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Research Article
Ellagic Acid Ameliorates Hepatic Functions Against the Hepatic Alterations Induced by Nitazoxanide (Antiprotozoal Drug) in Male Rats

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Abstract

Background and Objective: The liver plays an essential role in detoxifying any xenobiotic and regulating the main body functions. Prolonged exposure to antiprotozoal drugs such as nitazoxanide (NX) may develop antioxidant imbalance and thus induction of series of free radicals production and induction of oxidative stress, thus the main objective of the study focused on proving that the supplementation with active potent antioxidant compound such as ellagic acid (EA) may enhance the antioxidant defense and alleviate any signs of oxidative stress harm effect of anti-parasitic drugs. **Materials and Methods:** The 32 adult male rats were randomly assigned into 4 groups. Control group: Rats were fed by standard diet. The NX group: Rats were administrated NX at dosage of (18 mg/kg). The EA group: Rats were administered EA at a dosage of (20 mg/kg). The NX plus EA group: Rats were administrated by NX and then concurrent treatment of EA at the same dosages, all treatment was for successive 30 days. A lot of biochemical parameters were measured regarding the hepatic activities including liver enzymes AST, ALT, ALP and, LDH, besides lipid profile, tumor and inflammation markers (CRP, TNF-α and IL-6), antioxidant enzymes in liver tissue homogenates (SOD, CAT and GPx) and lipid peroxidation marker (MDA), besides histological sections of the hepatic tissues. **Results:** The NX group showed a significant elevation in hepatic enzymes with the elevation of lipid markers, besides decline of antioxidant enzymes with the elevation of (MDA) and inflammatory markers with induction of hepatic structure alterations. In contrast, the combined group of NX and EA demonstrated a significant attenuation of the previously mentioned parameters with elevation of the antioxidant enzymes. It is noteworthy that the combination of NX and EA exhibited significantly better ameliorative effects than either drug alone. **Conclusion:** The hepatic structure changes and redox imbalance brought on by NX treatment alone were reduced significantly by treatment by both NX and EA.

Key words: Parasitic, protozoal, nitazoxanide, oxidative stress, hepatic functions

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Competing Interest: The author has declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Nowadays, anti-parasitic medications that induce oxidative stress-such as elevated reactive oxygen species production and redox enzyme inhibition-are thought to be therapeutic candidates used excessively for the management of a lot of parasitic diseases[1]. The anti-parasitic drug NX is a member of the thiazolide nitro heterocyclic class[2,3].

Nitazoxanide (NX) is an oral antiprotozoal medication with good bioavailability and safety profile, which has FDA approval. Since 2004, the situation has been further used in clinical settings to treat diarrhea in adults and children caused by *Giardia* and *Cryptosporidium*[4]. The NX is a broad-spectrum anti-parasitic drug that is mainly used for protection against a lot of protozoal, viral and helminthic infections[5].

The NX can prevent resistance mechanisms and is more selective against parasites than bacterial or fungal infections when compared to other alternative medications[6].

The NX exhibits broad-spectrum activity of anaerobic bacteria and parasites. Some anaerobic bacteria and parasites have been demonstrated to be strongly inhibited by NX[5]. About 1/3 of the oral dosage of NX is eliminated in urine and 2/3 in feces after it is absorbed from the gastrointestinal tract. The NX is quickly hydrolyzed into its desacetyl derivative, tizoxanide (desacetyl-nitazoxanide), in blood by plasma esterase[7].

Abdominal pain, diarrhea, vomiting, headache, dizziness, insomnia, sweating and pale yellow eye discoloration, myalgia, leg cramps, some bone fractures, tachycardia and hypertension, as well as anemia were among the side effects of NX[8].

The only detectable species in plasma and the active metabolite *in vivo* is tizoxanide. After taking NX orally, plasma, urine, bile and feces have all been reported to contain tizoxanide and tizoxanide glucuronide has also been detected in these fluids[9]. Since tizoxanide does not inhibit cytochrome P450 enzymes *in vitro*, concurrent administration of tizoxanide and other agents that have been metabolized or inhibited by cytochrome P450 enzymes has not been expected to result in any notable interactions. Because tizoxanide has a high plasma protein binding (>99.9%), concurrent use of other drugs with small therapeutic index and high plasma protein binding (>99.9%) ensures patient safety. The NX did not seem to change the drug's kinetics when the two drugs were administered concurrently[10].

The liver is the largest internal organ in the body, making up around 2% of an adult's body weight. The liver is made up of hepatocytes, which make up nearly 80% of the liver's volume and carry out most of its functions, sinusoidal endothelial cells and Kupffer cells[11]. This organ absorbs nutrients, functions as a store or supplier for other organs, metabolizes a broad range of nutrients and foreign substances and excretes medications, cholesterol, bile pigments and bacterial products[12,13]. As a result, this organ is extremely vulnerable to the harmful effects of toxins[14,15].

One of the main processes in the etiology and development of liver diseases is oxidative stress, which is brought on by an imbalance between the antioxidant defenses of the cell and the actions of pro-oxidant agents[16,17]. These pro-oxidants, which include superoxide radical ($O_2^{\bullet-}$), hydroxyl radical ($HO^{\bullet}$), hydrogen peroxide (H_2O_2) and nitric oxide ($NO^{\bullet}$), are generally referred to as reactive oxygen species (ROS)[18]. For this reason, using natural antioxidants in place of traditional therapies has become a viable alternative for preventing or lessening liver damage[19-22]. Numerous plant extracts and their secondary metabolites have been linked to antioxidant qualities and hepatoprotective qualities[23,24].

As a result, numerous studies have been suggested as adjuvants or therapeutic agents for the management of liver disease[25]. particularly, recent research has shown that the naturally occurring polyphenolic compound ellagic acid (EA) has remarkable pharmacological properties that protect against liver disease and toxicity.

It has been reported that EA, a naturally occurring plant phenol present in some vegetables, fruits and nuts, possesses antioxidant, anti-tumor and anti-inflammatory properties[26-29]. The EC suppressed the expression of LOX-1 and the generation of ROS mediated by oxidized LDL[30]. It is still unknown, though, how EA affects acute hepatic injury brought on by a lot of xenobiotics.

This paper's goal is to elucidate the potential ameliorative effect regarding the hepatoprotective effect of EA, this compound that may be used as a dietary supplement to prevent or lessen the liver damage brought on by side effects induced by NX or other xenobiotics.

MATERIALS AND METHODS

Study area: The current study was carried out based on the official ethical approval, with approval number (ZU-IACUC/1/F/103/2023) with carrying out the experiments at the Zoology lab and all the samples were taken upon ethical guidelines and animals care.

Experimental animals: The 32 male albino rats, weighing between 120 and 150 g and 2 months age. The male rats were kept in clean cages in the animal house area where they had unrestricted access to the usual control group's water and regular food. Ketamine-xylazine was administered at a very low dosage to induce anesthesia to prevent any potential pain, by the ethical approval that was obtained.

Ethical consideration: Under approval number (ZU-IACUC/1/F/103/2023), this experimental work was conducted. The approval's duration was from March 30, 2023, to March 30, 2025.

Experimental design: The 32 male rats were divided equally into 4 groups, 8 male rats/each group, experimental procedures and tissue preparation as shown in Fig. 1. Treatment groups were divided into 4 groups. Control group: Rats were fed a standard diet of normal equally balanced diet with water *ad libitum*. The NX treated group: Rats were administrated (18 mg/kg, orally once/day)[5]. The EA treated group: Rats were given EA at a dosage of (20 mg/kg) daily[23]. The NX plus EA treated group: Rats were administrated NX drug at the same previous dosage and then concurrent treatment of EA as the same required dosage. All the dosages were concurrently for successive 30 days.

Samples collection: The 32 blood samples were taken from the retro-orbital plexus and centrifuged for about 5 min at a speed of roughly 3000 rpm to obtain serum samples. The serum samples were quickly kept in a -20°C freezer so that more biochemical analysis could be performed. The experimental animals were quickly decapitated and the hepatic tissues were removed and divided into two sections after xylene/ketamine (i.p.) anesthesia. In saline phosphate buffer (PBS), a portion was homogenized. After that, the homogenate was used for a biochemical analysis. The residual hepatic tissues were preserved in 10% normal buffered formalin for histopathological examination.

Tissue homogenates preparation for measurement of redox state: Oxidative stress markers were analyzed using liver tissue sections (*0.45 g). To protect the extremely vulnerable hepatic antioxidant enzymes from oxidation, we added a protease inhibitor after perfusing hepatic tissues with buffer phosphate saline (50 mM, pH ¼ 7.4).

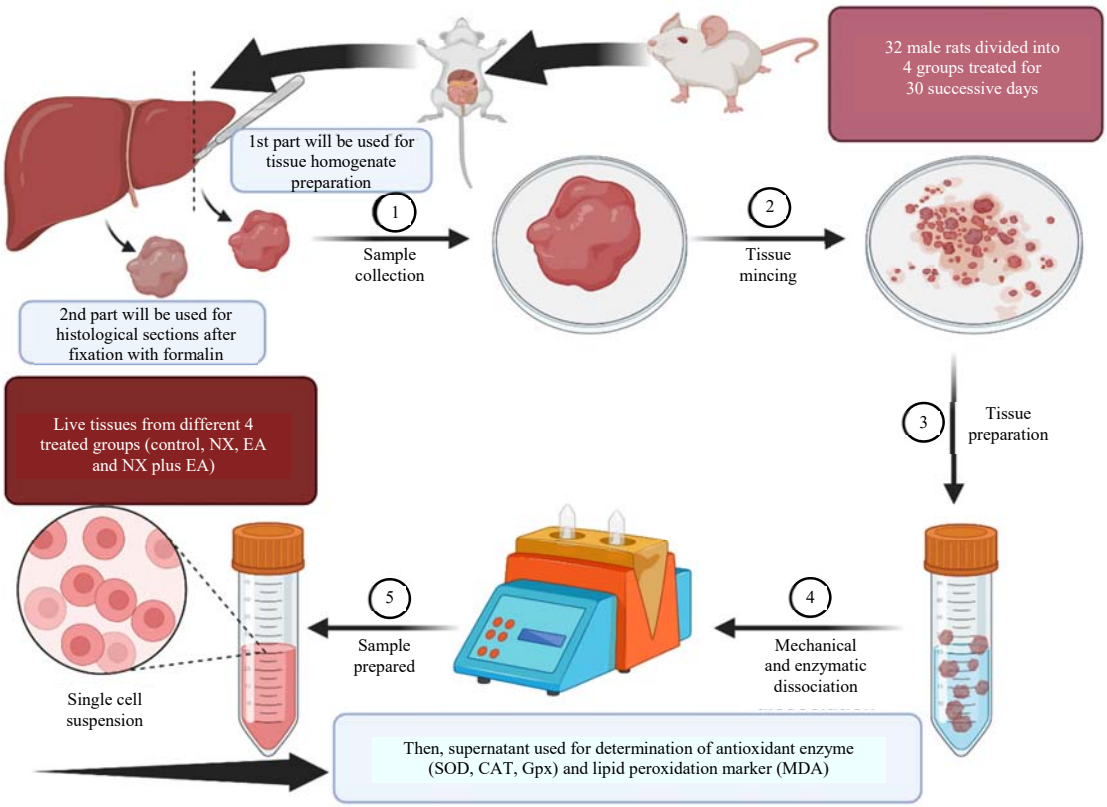

Fig. 1: Experimental procedures and tissue homogenates preparation

Biochemical investigation

Lipid profile: Total cholesterol (TC) and triglycerides (TG) were estimated by Carr *et al.*[31]. The HDL-C estimate was calculated by Descamps *et al.*[32], measured high, low and very low-density lipoprotein levels (HDL-C, LDL-C and VLDL-C).

Liver function biomarkers: The enzymes Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT)[33]. According to Choi *et al.*[34], ALP was measured using a commercial kit known as "Biodiagnostic kits". Vassault41 used the "BioVision Colorimetric Assay Kit", which is based in Milpitas, California, USA, to measure Lactate Dehydrogenase (LDH).

Inflammation marker (CRP) activity: The C-Reactive Protein (CRP) levels were measured using an ELISA kit in compliance with King *et al.*[35].

Assessment of the oxidative and antioxidant biomarkers activity: Using ice alkaline buffer, a small amount of hepatic tissues (about ~0.25 g) were homogenized and the supernatant was extracted for use in the antioxidant tests. The malondialdehyde (MDA) concentrations were ascertained in compliance with Ohkawa *et al.*[36]. Superoxide dismutase (SOD) enzyme activity was determined in compliance with Marklund and Marklund[37]. The hydrogen peroxide breakdown rate was measured at 240 nm using a Spectrophotometer SP-2200 from Bioespectro[38]. The catalase (CAT) activity was computed using the Aebi method. The outcomes were given in terms of units (U/g). Glutathione peroxidase (GPx) activity was quantified[39].

TNF-α and IL-6 activities in the liver homogenates: The cytokine levels of Interleukin-6 (IL-6) and Tumor Necrosis Factor-Alpha (TNF-α) in the homogenate of hepatic tissues were measured using an Enzyme-Linked Immunosorbent Assay (ELISA) kit that was purchased from Immuno-Biological Laboratories, USA.

Histological assessment of the hepatic tissues: The Hematoxylin and Eosin (H&E) staining was the method used to process hepatic tissues that had been fixed. Using a light microscope (Olympus Co., Japan), photomicrographs of the hepatic tissue samples were taken so that the stained slides could be seen.

Statistical analysis: The data were expressed statistically as (Mean±SE). One-way ANOVA is used. Valuation is significant at $p \leq 0.05$. Assumed SOD levels in the NX-treated group were 2.17±0.56, while those in the NX+ EA group were 6.58±0.95. The sample size at 80% power and 95% confidence level is 32 (8/each group). The OPEN EPI software was used to estimate this statistical ratio and then pairwise relations were plotted[40].

RESULTS

Effect on liver function parameters: Hepatic alterations were observed in the hepatic antioxidant enzymes in the male rats treated with NX. The current results manifested an increment in hepatic enzyme levels in the NX-treated group as demonstrated by the elevation of AST, ALT, ALP and LDH levels quantified as (U/L). Administration of EA with NX mitigated significantly the increased levels of the hepatic enzymes (Fig. 2).

Effect on lipid profile: Administration of EA significantly alleviated any hyperlipidemia in male rats treated with NX, as treatment with NX only elevated the lipid biomarkers. The EA significantly increased the beneficial cholesterol levels quantified as (mg/dL) and declined the other low molecular density cholesterol levels as shown in Fig. 3.

Effect on antioxidant enzymes and oxidative stress markers: Administration of EA effectively elevated the antioxidant enzymes (SOD, CAT and GPx) in male rats in comparison with the declined antioxidant levels in NX treated group. The group treated with a combination of EA and NX greatly enhanced the antioxidant enzyme levels and declined the final marker of lipid peroxidation (MDA). Alterations were noticed in the hepatic antioxidant enzymes' status in male rats treated with NX. The current results afforded an increment in MDA levels in the liver of the NX-treated group as revealed by the elevation of MDA levels, all were quantified as (U/g). The EA Administration with NX significantly modulated MDA levels (Fig. 4).

Effect on inflammatory markers (CRP, IL-6) and Tumor Necrosis Factor (TNF-α): Treatment of mature male rats with EA declined greatly the inflammatory markers (CRP and IL-6) levels as compared with NX treated group only. Meanwhile, a combined treated group with both NX and EA induced significant amelioration in inflammatory markers as compared with the NX-treated group. Meanwhile, NX induced significant cytokine storm and inflammation markers via increment of CRP and IL-6 concurrent with elevation of TNF-α levels, which

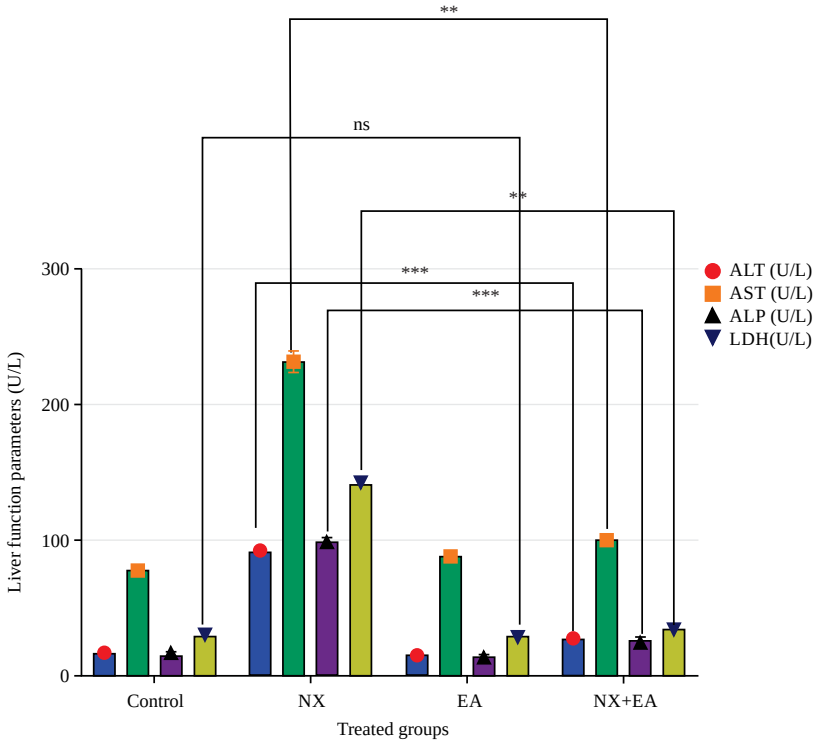

Fig. 2: Effect of EA and/or NX on liver enzymes levels
**Significant, ***Highly significant and ns: Non-significant

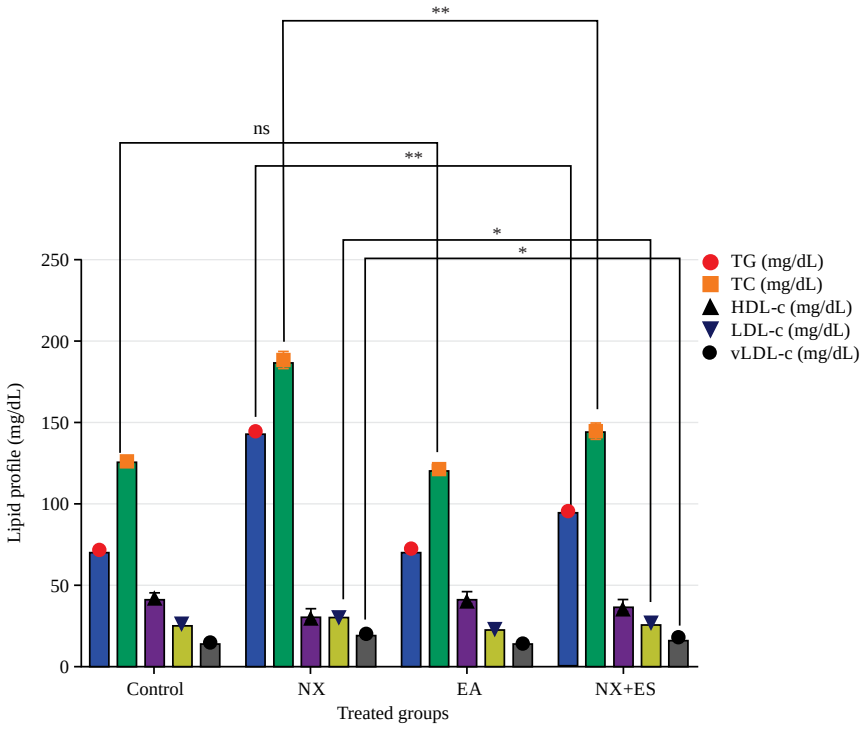

Fig. 3: Effect of EA and/or NX on lipid profile biomarkers
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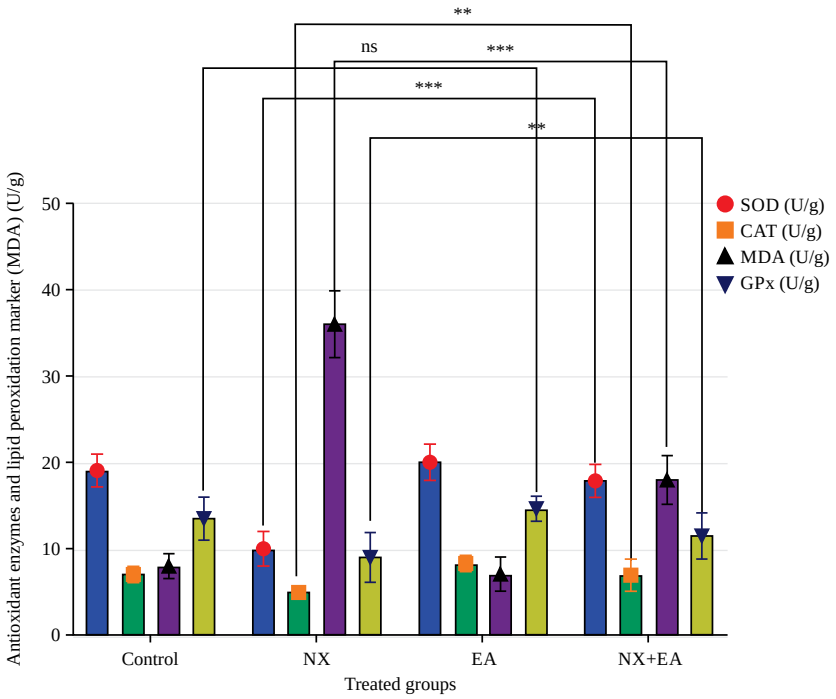

Fig. 4: Effect of EA and/or NX on antioxidant biomarkers and marker of lipid peroxidation (MDA)
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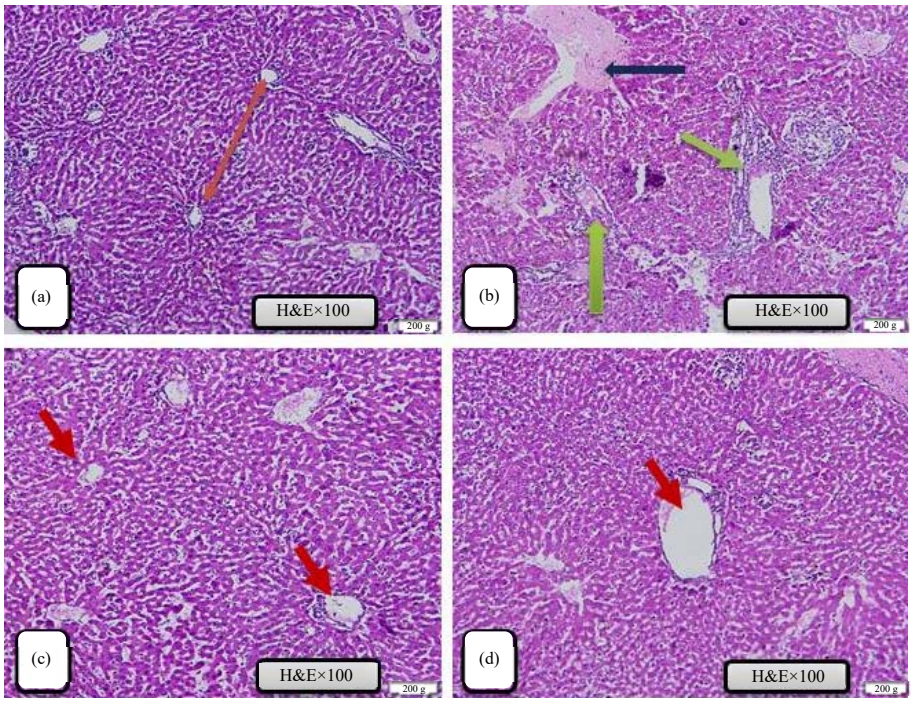

Fig. 5(a-d): Histological sections of the hepatic tissues of different treated groups (H&EX100), (a) Normal control group, (b) NX treated group, (c) EA treated group and (d) NX plus EA treated group
(a) Hepatic tissues were normal in appearance with a normal distance of the hepatic central veins (Two-headed orange arrow) (H&EX100), (b) Showed marked congestion of the central vein (Blue arrow), with marked fibrosis and inflammation (Green arrows), (c) Showed presence of normal sized central veins (Red arrow) and (d) Showed marked alleviation of the hepatic toxicity with some dilation of the central vein (Red arrow)

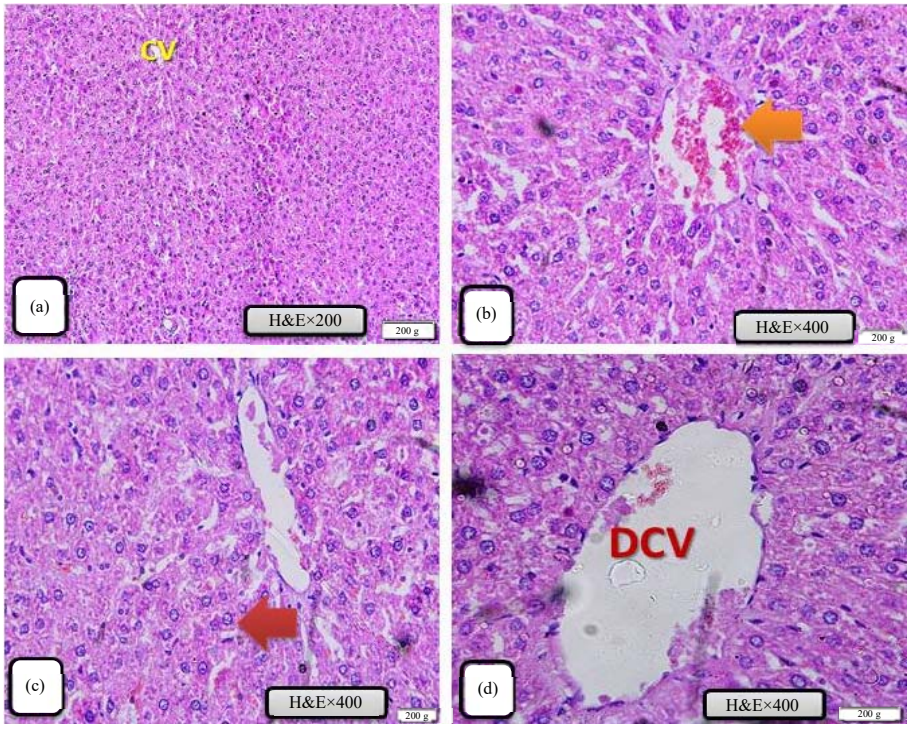

Fig. 6(a-d): Histological sections of the hepatic tissues of different treated groups (H&EX200 and X400), (a) Control group, (b) NX treated group, (c) EA treated group upon higher resolution and (d) NX plus EA treated group

(a) Showed normal hepatic structure with normal sized central vein (CV), (b) Hepatic congestion (Orange arrow), (c) Upon higher resolution, the hepatic tissues showed normal hepatic architecture with white spaces and marked hepatic nuclei (Red arrow) and (d) Showed normal hepatic structure and normal hepatic architecture

Table 1: Inflammatory markers (CRP and IL-6) and Tumor Necrosis Factor-α (TNF-α)

Group/marker	CRP (mg/L)	IL-6 (Pg/g) tissue	TNF-α (Pg/g) tissue
I-control group	10.05±1.69^{c}	15.87±1.87^{bc}	11.68±1.87^{c}
II-NX group	45.98±3.88^{a}	65.98±2.87^{a}	31.68±3.87^{a}
III-EA group	10.45±2.35^{c}	15.02±2.45^{bc}	10.98±1.78^{c}
IV-NX+EA group	14.69±2.98^{b}	17.97±2.36^{c}	18.87±3.87^{b}

Means within the same group (Mean±SE) presenting a lot of different letters which are highly significant at $p \leq 0.05$, whereas the highest value with symbol (a) and declining alphabetically

confirmed the ameliorative effect of EA in mitigating the effect of NX on inflammatory markers, as shown in (Table 1).

Histological evaluation: The hepatic tissues were normal in appearance with normal hepatic cords in the normal control group with a normal distance of the hepatic central veins (Two-headed orange arrow) (H&E×100) as shown in (Fig. 5a). The NX treated group showed fatty changes in the hepatic lobules (green arrows), with clear hepatic congestion (dark blue arrow) as shown in (Fig. 5b) (H&E×100). The EA treated groups showed normal hepatic structure and normal hepatic architecture about to be normal in appearance with the presence of normal-sized central veins (Red arrow) in both (Fig. 5c) (H&E×100 and 400). The NX plus EA treated group showed marked alleviation of the hepatic toxicity and amelioration of the fatty change case induced by NX with some dilation of the central vein as shown in (Fig. 5d) (H&E×100).

Appearance of normal hepatic architecture and central vein (CV) (H&E×200) as shown in (Fig. 6a). Upon higher resolution, the hepatic tissues of NX treated group showed marked congestion (Orange arrow) with the appearance of hepatic fatty changes with clear white spaces inside the hepatic tissues as shown in (Fig. 6b) (H&E×400). The EA-treated groups showed normal hepatic structure and normal hepatic architecture about to be normal in appearance with the presence of normal-sized central veins with marked nuclei (red arrow) in (Fig. 6c) (H&E×400). The NX plus EA-treated group showed marked alleviation of the hepatic toxicity and dilated central vein (DCV) as shown in (Fig. 6d) (H&E×400).

DISCUSSION

The liver is a vital organ that is involved in many physiological and biochemical functions. The liver plays a major role in metabolism, secretion and storage, among other essential processes. It is also highly effective at breaking down toxic substances and creating beneficial compounds. Certain medications have been shown to cause significant hepatic damage[41-43].

The current results revealed that NX at the therapeutic dose caused a significant increment in ALT, AST, LDH and ALP levels after successive treatments for 30 days; these findings of this investigation were consistent with those of Stockis *et al.*[8], who discovered that increased ALT and AST were among the effects of nitazoxanide at higher dosage levels.

Due to the severe fatty changes, the histopathological results of the current investigation showed significant dilated congested central veins which are surrounded by rows and some cords of hepatocytes with clear cytoplasm. The hepatocytes' fatty change and dilated, congested central vein were observed as previously proved by Shams *et al.*[5].

Disorders related to lipid metabolism comprise a range of conditions such as hyperlipidemia and atherosclerosis. These conditions are closely linked to the development of diabetes mellitus and cardiovascular incidents[39-46]. Given the function of mitochondrial uncoupling in the control of metabolism[47-50], drug developers have found small chemical mitochondrial functions to be highly appealing for the development of treatments for metabolic disorders. The primary obstacle to the clinical drug development of mitochondrial uncouplers is their limited therapeutic window, which occurs between toxicity and efficacy, these findings were in great agreement with the current finding and approved the potent antioxidant role of EA in mitigating the possible hyperlipidemic effect of NX.

Here, nitazoxanide drug was proved previously as discussed by Li *et al.*[42] study to induce mitochondrial uncoupling and activated AMPK in HepG2 cells *in vitro*. Therefore, it may explain the significant elevation of the liver enzymes[42] and some hyperlipidemia that had occurred in NX treated.

Additionally supporting the current findings, Hamza and Al-Baqami[23], reported that EA induced a noteworthy reduction in the generation of MDA and free radicals in diabetic rats. The current results revealed the potent antioxidant activity of EA against NX which induced some oxidative stress series and this concept regarding the effect of EA was significantly proved previously by Gupta *et al.*[43], who showed that EA could control the amount of GSH in mouse brain tissues.

The EA is proposed as a potent antioxidant agent that functions to shield tissues from oxidative damage consequences. Cell cytosol contains antioxidant enzymes called glutathione peroxidases. Utilizing glutathione as a substrate, these enzymes' primary job is to decrease alkyl peroxidases and soluble hydrogen peroxide. The H_2O_2 is converted to water by Abu-El-Zahab *et al.*[49]. The GPx when oxidized glutathione is present[50]. The observed reduction in SOD, CAT and GPx enzyme levels in the NX-treated group in this study could potentially revealed that EA could up-regulate and enhance the level of the antioxidant enzymes in the hepatic tissues of male rats. Also, EA induced a significant decline in MDA. The present study recorded data are in close agreement with Firdaus *et al.*[51], who demonstrated that EA administration attenuated the hepatic damage induced by as metal.

Histologically, the current hepatic tissues were confirmed to have normal structure in case of treatment with EA and results recorded great amelioration of hepatic tissues in the NX group treated with EA as reported previously by Hamza and Al-Baqami[23], who confirmed the ameliorative effect of EA in the protection of testicular tissues against oxidative stress and declining the free radicals production.

CONCLUSION

The NX elevated significantly the oxidative stress series in hepatic tissues, leading to the hepatic histological architecture alterations by the presence of congestion and fatty change in the hepatic cords. The NX downregulated the antioxidant enzymes (SOD, CAT and GPx) and up-regulated the final markers of lipid peroxidation (MDA). The present study manifested that EA has a hepato-ameliorative role in against NX-induced hyperlipidemia and oxidative stress. Its enhancement of the biochemical parameters paralleled with the improvement of the hepatic cellular structure. Consequently, it's critical to educate the whole public about EA's beneficial effects on hepatic functions and its ability to reduce oxidative stress caused by xenobiotics.

SIGNIFICANCE STATEMENT

Nowadays, anti-parasitic medications induce oxidative stress with elevation of the reactive oxygen species production and inhibition of the antioxidant enzymes used excessively. The anti-parasitic drug NX is a member of the thiazolide nitro heterocyclic class and in the current study, EA was used as a natural active compound with high antioxidant abilities to treat any oxidative series production induced by NX and elevated the antioxidant enzymes of the treated animals

which is a natural potent anti-parasitic alternative for any medications with any prospective oxidative stress series.

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REFERENCES

1. Bashir, S., K. Shabbir, Fakhar ud Din, S.U. Khan and Z. Ali *et al*., 2023. Nitazoxanide and quercetin co-loaded nanotransfersomal gel for topical treatment of cutaneous leishmaniasis with macrophage targeting and enhanced anti-leishmanial effect. Heliyon, Vol. 9. 10.1016/j.heliyon.2023.e21939.
2. White Jr., A.C., 2004. Nitazoxanide: A new broad spectrum antiparasitic agent. Expert Rev. Anti-Infect. Ther., 2: 43-49.
3. Khalid, H., S. Batool, Fakhar ud Din, S. Khan and G.M. Khan, 2022. Macrophage targeting of nitazoxanide-loaded transethosomal gel in cutaneous leishmaniasis. R. Soc. Open Sci., Vol. 9. 10.1098/rsos.220428.
4. Miyamoto, Y. and L. Eckmann, 2015. Drug development against the major diarrhea-causing parasites of the small intestine, *Cryptosporidium* and *Giardia*. Front. Microbiol., Vol. 6. 10.3389/fmicb.2015.01208.
5. Shams, G.E.D., A.E.A. Fouad and N. Naiem, 2018. Nitazoxanide adverse effects on biochemical markers of liver & kidney injury and antioxidant enzymes on rats. Int. J. Pharm. Res. Allied Sci., 7: 1-6.
6. Gurgen, J., D. Hogan, E. Grace and D. Johnson, 2011. Nitazoxanide in the treatment of chronic cutaneous leishmaniasis resistant to traditional sodium stibogluconate. J. Am. Acad. Dermatol., 64: 202-203.
7. Broekhuysen, J., A. Stockis, R.L. Lins, J. de Graeve and J.F. Rossignol, 2000. Nitazoxanide: Pharmacokinetics and metabolism in man. Int. J. Clin. Pharmacol. Ther., 38: 387-394.
8. Stockis, A., A.M. Allemon, S. de Bruyn and C. Gengler, 2002. Nitazoxanide pharmacokinetics and tolerability in man using single ascending oral doses. Int. J. Clin. Pharmacol. Ther., 40: 213-220.
9. Guo, S., F. Li, B. Wang, Y. Zhao and X. Wang *et al*., 2020. Analysis of tizoxanide, active metabolite of nitazoxanide, in rat brain tissue and plasma by UHPLC-MS/MS. Biomed. Chromatogr., Vol. 34. 10.1002/bmc.4716.
10. Lokhande, A.S. and P.V. Devarajan, 2021. A review on possible mechanistic insights of nitazoxanide for repurposing in COVID-19. Eur. J. Pharmacol., Vol. 891. 10.1016/j.ejphar.2020.173748.
11. García-Niño, W.R. and C. Zazueta, 2015. Ellagic acid: Pharmacological activities and molecular mechanisms involved in liver protection. Pharmacol. Res., 97: 84-103.
12. Kmieć, Z., 2001. Cooperation of Liver Cells in Health and Disease. Springer, Berlin, Heidelberg, ISBN: 978-3-642-56553-3, Pages: 151.
13. Ramadori, G., F. Moriconi, I. Malik and J. Dudas, 2008. Physiology and pathophysiology of liver inflammation, damage and repair. J. Physiol. Pharmacol., 59: 107-117.
14. Nagy, P., S.S. Thorgeirsson and J.W. Grisham, 2009. Organizational Principles of the Liver. In: The Liver: Biology and Pathobiology, Arias, I.M., H.J. Alter, J.L. Boyer, D.E. Cohen, D.A. Shafritz, S.S. Thorgeirsson and A.W. Wolkoff (Eds.), John Wiley & Sons Ltd., Hoboken, New Jersey, ISBN: 9781119436812, pp: 1-13.
15. Singh, A., T.K. Bhat and O.P. Sharma, 2011. Clinical biochemistry of hepatotoxicity. J. Clin. Toxicol., Vol. S4. 10.4172/2161-0495.S4-001
16. Cichoż-Lach, H. and A. Michalak, 2014. Oxidative stress as a crucial factor in liver diseases. World J. Gastroenterol., 20: 8082-8091.
17. Tell, G., C. Vascotto and C. Tiribelli, 2013. Alterations in the redox state and liver damage: Hints from the EASL Basic School of Hepatology. J. Hepatol., 58: 365-374.
18. El-Megharbel, S.M., S.H. Qahl, B. Albogami and R.Z. Hamza, 2024. Chemical and spectroscopic characterization of (artemisinin/querctin/zinc) novel mixed ligand complex with assessment of its potent high antiviral activity against SARS-CoV-2 and antioxidant capacity against toxicity induced by acrylamide in male rats. PeerJ, Vol. 12. 10.7717/peerj.15638.
19. Albogami, B., 2023. Evaluation of the antiparasitic, antihepatotoxicity, and antioxidant efficacy of quercetin and chitosan, either alone or in combination, against infection induced by *Giardia lamblia* in male rats. Life, Vol. 13. 10.3390/life13122316.
20. Albogami, B., 2023. Ameliorative synergistic therapeutic effect of gallic acid and albendazole against *Trichinella spiralis* muscular phase infection and assessment of their effects on hepatic and cardiac tissues in male mice. Saudi J. Biol. Sci., Vol. 30. 10.1016/j.sjbs.2023.103763.
21. Hamza, R.Z. and K. Alsolami, 2023. Ameliorative effects of orlistat and metformin either alone or in combination on liver functions, structure, immunoreactivity and antioxidant enzymes in experimentally induced obesity in male rats. Heliyon, Vol. 9. 10.1016/j.heliyon.2023.e18724.
22. Hamza, R.Z., N.A. Al-Malki, S. Alharthi, S.A. Alharthy, B. Albogami and S.M. El-Megharbel, 2022. Chemical characterization of Taif rose (*Rosa damascena*) methanolic extract and its physiological effect on liver functions, blood indices, antioxidant capacity, and heart vitality against cadmium chloride toxicity. Antioxidants, Vol. 11. 10.3390/antiox11071229.

23. Hamza, R.Z. and N.M. Al-Baqami, 2019. Testicular protective effects of ellagic acid on monosodium glutamate-induced testicular structural alterations in male rats. Ultrastruct. Pathol., 43: 170-183.
24. Al-Baqami, N.M. and R.Z. Hamza, 2021. Synergistic antioxidant capacities of vanillin and chitosan nanoparticles against reactive oxygen species, hepatotoxicity, and genotoxicity induced by aging in male Wistar rats. Hum. Exp. Toxicol., 40: 183-202.
25. Han, D.H., M.J. Lee and J.H. Kim, 2006. Antioxidant and apoptosis-inducing activities of ellagic acid. Anticancer Res., 26: 3601-3606.
26. Gu, L., W.S. Deng, Y. Liu, C.H. Jiang and L.C. Sun *et al*., 2014. Ellagic acid protects lipopolysaccharide/D-galactosamine-induced acute hepatic injury in mice. Int. Immunopharmacol., 22: 341-345.
27. Rogerio, A.P., C. Fontanari, M.C.C. Melo, S.R. Ambrosio and G.E.P. de Souza *et al*., 2006. Anti-inflammatory, analgesic and anti-oedematous effects of *Lafoensia pacari* extract and ellagic acid. J. Pharm. Pharmacol., 58: 1265-1273.
28. Kassim, M., M. Achoui, M.R. Mustafa, M.A. Mohd and K.M. Yusoff, 2010. Ellagic acid, phenolic acids, and flavonoids in Malaysian honey extracts demonstrate *in vitro* anti-inflammatory activity. Nutr. Res., 30: 650-659.
29. Kim, S., S.K. Nishimoto, J.D. Bumgardner, W.O. Haggard, M.W. Gaber and Y. Yang, 2010. A chitosan/β-glycerophosphate thermo-sensitive gel for the delivery of ellagic acid for the treatment of brain cancer. Biomaterials, 31: 4157-4166.
30. Lee, W.J., H.C. Ou, W.C. Hsu, M.M. Chou and J.J. Tseng *et al*., 2010. Ellagic acid inhibits oxidized LDL-mediated LOX-1 expression, ROS generation and inflammation in human endothelial cells. J. Vasc. Surg., 52: 1290-1300.
31. Carr, T.P., C.J. Andresen and L.L. Rudel, 1993. Enzymatic determination of triglyceride, free cholesterol and total cholesterol in tissue lipid extracts. Clin. Biochem., 26: 39-42.
32. Descamps, O.S., M.T.Cooney, G. de Backer and I. Graham, 2012. A simple multiplier to calculate the impact of HDL cholesterol on cardiovascular risk estimation using SCORE. Atherosclerosis, 222: 564-566.
33. Reitman, S. and S. Frankel, 1957. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am. J. Clin. Pathol., 28: 56-63.
34. Choi, Y.D., K.H.O. Rha and H.K.I. Choi, 1999. *In vitro* and *in vivo* experimental effect of Korean red ginseng on erection. J. Urol., 162: 1508-1511.
35. King, D.E., A.G. Mainous, T.A. Buchanan and W.S. Pearson, 2003. C-reactive protein and glycemic control in adults with diabetes. Diabetes Care, 26: 1535-1539.
36. Ohkawa, H., N. Ohishi and K. Yagi, 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem., 95: 351-358.
37. Marklund, S. and G. Marklund, 1974. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur. J. Biochem., 47: 469-474.
38. Hafeman, D.G., R.A. Sunde and W.G. Hoekstra, 1974. Effect of dietary selenium on erythrocyte and liver glutathione peroxidase in the rat. J. Nutr., 104: 580-587.
39. Aebi, H., 1974. Catalase. In: Methods of Enzymatic Analysis, Bergneyer, H. (Ed.), Academic Press, Weinham, New York, pp: 673-684.
40. Filho, W.J., C.C. Lima, M.R.R. Paunksnis, A.A. Silva and M.S. Perilhão *et al*., 2018. Reference database of hematological parameters for growing and aging rats. Aging Male, 21: 145-148.
41. Hamza, R.Z., H.M. Al-Yasi, E.F. Ali, M.A. Fawzy, T.G. Abdelkader and T.M. Galal, 2022. Chemical characterization of Taif rose (*Rosa damascena* Mill var. *trigentipetala*) waste methanolic extract and its hepatoprotective and antioxidant effects against cadmium chloride ($CdCl_2$)-induced hepatotoxicity and potential anticancer activities against liver cancer cells (HepG2). Crystals, Vol. 12. 10.3390/cryst12040460.
42. Li, F., M. Jiang, M. Ma, X. Chen and Y. Zhang *et al*., 2022. Anthelmintics nitazoxanide protects against experimental hyperlipidemia and hepatic steatosis in hamsters and mice. Acta Pharm. Sin. B, 12: 1322-1338.
43. Gupta, A., A.K. Singh, R. Kumar, S. Jamieson, A.K. Pandey and A. Bishayee, 2021. Neuroprotective potential of ellagic acid: A critical review. Adv. Nutr., 12: 1211-1238.
44. Geisler, J.G., 2019. 2,4 Dinitrophenol as medicine. Cells, Vol. 8. 10.3390/cells8030280.
45. Goedeke, L., L. Peng, V. Montalvo-Romeral, G.M. Butrico and S. Dufour *et al*., 2019. Controlled-release mitochondrial protonophore (CRMP) reverses dyslipidemia and hepatic steatosis in dys-metabolic nonhuman primates. Sci. Transl. Med., Vol. 11. 10.1126/scitranslmed.aay0284.
46. Samuel, V.T. and G.I. Shulman, 2018. Nonalcoholic fatty liver disease as a nexus of metabolic and hepatic diseases. Cell Metab., 27: 22-41.
47. Harald, C., M.C.W. Myhrstad, M.J. Øivind, B. Rune and T. Magne, 2003. *Research Communication*: Berry intake increases the activity of the γ-glutamylcysteine synthetase promoter in transgenic reporter mice. J. Nutr., 133: 2137-2140.
48. Hamza, R.Z., M.S. Al-Harbi and N.S. El-Shenawy, 2017. Ameliorative effect of vitamin E and selenium against oxidative stress induced by sodium azide in liver, kidney, testis and heart of male mice. Biomed. Pharmacother., 91: 602-610.

49. Abu-El-Zahab, H.S.H., R.Z. Hamza, M.M. Montaser, M.M. El-Mahdi and W.A. Al-Harthi, 2019. Antioxidant, antiapoptotic, antigenotoxic and hepatic ameliorative effects of L-carnitine and selenium on cadmium-induced hepatotoxicity and alterations in liver cell structure in male mice. Ecotoxicol. Environ. Saf., 173: 419-428.
50. Pavlova, E.L., L.S. Simeonova and G.A. Gegova, 2018. Combined efficacy of oseltamivir, isoprinosine and ellagic acid in influenza A(H3N2)-infected mice. Biomed. Pharmacother., 98: 29-35.
51. Firdaus, F., M.F. Zafeer, E. Anis, M. Ahmad and M. Afzal, 2018. Ellagic acid attenuates arsenic induced neuro-inflammation and mitochondrial dysfunction associated apoptosis. Toxicol. Rep., 5: 411-417.