E. Shabana[c], Ghada M. Elqattan[a], Mahitab I. EL-Kassaby[a], Howida S. Abou- Seif[a]

[a]Medical Physiology Department, Medical Research and Clinical Studies Institute, the National Research Centre, [b]Physiology Division, Zoology Department, Faculty of Science, Beni-Suef University, Beni-Suef, [c]Pathology Department, Medical Research and Clinical Studies Institute, the National Research Centre, Cairo, Egypt

Correspondence to Howida S. Abou- Seif, (PhD), Assistant Professor, Medical Physiology Department, Medical Research and Clinical Studies Institute, National Research Centre, Beni Suaif Governorate, Beni Suaif City, Post/zip code: 2711860, Dokii, Cairo, Egypt. Tel: +0128 846 2008; e-mail: drhoidaabouseif@gmail.com

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Background
Diethylnitrosamine (DEN) is a potent environmental toxin that can reach humans through the food chain. It induces proliferative, degenerative and cancerous lesions in the liver and kidneys.
Objective
The principal goal of the existing research was to assess the preventive impacts of ferulic acid (FA) versus DEN- provoked hepato-renal damage and malfunction.
Materials and methods
Adult male rats were divided into four groups: group 1 (normal control) animals orally received saline every day for 14 weeks; group 2 (DEN) animals intraperitoneally received DEN (150 mg/kg twice a week) for 2 weeks; group 3 (DEN + FA) animals were injected intraperitoneally twice a week with DEN for 2 weeks besides to oral administration of FA (100 mg/kg/day) for 14 weeks; group 4 (FA) animals were given a similar dose of FA for a similar period.
Results
The results revealed that FA treatment reversed the DEN-mediated elevation in serum values of the liver enzymes activities as well as urea and creatinine levels; it also augmented the hepato-renal antioxidant system that overcame DEN-induced oxidative stress deteriorations. Moreover, FA markedly reduced the DEN-induced elevated hepato-renal levels of immuno-inflammatory markers (IL-1β and TNF-α) as well as downregulated the inflammatory mediators (Bcl-2, NF-κB, and nuclear factor erythroid related factor-2 (Nrf-2), reflecting its protective potential.
Conclusion
The existing results elucidate that ferulic acid could prevent and ameliorate DEN-induced hepato-renal toxicological changes and can restore livers and kidneys' functions; this effect could be mechanized through activation of anti-inflammatory and antioxidant systems, as well as regulation of NF-κB, Bcl2, and nuclear factor erythroid related factor-2 expression.

Keywords:
apoptosis, diethylnitrosamine, hepato-renal damage, oxidative stress

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Introduction

Hepatocellular carcinoma ranks as the fifth most common cancer globally and the third leading cause of cancer-related death [1]. Although the liver can regenerate and fully recover from most acute noniterative circumstances [2], this ability of hepatocytes can be rendered dysfunction by several conditions such as hepatitis, chronic alcohol use, frequent use of antibiotics-associated drugs and nonalcoholic fatty liver disease [3].

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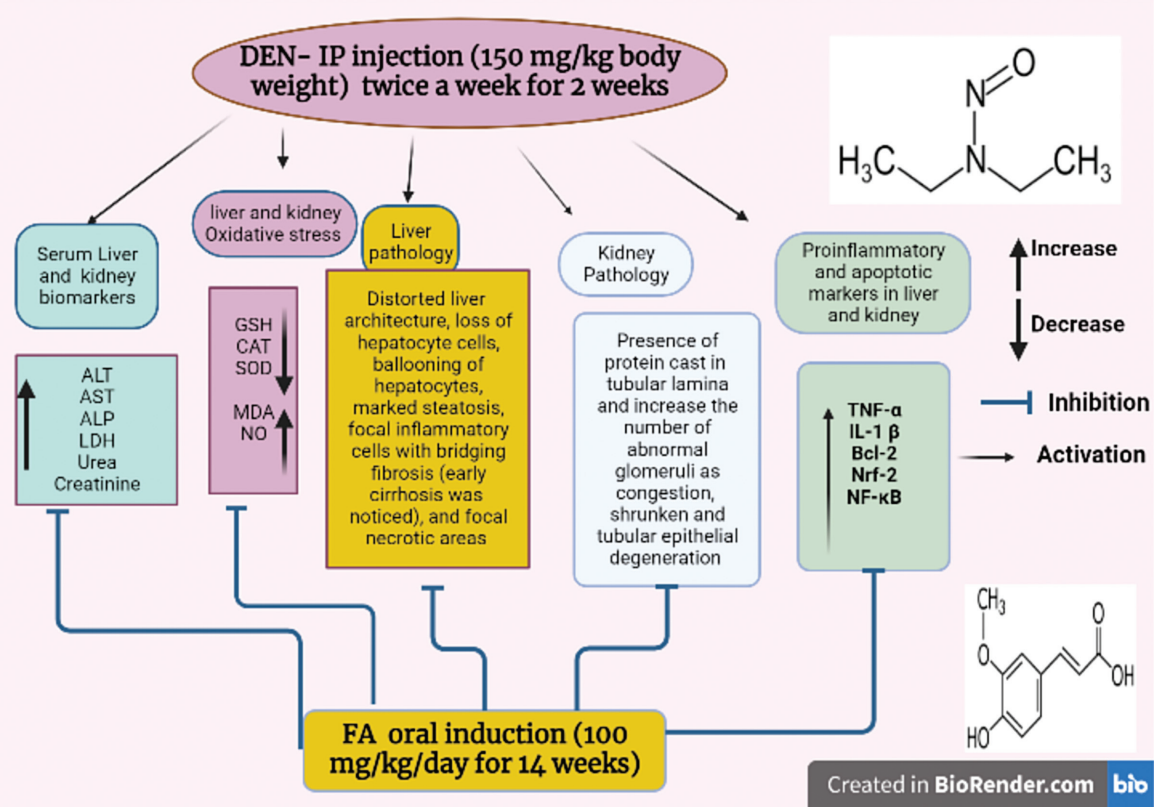
'The authors declare that all data were generated in-house and that no paper mill was used'



When exposed to medications, xenobiotics, or toxins, the kidney is frequently the target organ for toxicity and damage [4]. The exact pattern of kidney toxicity and damage depends on the physiochemical characteristics of the medications and their dose, chemicals, or xenobiotics, toxicokinetic properties, renal clearance profile, local kidney tissue concentration, metabolic characteristics and length of exposure [5]. Kidney injury can be caused by endogenous nephrotoxic chemicals being produced, immunological processes, changes in kidney hemodynamics, immunological processes, or direct cytotoxic harm to the renal structures from environmental toxins [6]. Due in part to transporter expression that controls the release and reabsorption of xenobiotics, the renal tubules were also toxicity targets [7–9].

Mittal and colleagues [10] reported that a powerful environmental carcinogen from the nitrosamine class that enters the food chain is diethylnitrosamine (DEN). Endogenous sources of DEN include industrial environments, tobacco smoke, processed meat, alcoholic beverages, agricultural chemicals, cosmetic products and pharmaceuticals and agricultural pesticides [11]. Under typical physiological conditions, mitochondria and NADPH oxidase were the principal contributors to the moderate amounts of reactive oxygen species (ROS) that were produced. Oxidative stress is formed when the amount of ROS produced is greater than what the cell's internal antioxidant systems can handle. A significant way that DEN contributes to the development of kidney injury is by the excessive generation of ROS and/or the depletion of endogenous antioxidants [12]. According to reports, DEN's metabolized end-product, which plays a role in oxidative stress and cell injury, causes the formation of free radicals; it also results from the metabolism of several medicines [13]. It results in liver lesions that are proliferative, degenerative, and malignant. It can alkylate DNA molecules while being transformed into a highly reactive molecule by oxygenase that depends on CYP450 and produces reactive oxygen species (ROS) that cause oxidative stress. In the liver, DEN creates alkyl DNA adducts that cause chromosomal abbreviations, micronuclei, and chromatid exchanges. These liver mutations caused by DEN are what cause liver cancer to arise [14,15].

Through mechanisms that scavenge ROS, the antioxidants lower the levels of oxidative stress [16]. The functionalities of lipids, nucleic acids and proteins may change as a result of ROS damage. When the balance between ROS production and antioxidant defense is deactivating, oxidative stress results [17]. Unchecked and ongoing liver imbalances between ROS production and ROS elimination by defense mechanisms (antioxidants) cause chronic illness and harm to crucial macromolecules and cells [18]. Phytonutrients are plant-based nutrients or

phytochemicals that may have health advantages to maintain bodily health and function as well as lengthen life [19].

Chemo-preventive agents are recognized for their antimutagenic, anti-inflammatory and antioxidant activities that can decrease proliferation and induce apoptosis, which are crucial components of their anticancer efficacy. Chemoprevention has made significant strides in recent years, which is quite encouraging. Currently, the benefits of a plant-based diet are being assessed for ameliorating various chronic illnesses, including those of the liver, such as cirrhosis, hepatic ulcerative syndrome, and fibrosis. Natural phenolics, abundant in tea, red wine, whole grains, fruits, vegetables, coffee, chocolate and legumes are frequently found in plant-based diets [20]. The consumption of polyphenols has been directly correlated with a lower incidence of numerous liver diseases in humans, including hepatocellular carcinoma. Polyphenols function as natural scavengers for hazardous substances because they are rich in antioxidants [21]. They also possess anti-proliferative properties and may induce apoptosis in cancer cells via increased intracellular calcium, resulting in these individuals in lower tumor growth and a higher likelihood of recovery [22]. Ferulic acid (FA) is a phenolic compound present in the cell wall of plants like rice and barley, as well as in the seeds of fruits like apples, oranges and grapes. FA has neuroprotective, reno-protective, hepatoprotective, antitumor, anti-inflammatory, antiapoptotic, antioxidant and antiaging properties [23]. FA has become a part of various medications, supplements and functional foods since it can be digested and eliminated without accumulating in the body through the urine [24]. It has been used for treating vascular endothelial damage, platelet aggregation, cancer, inflammation, fibrosis, apoptosis, and oxidative stress [4]. FA's anticancer activity is mostly associated with its ability to promote apoptosis [25]. In an experimental animal model, it has been found that FA dramatically lowers the plasma levels of liver biomarkers and the lipid peroxidative index in the hepatic and renal tissue after exposure to carbon tetrachloride (CCl4). The antioxidants (SOD, CAT, GPx and GSH) that were depleted in CCl4 treated animals, were greatly enhanced by FA therapy. FA reduces the toxicity of some chemicals, including formaldehyde, acetaminophen, diosbulbin B, carbon tetrachloride and others that cause hepatocytic inflammation [26]. Ferulic acid is a chemical with activity that exhibits antioxidant properties as well as the ability to stop the proliferation of cancer cells. Previous research looked at how ferulic acid, a dietary supplement, affected hepatocellular carcinoma cells' ability to proliferate and induce apoptosis [27].

The current study aimed to explore the chemo-preventive effectiveness, immune modulatory, and antioxidative regulatory response of ferulic acid against DEN-induced hepato- and renal toxicities via its antioxidant, anti-inflammatory and chemical detoxifying activities.

Materials and methods

Experimental animals

Age-matched (8-9 weeks) 40 male Wistar rats (150–180 g) were obtained from the Animal Colony, National Research Centre, Giza, Egypt and housed in standard cages under standard conditions for 10 days before starting the experiment for acclimatization. The animals were supplied with a standard diet and had free access to drinking water. All experiments were performed in line with the ethical guidelines approved by the Medical Research Ethics Committee of the National Research Centre, Giza, Egypt that approved the proposal of experimental study (No. 2020-20150).

Chemicals

DEN was purchased from Sigma-Aldrich (St. Louis, MO, USA); FA was purchased as pure powder from Alpha Aesar, Germany.

Experimental protocol and drug administration

After the acclimatization, animals were randomly categorized (Fig. 1) into four groups (10 rats each) as follows:

Group I (control): rats received saline solution daily for 14 weeks.

Group II (DEN): rats injected intraperitoneally with DEN (150 mg/kg twice a week) for 2 weeks [28].

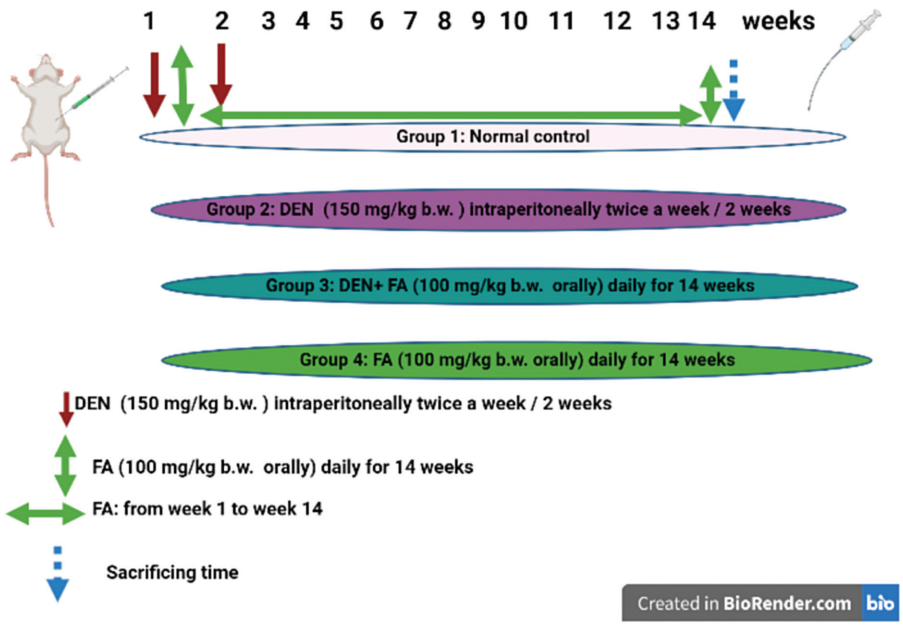
Group III (DEN + FA): rats injected intraperitoneally with DEN (150 mg/kg twice a week) for 2 weeks concomitant with the oral administration of FA (100 mg/kg/day) for 14 weeks [29].

Group IV (FA): rats orally received 100 mg/kg body weight of FA daily for 14 weeks [29].

Blood and tissue sampling

24 h after the last treatment and under light anesthesia, blood samples were collected and left for coagulation

Figure 1



Schematic Illustration of the animal grouping and experimental design.

and cool-centrifuged (Hettich centrifuge, NEWTOWN CT USA, at 3000 rpm for 10 min); then the sera were separated and preserved at −80°C. Post blood collection, the animals were sacrificed by sudden decapitation and the livers and kidneys were excised, washed in ice-cold phosphate-buffered saline; then one part of liver and kidney tissues was homogenized (SONICS homogenizer, FRANCE) in Tris-HCl buffer (pH 7.4) at concentration of 10% (w/v). The homogenates were cool-centrifuged at 3000 rpm for 20 min; then the supernatants were separated and frozen at −80°C. Other parts of the livers and kidneys were immediately immersed and fixed in buffered formalin saline solution (10%) for 48 h, then dehydrated in ascending grades of alcohol, cleared in xylol, and finally embedded in paraffin blocks.

Detection of hepato-renal function

Spectrophotometrically (MY 1345003 spectrophotometer, China) and using kits purchased from Biodiagnostic (Egypt), serum alkaline phosphatase (ALP) activity was estimated kinetically according to the method described by Kind and King [30]; serum lactate dehydrogenase (LDH) activity was measured according to Young's method [31]; alanine and aspartate aminotransferases (ALT and AST) activities were estimated as described by Reitman and Frankel's method [32]; urea and creatinine levels were estimated according to the methods of Patton and Crouch [33] and Bowers and Wong [34], respectively.

Estimation of pro-inflammatory cytokines and survival markers

The hepato-renal levels of interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), nuclear factor-kappa B (NF-κB), nuclear factor erythroid related factor-2 (Nrf-2), and B-cell leukemia/lymphoma 2 (Bcl-2) were measured in livers and kidneys homogenates using ELISA technique (UV-2401 Shimadzu, Japan) and rats ELISA reagent kits purchased from SinoGeneClon Biotech Co., Ltd, China.

Measurement of oxidative stress and antioxidant biomarkers

Using reagent kits obtained from Biodiagnostic (Egypt) and spectrophotometrically, hepato-renal level of reduced glutathione (GSH) was measured according to Beutler and colleagues [35]; lipid peroxidation/malondialdehyde (LPO/MDA) level was measured according to Ohkawa and colleagues [36], Catalase (CAT) activity was determined following the method of Aebi [37]; Nitric oxide (NO) level and superoxide dismutase (SOD) activity were estimated according to Grisham [38] and Marklund and Marklund [39], respectively.

Histological examination

After embedding the tissues in paraffin blocks, serial sections (5 µm thick) were mounted on glass slides, washed in a water bath and left in an oven for dewaxing. Finally, the sections were stained with hematoxylin and eosin. Histological changes were assessed under an electrical light microscope (Olympus CX 41 RF,

TOKYO, JAPAN) Adobe Photoshop version 8.0 was used for processing the photomicrographs.

Statistical analysis

The obtained data were analyzed post–hoc by one-way analysis of variance followed by Duncan-Kramer methods and presented as mean ± standard error. Data were considered statistically significant when p less than or equal to 0.05, as calculated by the GraphPad Prism 5 software for statistical analysis (San Diego, CA, USA). Percentage of change was calculated using the formula $\%\ \text{change} = \frac{treated - Control}{control} * 100$..

Results

FA alleviate serum hepatic and renal biomarkers in rats receiving DEN

Compared with the normal group, serum AST, ALT, ALP and LDH activities, as well as urea and creatinine levels of the achieved results were markedly increased ($P \leq 0.05$) after DEN injection, while ingestion of normal rats with FA only did not disturb the mentioned measurements. Interestingly, rats treated with FA besides to DEN intoxication showed a marked reduction in AST, ALT, ALP and LDH activities as well as urea and creatinine levels when compared with the DEN-intoxicated group (Table 1).

FA prevents DEN-induced hepato-renal oxidative stress in rats

Comparing with the control group, the study showed that DEN-injection resulted in a significant elevation ($P \leq 0.05$) in the levels of hepatic and renal oxidative markers (MDA and NO) coupled with a remarkable reduction in the values of the antioxidative markers (GSH, CAT, and SOD), while administration of animals with FA alone did not negatively affect the oxidative stress status of both liver and kidney tissues. In a favorable manner, cotreatment of rats with FA plus DEN showed a marked decrease in the levels of NO and MDA matched with a notable raise in the level of GSH and the activities of CAT and SOD in hepatic and renal homogenates compared with those of DEN-injected group (Table 2).

FA ameliorates inflammatory markers and mediators in DEN-treated rats

The obtained results showed that FA ingestion neither disturb the hepatic nor the renal levels of inflammatory

Table 1 Effect of DEN on serum AST, ALT, ALP and LDH activities, urea and creatinine levels and the ameliorative effect of FA

	G1	G2		G3		G4	
	Control	DEN	% Change	DEN+ FA	% Change	FA	% Change
AST (U/l)	65.13^{a}±1.9	115.88^{c} ±7.2	77.92	99.25^{b}±4.3	52.39	63.75^{a}±3.1	–2.12
ALT (U/l)	25.38^{a}±2.2	42.75^{b}±1.7	68.44	34.71^{b} ±0.9	36.76	24.88^{a}±0.5	–1.97
ALP (U/l)	35.96^{a}±2.2	45.16^{b}±2.8	25.59	30.56^{a}±3.3	–15.02	32.2^{a}±2.0	–10.46
LDH (U/l)	55.08 ^{a}±2.5	78.56^{b}±2.4	42.63	59.16 ^{a}±8.0	7.41	54.76 ^{a}±4.5	–0.581
Urea (mmol/l)	25.52^{a}±1.1	39.36^{b}±1.2	54.23	32.85^{b}±2.3	28.72	23.11^{a}±0.8	–9.44
Creatinine (mmol/l)	1.07^{a}±0.1	1.43^{b}±0.1	33.64	1.01^{a}±0.1	–5.61	0.98^{a}±0.1	–8.41

Data are presented as mean±SEM (n=8). Within the same raw, means with different superscript. Letters are significantly different at $P \leq 0.05$. % change = [Treated Value – Control Value/Control Value]×100.

Table 2 Effect of DEN on the levels of liver and kidney MDA, GSH, NO and the activities of CAT and SOD and the ameliorative effect of FA

	G1	G2		G3		G4	
	Control	DEN	% change	DEN+ FA	% change	FA	% change
Liver							
MDA (nmol/g)	6.68^{a}±1.2	14.05^{b}±1.7	110.33	8.01^{a}±1.2	19.91	6.05^{a}±1.7	–9.01
NO (μmol/g)	0.308^{a}±0.02	0.547^{b}±0.08	77.60	0.391^{c} ±0.1	26.94	0.290^{a}±0.04	–5.84
GSH (mmol/g)	26.11^{b}±4.6	19.15^{c}±3.1	–26.66	22.55^{a} ±3.1	–13.63	26.36^{b}±4.1	0.96
CAT (U/g)	64.14^{a}±5.8	34.56^{b}±0.6	–46.11	59.21^{a} ±1.2	–7.69	61.87^{a} ±1.7	–3.54
SOD (U/g)	7.42^{b}±0.4	4.12^{a}±0.9	–44.47	6.17^{b}±0.5	–16.85	7.61^{b}±0.3	2.56
Kidney							
MDA (nmol/g)	8.28^{a}±2.4	12.65^{b}±3.5	52.78	9.04^{a}±1.4	9.18	8.01^{a}±1.00	–3.26
NO (μmol/g)	0.293^{a} ±0.01	0.562^{b} ±0.02	91.81	0.374^{c} ±0.05	27.65	0.281^{a}±0.5	–4.09
GSH (mmol/g)	6.49^{a}±1.4	2.34^{b}±1.2	–63.94	4.67^{c}±1.0	–28.04	6.55^{a}±0.1	0.92
CAT (U/g)	44.81^{a} ±5.8	21.34 ^{c} ±3.1	–52.38	33.51^{b} ±6.0	–25.22	45.30^{a}±3.7	1.09
SOD (U/g)	18.69^{a}±2.6	13.85^{c}±1.9	–25.89	16.95^{b}±2.3	–9.31	20.03^{a}±1.1	7.16

Data are presented as mean±SEM (n=8). Within the same raw, means with different superscript. Letters are significantly different at $P \leq 0.05$. % change = [Treated Value – Control Value/Control Value]×100.

markers (TNF- α and IL-1β) or inflammatory mediators (NF-κB, Bcl-2, and Nrf2) levels; while DEN injection led to a significant elevation ($P \leq 0.05$) in the hepatic and renal inflammatory markers and mediators levels compared with the corresponding values of the normal group. Interestingly, animals orally administered with FA concomitant with DEN injection showed a considerable down regulation in the levels of both inflammatory markers (TNF- α, IL-1β) and mediators (NF-κB, Bcl-2 and Nrf2) when compared with DEN-intoxicated group (Table 3).

Histopathological changes

The histopathological results revealed that control group showed normal hepatic architecture with central vein that surrounded by radiating hepatic cord (Fig. 2a); while liver sections of DEN-administered group showed histological abnormalities as distorted liver architecture, loss of hepatocyte cells, ballooning of hepatocytes, marked steatosis, focal inflammatory cells with bridging fibrosis (early cirrhosis was noticed), and focal necrotic areas (Fig. 2b & c). Treatment with FA alone did not negatively affect the normal hepatic architecture and showed normal appearance (Fig. 2d). Animal cotreated with DEN together with FA showed obvious improvement in the hepatic histological pattern, and restored to normal structure with mild steatosis, dilated portal tract surrunded by periportal fibrosis and dilated sinusoidal spaces (Fig. 2e & f).

Regarding the microscopic examination of the kidneys' sections, the histological findings illustrated that normal rats showed normal tubular architecture and normal glomeruli (Fig. 3a). Dramatically, DEN-treated animals showed histological abnormalities as presence of protein cast in tubular lamina and increase the number of abnormal glomeruli as congestion, shrunken and tubular epithelial degeneration (Fig. 3b & c). FA-administrated rats performed a clear and normal kidney structure (Fig. 3d). Favorably, cotreatment of rats with FA plus DEN resulted in a pronounced improvement in renal tubules and glomeruli with mild protein cast deposition and normal appearance of kidney (Fig. 3e & f).

Discussion

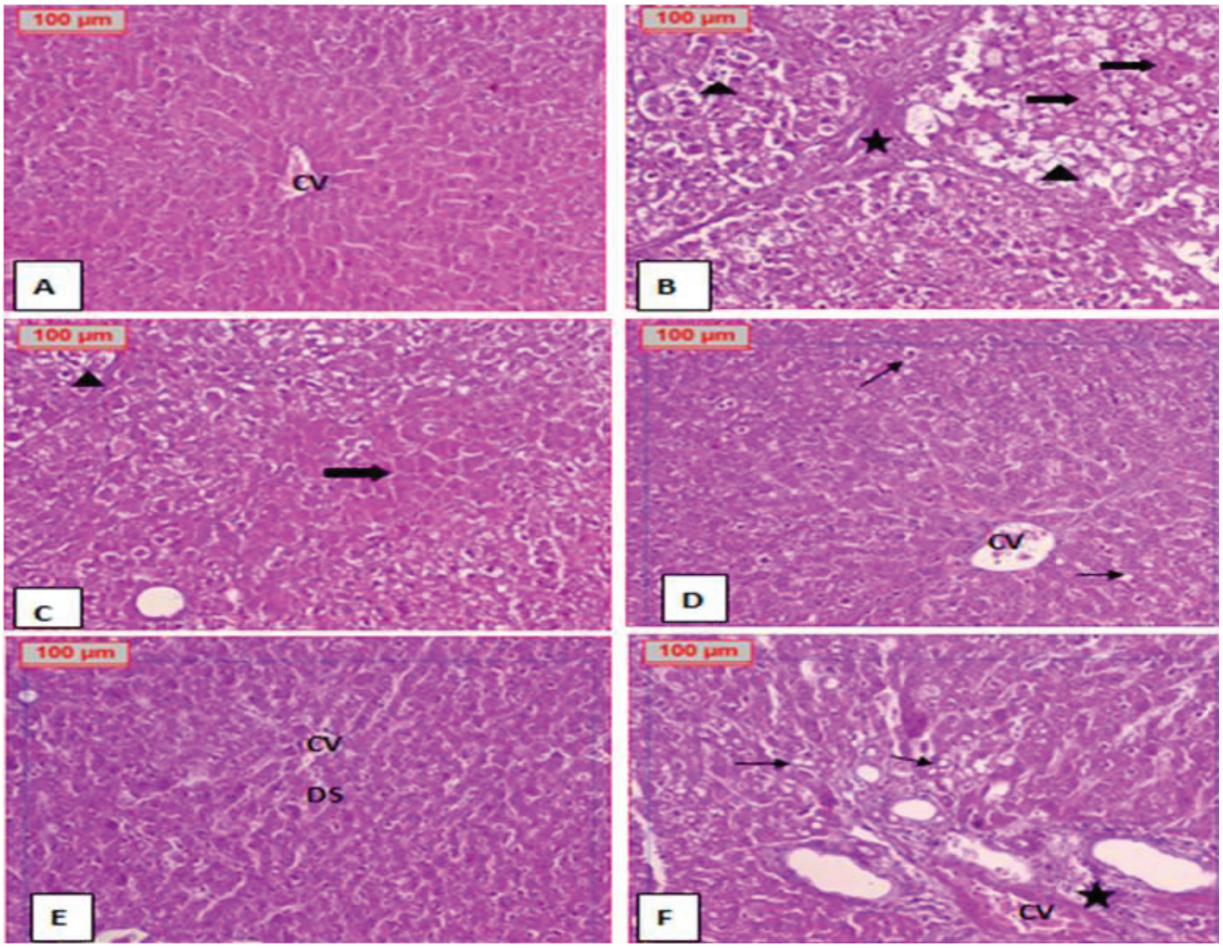
The most sensitive markers of liver injury, serum AST, ALT, ALP and LDH activities are markedly elevated in the presence of hepatocellular damage. The amount of these enzymes in the blood stream reflects the degree of liver damage. Because DEN-induced hepatic damage caused the release of these cytosolic enzymes into the blood stream, the rats in the current investigation with DEN-induced liver lesions had considerably greater serum biomarker levels than the control rats. Due to liver hypofunction and decreased integrity of hepatocyte cell membranes, this signals the start of hepatocellular destruction Naughton, Mittal and colleagues [11,13]. FA, on the other hand, demonstrated hepatoprotective benefits by lowering the elevated serum levels of these indicators. A phenolic substance called FA possesses an unsaturated side chain. They are hydroxyl and the electrons from the phenoxyl groups in its phenolic nucleus that neutralize the free radicals. It has been demonstrated that the treatment with FA considerably reduces the activity of these marker enzymes Banchroft and colleagues [40]. The carboxylic acid group with

Table 3 Effect of DEN on liver and kidneys' TNF-α, IL-1β, Bcl-2, Nrf-2 and NF-κB protein expression levels and the ameliorative effect of FA

	G1	G2		G3		G4	
	Control	DEN	% change	DEN+ FA	% change	FA	%change
Liver							
TNF-α (ng/g)	145^{c}±2.8	481^{a}±3.9	331.7	271^{b}±2.5	86.9	141^{c}±2. 7	−2.75
IL-1β (ng/g)	121^{c}±0.8	336^{a}±0.2	177.7	208^{b}±0.2	71.9	119^{c}±0.2	−1.65
Bcl-2 (ng/g)	36.99^{a}± 1.1	107.9^{c} ±3.1	191.7	55.74^{b}±2.6	50.68	34.77^{a}±1.4	−6.01
NF-κB (ng/g)	147^{c}±0.8	336^{a}± 3.1	128.6	198^{b}± 1.6	34.69	143^{c}±1.8	−2.72
Nrf-2 (pg/g)	808^{a}±4.9	2036^{c} ±12.7	151.9	1458^{b}± 7.0	80.3	794^{a}±3.0	1.73
Kidney							
TNF-α (ng/g)	71.3^{c}±1.7	183.4^{a}±3.2	157.2	103.3^{b}±1.7	44.9	72.4^{c}±1.6	1.54
IL-1β (ng/g)	107^{c}±1.2	191^{a}±2.3	78.5	123^{b}± 0.3	14.95	103^{c}±0.1	−3.73
Bcl-2 (ng/g)	22.3^{a}±1.1	60.7^{c}±0.8	172.2	33.2^{c}±0.4	48.87	21.6^{a}±0.3	−3.14
NF-κB (ng/g)	117.6^{c}±1.6	245.2^{a}±4.2	108.5	152.4^{b}±2.7	29.6	115.6^{c}±3.2	−1.7
Nrf-2 (pg/g)	267.9^{c}±2.2	2434^{a} ±11.5	808	854^{b}±9.7	218.8	265^{c}±4.0	−1.1

Data are presented as mean ± SEM (n=8). Within the same raw, means with different superscript. Letters are significantly different at $p \leq 0.05$. % change = [Treated Value − Control Value / Control Value]×100.

Figure 2



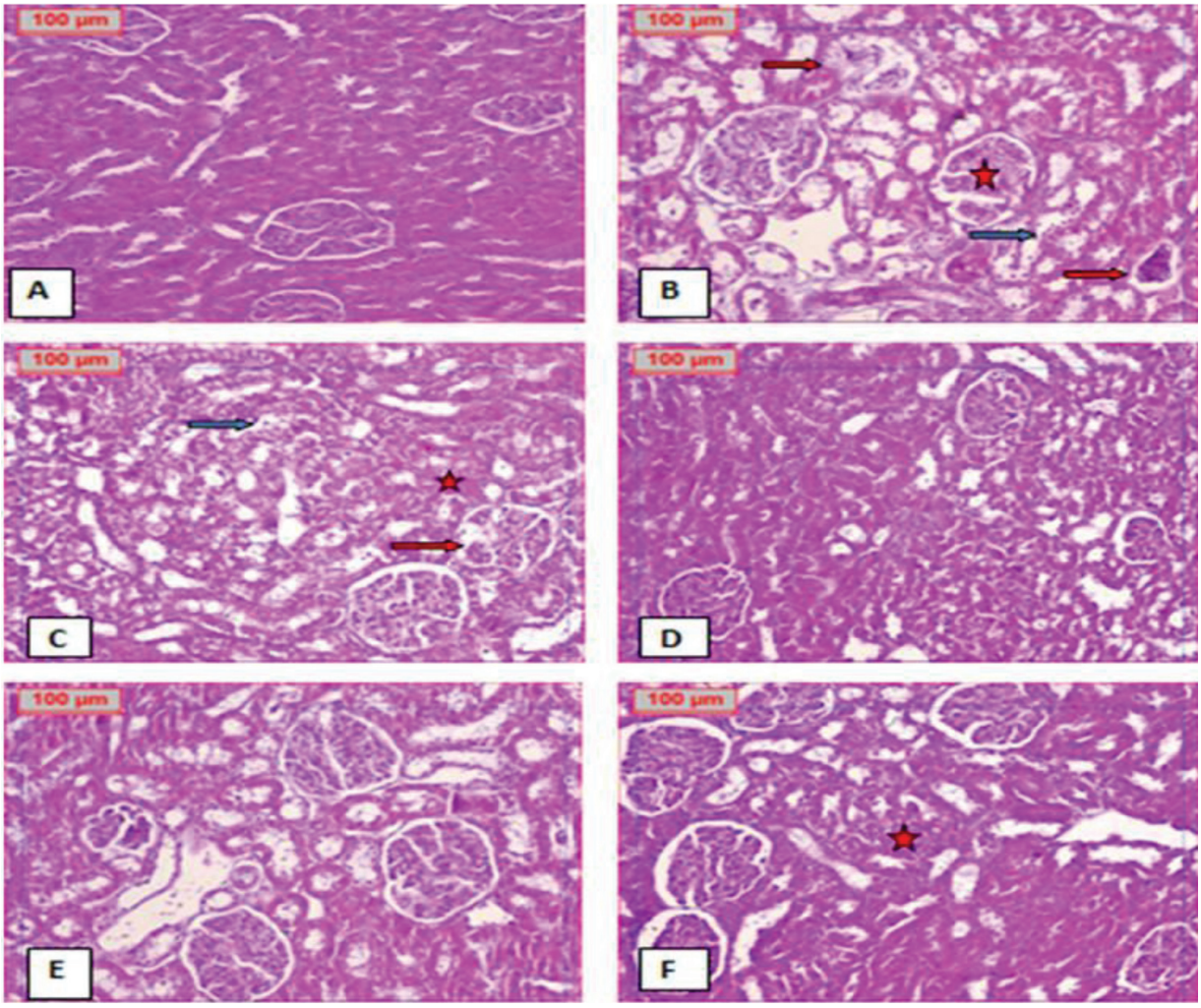
Photomicrograph of hepatic tissues show (a): normal control show normal liver architecture with radiating hepatocytes around central vein. (b & c): DEN-injected group shows distorted liver architecture with lobular cirrhotic pattern, fibrosis with infiltrating inflammatory cells (star), balloning degeneated hepatic cells (arrow head) and scattered necrotic hepatic cells (thick arrow). (d): FA treated group shows few scattered fat cells (thin arrow). (e & f): FA + DEN treated group shows dialed portal tract surrunded by periportal fibrosis (star), scattered fat cells and dilated sinusoidal (DS) spaces (H&E; x200).

unsaturated chain attached draws free radicals and stops them from damaging cell membranes. The electron donating groups on the benzene ring stop free radical chain reactions. By squelching free radicals and keeping them from damaging cell membranes, FA prevents the leaking of liver markers into circulation Tolba and colleagues [41].

The increased levels of serum indicators including urea and creatinine in the current results provided proof that DEN could cause kidney injury in rats. These results agree with many studies illustrated that the range of serum creatinine levels reveals the glomerular function and a rise in these levels signifies renal failure. By causing oxidative stress, cytochrome P450-dependent mono-oxidase systems metabolize DEN, starting the process of mutagenicity, carcinogenicity and cytotoxicity. As a result, DEN is known to cause kidney injury and cancer Vilarnau and colleagues, Ahmadipour and colleagues [42–44].

Oxidative stress is produced as a result of the metabolic activation and detoxification of DEN, which worsens liver damage. Rats treated with DEN in the current study had considerably higher levels of NO and lipid peroxidation and overall oxidative state, whereas their levels of GSH, SOD, and CAT were decreased (Fig. 4). FA administration restores these markers toward normal levels. These findings coincide with Niture and Jaiswal [45], who found that lipid peroxidation is connected to the harmony between oxidative stress and the body's antioxidant defenses. Additionally, numerous antioxidant enzymes as well as nonenzymatic antioxidants may change throughout this process. Among them, lipid peroxide is free radical-related Sivaramakrishana and colleagues, Jagadeesh and colleagues [16–18] and one of the primary end-products of lipid peroxide is malondialdehyde. It is thought that CAT and SOD are crucial components of cells' enzymatic defense against the harm caused by oxidative stress. By

Figure 3



Photomicrographs of kidney sections of the tested groups. (a): normal control shows normal histological structure of renal parenchyma, glomeruli. (b & c): DEN-injected group show distorted glomeruli (red arrow), scattered glomerular and tubular amyloid deposition (star) and hydrophic tubular degeneration (blue arrow). (d): FA treated group shows normal renal glomerular and tubular histological structure. (e & f): FA + DEN treated group shows normal appearance of kidney except for few amyloid (star) deposition (H&E; x200).

converting H_2O_2 into molecular oxygen and water without generating harmful free radicals [46]. According to its antioxidative properties, FA reduced the oxidative status of DEN-induced chronic liver injuries in rats Sadik and colleagues [47]. Hepatocytes need to be protected from oxidative stress and chemicals-induced damage and Nrf2 and its downstream proteins are essential for this Peskin and Cu [48]. A number of organic substances work to protect the liver by stimulating the Nrf2 signaling pathway Jelic and colleagues [49]. The antioxidant enzyme activities were enhanced by FA treatment and MDA levels were dropped. FA serves as an antioxidant system inducer, reduces ROS and guards against tissue annihilation. The majority of FA's anticancer activity is linked to its capacity to promote apoptosis Samuhasaneeto and colleagues [50]. Numerous studies have shown that oxidative stress is a key factor in the liver damage brought on by drugs like alcohol, carbon tetrachloride, acetaminophen and chemotherapy drugs Moselhy and Ali [51]. DEN is a potent hepatotoxin, mutagen and carcinogen. Reactive metabolites generated from its metabolism by cytochrome P450 2E1 are the ultimate toxicants responsible for DEN-induced hepatotoxicity Schett [52]. Repeated low doses of DEN administered to rats result in hepatic necrosis, chronic inflammation and liver and renal cancer. DEN-induced liver fibrosis models have various advantages, such as progressive and prominent pathological changes, high fibrosis reproduction rates and relatively low mortality in the experimental animals Parameswaran and colleagues [53].

Figure 4

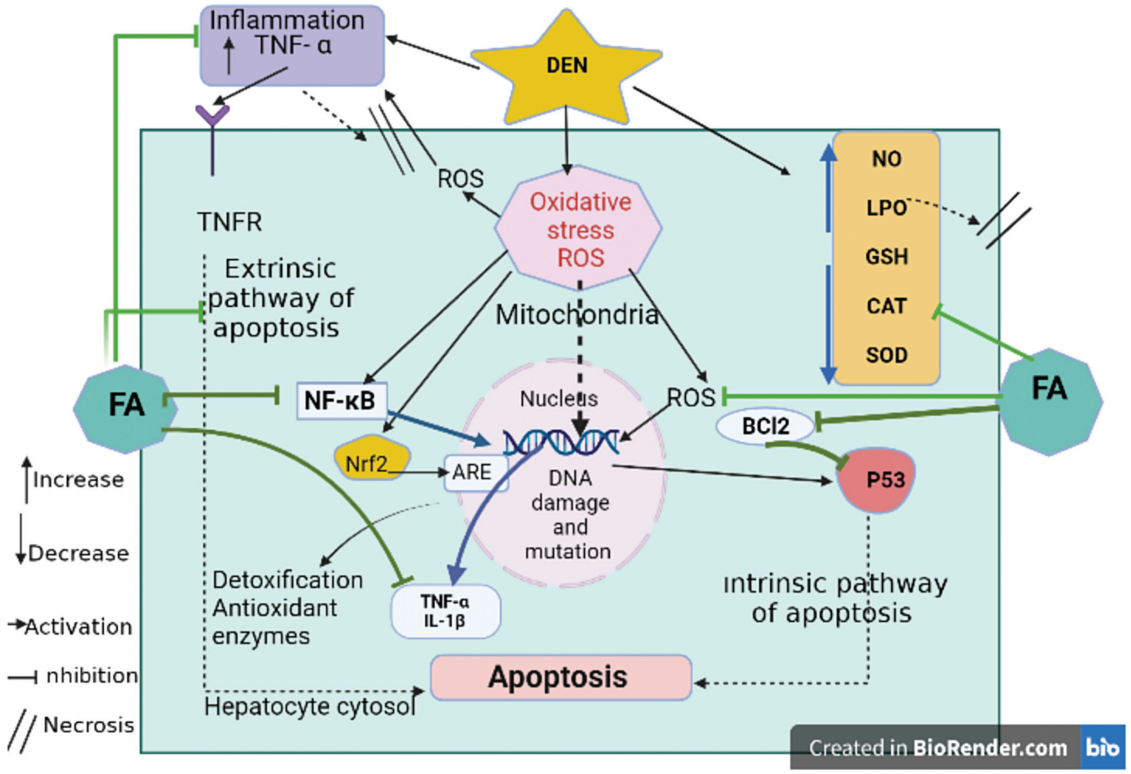


Diagram illustrating the processes through which ferulic acid affects oxidative stress, inflammation and apoptosis in rat liver treated with DEN.

In the existing study, FA successfully ameliorated TNF-α, Bcl2, IL-1B, Nrf2 and NF-κB levels that worsen due to DEN administration. Hepatocyte injury is followed by inflammation and elevated levels of cytokines, such as TNF-α and IL-1β, which are important proinflammatory mediators induced by monocytes and macrophages. TNF-α plays a pivotal role in inflammatory responses and the induction of apoptosis Hoesel and Schmid [54]. TNF-α and IL-1β contribute to the pathogenesis of liver diseases by activating the NF-κB signaling pathway Tawfik and colleagues [55]. NF-κB controls cell viability and inflammation in cancer Karthikeyan and colleagues [56]. DEN-induced hepatic cancer impacts Kupffer cells of the liver by triggering NF-κB and controlling the levels of various circulating inflammatory mediators like IL-6 and TNF-α. NF-κB activation and the subsequent upregulation of TNF-α and IL-1β in DEN-induced liver injury were inhibited by FA treatment, suggesting that FA exerts an anti-inflammatory effect by inhibiting NF-κB activation and suppressing the production of proinflammatory cytokines associated with hepatic cancer Fahrioglu and colleagues [57].

Treating DEN-intoxicated rats with FA activated Nrf2 and enhanced the expression level of heme oxygenase-1 and catalase activity. The cellular GSH levels in the hepatocytes might be relevant for upregulating the expression of GSH-related defense enzymes, such as GSH synthase and GSH peroxidase, which exist downstream of Nrf2 and contribute to protecting cells from oxidative stress. The improved oxidative status in DEN-intoxicated rats treated with FA may result from Nrf2 activation combined with the antioxidative effect of FA Limon-Pacheco and colleagues [58].

FA was reported to inhibit H_2O_2-induced Bcl-2-dependent apoptosis in cancer cells without affecting the expression of Bcl-2, resulting in better recognition of the cells by the immune system and apoptosis Romero and colleagues [59]. It has been shown to reduce cancer cell viability while inhibiting their proliferation, migration and invasion Bocchetti and colleagues [60].

FA prevents renal carcinoma in a concentration-dependent manner. It is anticancer and proapoptotic properties may be related to the upregulation of Bax and caspase 3 and the downregulation of Bcl-2 Lampiasi and Montana [61].

Yuan and colleagues [62] demonstrated that ferulic acid is a crucial anti-inflammatory agent in a variety of pathophysiological conditions, either through reducing

the expression of IL-6, IL-1b, TNF-α, MCP-1, etc. as a proinflammatory cytokines or through elevating the expression of anti-inflammatory cytokines, genes which are specified for stress and some antioxidant molecules modulating cell signaling pathways like metallothioneins as MT-1, MT-2, ...etc. Khanduja and colleagues [63]. Normal human peripheral blood mononuclear cells (PBMCs) were used in an anti-apoptotic and free radical scavenging investigation, which demonstrated how FA prevents lipid peroxidation and scavenges DPPH. In the presence of FA, PBMC can develop a resistance to H_2O_2-induced nucleosome damage and DNA fragmentation. When H_2O_2 induces Bcl-2 dependent apoptosis in cancer cells, polyphenols like FA prevent it without changing Bcl-2 expression. The inhibition of the translocase enzyme in the presence of FA results in the externalization of phosphatidyl serine. As a result, the immune system's detection of the threat is increased and the cells experience apoptosis Karimvand and colleagues [64]. Depend on its concentration, FA prevented renal cancer. The overexpression of Bax and caspase 3 and the downregulation of Bcl-2 may explain its anticancer and proapoptotic effects Peng and colleagues [65].

In the present study, DEN treatment cause destruction of hepatic cells with necrosis in many of these cells, hepatic fibrosis, inflammation, ballooning degeneated hepatic cells, and steatosis this in aggreement with Abdo and Al Bogami [66]. The study of Somade and colleagues [67] is in accordance with our result in affection of DEN on renal glomeruli and tubules. Treatment with FA causes significant improvement in deteriorations that occurred in liver or kidney by DEN in this study as decrease hepatic fibrosis, steatosis and necrosis and improvement of glomerular and tubular structures as previously proved by Yang and colleagues [68].

Conclusions

The present study provides compelling evidence for the adverse impacts of DEN on hepatic and renal cells and the substantial protective effects of FA, mediated via its antioxidant and anti- inflammatory properties. Treatment with FA suppressed DEN-induced hepatocellular and renal toxicities due to its ability to attenuate ROS generation, LPO and inflammation, while boosting antioxidant defenses. Our findings propose a potent and cost-effective preventive agent for people exposed to DEN toxicity. However, further investigations and clinical studies are required to elucidate other mechanisms by which FA improves liver and kidney function.

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Conflicts of interest

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