Journal of Functional Foods 124 (2025) 106626

Contents lists available at ScienceDirect

Journal of Functional Foods

journal homepage: www.elsevier.com/locate/jff

Mitigation of doxorubicin-induced liver toxicity in mice breast cancer model by green tea and *Moringa oleifera* combination: Targeting apoptosis, inflammation, and oxidative stress

Abdulrahman H. Laftah [a], Nawfal Alhelfi [a], Sadeq K. Al Salait [b], Ammar B. Altemimi [c,d], Mohammad Reza Tabandeh [e], Efstathia Tsakali [f,g], Jan F.M. Van Impe [g,*], Ahmed A. Abd El-Maksoud [h], Tarek Gamal Abedelmaksoud [i]

[a] *Department of Food Science, College of Agriculture, University of Basra, Basra, Iraq*
[b] *Hematologist, Oncology unit, Al-Sadr Hospital, Basra, Iraq*
[c] *Department of Food Science, College of Agriculture, University of Basrah, Basrah 61004, Iraq.*
[d] *College of Medicine, University of Warith Al-Anbiyaa, Karbala 56001, Iraq.*
[e] *Department of Biochemistry and Molecular Biology, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran*
[f] *Department of Food Science and Technology, University of West Attica, 12243, Egaleo, Greece*
[g] *Department of Chemical Engineering, BioTeC+ Chemical & Biochemical Process, KU Leuven, 9000 Gent, Belgium*
[h] *Dairy Science Department, Faculty of Agriculture, Cairo University, Giza 12613, Egypt,*
[i] *Food Science Department, Faculty of Agriculture, Cairo University, Giza 12613, Egypt*

ARTICLE INFO

Keywords:
Doxorubicin
Liver toxicity
Breast Cancer
Green tea
Moringa oleifera
Oxidative stress

ABSTRACT

This study evaluated the effect of green tea and *Moringa oleifera* extracts (at 1 % and 2 % concentration) on DOX-treated breast cancer mice. 36 Balb/C female mice with breast cancer were split into healthy mice, 4 T1 cell cancer-induced, and Doxorubicin-induced malignancies. Assessments included weight, tumor size, liver enzymes, antioxidant enzymes, oxidative stress markers, pro-inflammatory cytokines, gene expressions for apoptosis and inflammation (BAX, BCL2, NLRP3, NFKB), and liver tissues were histopathologically examined. DOX increased liver enzymes and may have damaged the liver. The hepatoprotective effects of these extracts, notably at 2 %, were apparent in their enzyme reduction. Liver CAT, GPX, and SOD activity increased and TOS and OSI levels decreased due to the extracts. Additionally, the herbal treatment lowered pro-inflammatory cytokine levels and regulated apoptosis-related gene expression, lowering **BAX** and increasing BCL2, promoting cell survival, and reducing inflammation. Herbal extracts can reduce NLRP3/NFKB expression in DOX-treated mice's livers, depending on dosage. Histopathological evaluation showed that highly-dose therapy reduced hepatocyte degradation, inflammatory cell infiltration, localized necrosis, and blood vessel congestion. Finally, green tea and *Moringa oleifera* extracts protect against DOX-induced hepatotoxicity.

1. Introduction

Doxorubicin (DOX), is a regularly utilized chemotherapy advertisers that exhibit antineoplastic homes against a colossal assortment of growths, which incorporate breast, prostate, uterus, ovaries, throat, stomach, liver, gallbladder, and breast cancer (Tian et al., 2020). Despite its cytotoxic effects on cancer cells, the use of DOX is restricted due to its potential damage to healthy tissues and organs (Renu, Abilash, & Arunachalam, 2018), especially, hepatotoxicity, nephrotoxicity and cardiotoxicity. The destructive consequences precipitated by way of DOX continue to be a massive situation for both sufferers and clinicians inside the subject of most cancer chemotherapy. (Jin et al., 2019; Lehuédé et al., 2019; Tian et al., 2020; Tormo et al., 2019). Several mechanisms have been mentioned for hepatotoxic results of doxorubicin. One of the primary mechanisms underlying DOX -brought about liver toxicity is the induction of oxidative stress. Further to oxidative stress, DOX is also implicated in the dysregulation of apoptotic pathways within the liver (Hussain et al., 2021). DOX up-regulates the pro-apoptotic genes which include BAX and the downregules the anti-apoptotic genes which include Bcl-2, resulting in the activation of caspase3-mediated apoptotic

* Corresponding author.
E-mail address: jan.vanimpe@kuleuven.be (J.F.M. Van Impe).

https://doi.org/10.1016/j.jff.2024.106626
Received 20 August 2024; Received in revised form 22 November 2024; Accepted 3 December 2024

pathways (Özgen, Erdinç, Kelle, Erdinç, & Nergız, 2022). The dysregulation of apoptotic signaling cascades contributes to hepatocyte death and liver dysfunction (Xu et al., 2020). Moreover, DOX-induced liver toxicity entails the activation of inflammatory pathways, notably the nuclear factor kappa B (NF-B) pathway and TNF-α production, as one example of pro-inflammatory cytokines. Activation of the NF-KB/TNF-α pathway promotes hepatic inflammation and exacerbates liver injury in response to DOX contact.

Functional foods, which encompass a huge variety of bioactive compounds and plant-primarily based nutrients, have received attention as probably useful supplements in cancer treatment. They provide multifaceted defense mechanisms, comprising a diverse range of protective moves, to mitigate the destructive effects induced by using chemotherapy. Herbs and spices are important additives of culinary customs across the world. Similarly, to improving the taste of meals, additionally they showcase healing attributes, rendering them crucial inside the field of purposeful foods (Alansari, 2020; Al-Temimi, Al-Garory, & Khalaf, 2020; Anyene et al., 2021; Cheon, Chung, & Park, 2021; Teibo et al., 2020). Green tea has obtained large interest for its hepatoprotective properties, typically due to excessive levels of polyphenol compounds, especially epigallocatechin gallate (EGCG) (Ohgitani et al., 2021). Several preceding studies have shown that EGCG and other catechins in green tea protect the liver from DOX-induced hepatotoxicity by exerting antioxidant and anti-inflammatory properties (Sojoodi et al., 2020). Furthermore, advanced studies have supplied compelling evidence concerning the capability of green tea to prevent the progression of liver cirrhosis, fibrosis, and inflammation which cause chronic liver disease. (Shareef, Ibrahim, Alzahrani, Al-Medhtiy, & Abdulla, 2022; Sojoodi et al., 2020; Wu et al., 2022).

Additionally, the *Moringa oleifera* tree, which has medicinal and nutritional value, has recently emerged as a potential addition to reduce DOX-induced liver damage. It was demonstrated that *Moringa oleifera* 's antioxidant and anti-inflammatory properties reduced liver damage caused by DOX and other harmful chemicals. Several previous studies found that extracts from *Moringa oleifera* improve liver function and provide protection in opposition to hepatotoxicity due to DOX via decreasing levels of cholesterol, oxidative pressure signs, and liver enzymes (Obayuwana et al., 2022; Abd-Elnaby et al., 2022a, 2022b; Aly et al., 2020). Following an analysis using Darweish, GabAllh, El-mashad, Moustafa, and Amin (2021), the consequences of rosemary and *Moringa oleifera* extracts on liver fibrosis introduced via thioacetamide (TAA) in rats were investigated. They found that the extract reduced the severity of TAA-induced liver damage in both groups.

The objective of this study was to investigate the potential association between the hepatoprotective effects of green tea and *Moringa oleifera* and the mitigation of liver damage produced by doxorubicin in a mouse model of breast cancer. This research aims to investigate the synergistic effects of green tea and *Moringa oleifera* in safeguarding the liver from doxorubicin-induced hepatotoxicity. Specifically, the study will analyze the impact of these two herbs on liver processes such as apoptosis, inflammation, and oxidative stress.

2. Materials and methods

2.1. Collection, authentication and preparation of plants

The plants were obtained from local retailers of Basra Governorate, South of Iraq. The plant was authenticated by a specialist (Haider S. S. Al- Jabir) in medicinal plants (Department of Horticulture, University of Basrah). The samples were in dry leaves, with no visible contaminants. It underwent a cleaning inspection for impurities and possible foreign objects. The herbs were processed using a manual grinder (DAMFOX, No: MK-Y599, German) to achieve a fine powder. Room temperature was used to store all samples until analysis.

2.2. Preparation of herbs water extracts

For preparation of water extracts about 60 g of each herbs powder were put in 300 mL of boiling DW for 30 min on magnetic stirrer. Then the samples were filtrated by a Buchner funnel using the filter paper (Whatman No. 1) with vacuuming, then the filtrate extraction was concentrated by the rotary evaporator (Stuart/ RE 300 dB, UK) at a temperature of 40 °C to dry the extracts. After that the samples were dried using a Freeze dryer (CHRIST, Germany) and the dried samples were stored in amber glass tube under freezing at −18 °C until use. Green tea and *Moringa oleifera* leaves water extract were mixed in a 1:1 ratio.

2.3. HPLC analysis

HPLC analysis was conducted according to the methodology outlined in the study Krstonošić, Hogervorst, Mikulić, and Gojković-Bukarica (2020). Briefly, the HPLC analysis was conducted using an SYKAM S500 HPLC system analysis (*SYKAM*, Germany) with a PDA detector in a C18-ODS column (particle 25 cm × 4.6 mm, Zorbax Eclipse Plus). The mobile phase was DW with solvent A (0.1 % glacial acetic acid) and solvent B (acetonitrile with 0.1 % glacial acetic acid) from (0–5 min, A = 40 %, B = 60 %) (6–14 min, A = 80 %, B = 20 %). HPLC profiling was performed at 30 °C at a constant flow rate of 1 mL/min. Equal volumes of 10 µL were injected into the system for all samples, and 280 nm wavelength was selected for analysis. Gallic acid, Caffeic acid, P-coumaric acid, Chlorogenic acid, Ferulic acid, Tannic acid, Rutin, Apigenin have been purchased from Germany (Sigma-Aldrich) and used standard **(Fig. S1A)**.

2.4. Determination of total phenolic content (TPC)

Folin-Ciocalteu (Avonchem, UK) a procedure that was used to determine TPC with some modification (Kumar, Reddy, Prakash, & Kumar, 2018). Briefly, Folin-Ciocalteu reagent was diluted 10 times and 0.5 mL with 500 µL of each extract was mixed. 4.5 mL of DW and 0.5 mL of Na_2CO_3 (*w/v*) 7.5 % were added to the mixture after being kept at room temperature for 5 min, and then was vortexes, and incubated for 120 min in a dark place. By using A Metstar MUV-61PCS spectrophotometer (Optima, England), measured the optical density at 760 nm in comparison to a blank. A calibration curve for Gallic acid (BHD, England) was used to measure the TPC. At different concentrations (0–300 µg/mL). Results were presented as µg of Gallic acid equivalent (GAE) per g dry weight.

2.5. Determination of total flavonoids content TFC

TFC was measured by using $AlCl_3$ (BHD, England) colorimetric assay as mentioned in Pandey and Rajbhandari (2014). Each (1 mL) aqueous extract (1 mg in 1 mL DW) was mixed with 4 mL of double DW and 0.3 mL of 5 % (w/v) NaNO2 (BHD, England). The mixture was kept for 5 min at room temperature. After that 10 % (w/v) AlCl3 (BHD, England) has been added to the mixture. 2 mL of 1 M NaOH (VWR Chemicals, USA) was added immediately, and the volume was increased to 10.0 mL with double DW. VIS double-beam Spectrophotometer (Optima England) was used to measure the absorbance at 510 nm after the solution had been fully mixed. Quercetin solutions at (0–1000 µg/mL) were prepared as standard for flavonoid assessment. Total flavonoid content was presented as µg of Quercetin equivalents (QE) per g dry weight.

2.6. Evaluation of antioxidant potential

DPPH is a method of free radical scavenging activity, known as 2,2′-diphenyl-1-picrylhydrazyl (Sigma-Aldrich, Germany), utilized to estimate the antioxidant activity of the extracts. Which was describe by Phuyal, Jha, Raturi, and Rajbhandary (2020). For 30 min and at a dark room temperature, 0.5 mL of the extracts (200 and 400 µg/ mL) were

mixed with 1 mL DPPH solution.1 mL methanol and 1 mL DPPH were mixed in preparation as a control. Finally, by using a spectrophotometer (Optima England) at 517 nm, the solutions were measured. *Butylated Hydroxytoluene* (BHT) (Samchun, Korea) at a concentration of 200 and 400 μg/mL was used as the standard. Radical scavenging potential was determined applying the following relationship formula:

$$\text{DPPH\%} = 1 - \frac{\text{A sample}}{\text{A control}} * 100$$

where, **A** control is a control sample absorbance and **A** sample is the extract absorbance.

2.7. Ferric reducing antioxidant power (FRAP) activity

FRAP was measured as reported by Védékoi et al. (2019). Buffer of sodium phosphate 2 mL of (0.2 M, pH 6.6) (Ino Lab pH 7110 WTW, UK) and was mixed with 2 mL of 1 % potassium ferricyanide and 1 mL of various concentrations of samples (200 to 400 μg/ mL) in a test tube. For 10 min and at 3000 rpm, the mixture was centrifuged (Harmonic Series, Taiwan) after being incubated for 20 min in a water bath (50 °C). 10 % trichloroacetic acid (2 mL) was then added to the mixture. 2 mL of solution upper layer was mixed with 2 mL DW and 0.5 mL of 1 % ferric chloride (BHD, England), (FeCl3) was prepared freshly in some DW and then, using a spectrophotometer UV-VIS, at 700 nm the absorbance of the reaction mixture was measured. (Optima England). A greater absorbance of the reaction mixture indicates a significant capacity for reduction. As a means of comparison, Tochopherol was used as a positive control, and the total antioxidant content was quantified and reported as absorbance values.

2.8. Metal chelating ability

The effect of chelating on ferrous ions was measured as described by Gulcin and Alwasel (2022). In brief, the solution was prepared at different concentrations (200–400 μg/mL) by mixed the extract 0.25 mL with $FeCl_2$ (2 mM) 0.05 mL. The process was followed by added 0.2 mL of Ferrozine (2 mM) (Sigma-Aldrich, Germany). The mixture has stirred and left for 10 minuits at 25 °C. Then, absorbance values of solution were recorded at 562 nm. EDTA was used as a control and results of total anioxidant content were expressed as percentage. The percentage (%) of chelating effect was calculated from the formula:

Metal chelating effect % = Abs (Control) – Abs (Sample)/Abs (Control) × 100.

2.9. Experimental animals

A 36 female mice BALB/c wild-type in all (8 weeks old, 20–22 g) were obtained from the StemGene Biohealth laboratory (Iran, Ahvaz). Under standard laboratory conditions of temperature (22 ± 2 °C), mice were maintained, humidity, and a 12 h cycle light/dark, with ad libitum access to food (Parsfeed Co, Iran) and water.

The animal care was conducted by the procedures specified in the "Guide for Care and Use of Laboratory Press" (Washington, DC, USA).

2.10. Breast cancer 4 T1 mouse model

24 female BALB/c mice in all were used to develop the 4 T1 breast cancer mouse model. 4 T1 cell line was obtained from the StemGene Biohealth laboratory (Iran, Ahvaz). Cells were cultured in a DMEM-HG medium (Bioidea, Iran), supplemented with 10 % fetal bovine serum (FBS, Biosera, France) and 100 unit/mL penicillin/streptomycin (Bioidea, Iran). Cells were incubated at 37 °C with 5 % CO_2 and 95 % humidity for 36 h. approximately, third mammary fat pad of the BALB/c mice has been induced subcutaneously with 1 × 105 4 T1 cells (100 μL) after isolation as described before. At a similar site of control mice, 100 μL of PBS was introduced (Rajaratinam et al., 2022). Body weight, appearance, and size of tumors were measured once a week. Also, the tumors's length and width, by a caliper, were measured and the final volume was determined by using the formula: 0.52 × width^2 × diameter (Sauter, Martinet, Zhang, Mandeli, & Woo, 2000). They were also monitored daily for water and, food consumption, and behavior.

2.11. Experimental design

When tumors became palpable and the tumor size reached 100 mm, tumor-bearing, and healthy mice were divided into 6 groups (each group has six), as follows:

- Control or sham group (H): Healthy mice received normal saline (0.2 mL once a day) by gavage method and a single intraperitoneal of normal saline (0.1 mL) was received once a week for five weeks.
- Tumor group (BC): Tumor bearing mice received normal saline (0.2 mL once a day) by gavage method and a single intraperitoneal of normal saline (0.1 mL) was received once a week for five weeks.
- Control + DOX group (HD): Healthy mice received a single intraperitoneal was received Doxorubicin (DOX-Cell, Germany) (5 mg/kg) (Zeiss et al., 2019) once a week for five weeks.
- Tumor + DOX Group (BCD): Tumor bearing mice received a single intraperitoneal injection of Doxorubicin (5 mg/kg) weekly for five weeks.
- Tumor+ DOX+ Low dose of herb mixture (BCDLH): Tumor bearing mice received a single intraperitoneal injection of Doxorubicin (5 mg/kg) weekly for five weeks and then treated with 1 % of formula (0.5 g of green tea and 0.5 g of *Moringa oleifera*) dissolved in 100 mL DW.
- Tumor + DOX + High dose of herb mixture (BCDHH): Tumor bearing mice received a single intraperitoneal injection of Doxorubicin (5 mg/kg) weekly for five weeks and then treated with formula (1 g of green tea and 1 g of *Moringa oleifera*) dissolved in 100 mL DW.

2.12. Sampling

Xylazine and Ketamine hydrochloride (5 mg/ kg and 50 mg/kg respectively) were used at the end of the experiment to anesthetize the mice before decapitation. For histological evaluation, liver tissue was fixed after removal in a 10 % formalin buffer immediately after removal. For gene expression and antioxidant indices analyses, liver tissue was collected Serum samples were collected and stored at −70 °C. Serum samples were obtained by centrifuging the blood from each mouse at 2700 g for 15 min.

2.13. Tissues preparation

The tissues were homogenized before analysis of inflammatory and antioxidant/oxidant factors According to Amiri, Tabandeh, and Hosseini (2021). In a total volume of 1000 μL, the RIPA lysis buffer contains the following components: NaCl (150 mM), SDS (0.1 %), Tris (25 mM, pH 7.4), NaF (1 mM), Phenylmethylsulphonyl fluoride (1 mM), Sodium Fluoride (50 mM), and a protease inhibitor cocktail from Sigma-USA. liver specimen was homogenized by Heidolph, Germany homogenizer at a1:5 ratio. at 10000 × RPM for fifteen minutes at 4 °C, Homogenate tissues were centrifuged. Clear supernatants were carefully collected and stored at −70 °C until use immediately. Bradford protein assay was a kit to measure the protein concentration (AvinStemgene Biohealth Co, Iran).

2.14. Antioxidant enzymes assay

Enzymes of antioxidant activities consisting of SOD, GPX, and CAT (catalase, Glutathione peroxidase, and superoxide dismutase respectively), were determined using the commercial kits SOD (sensitivity

range is 0.044 U/mL the kit code is ZX-44108-96) GPX (Sensitivity range is 0.044 U/mL and the kit code is *E*-BC-K096-M), as recommended by the manufacturer (KiaZist Co, Iran). The activities of antioxidant enzymes were expressed as U/mg of tissue protein.

2.15. Tissue cytokine measurement

The tissue concentration of IL1-β (sensitivity is 1 pg/mL, SLD001Hu) and TNF-α (sensitivity is 5.5 pg/mL, SLD004Hu) were measured by using mice Special ELISA kits (Sunlong Biotech, China) and expressed as pg/mg of protein tissue.

2.16. Analysis of oxidative stress index OSI

OSI was determined by calculation the total oxidant status (TOS)/ total antioxidant capacity (TAC) ratio. Measuring of total oxidative status (TOS) with hydrogen peroxide (H_2O_2) as a standard was done by a semiautomatic microplate colorimetric method. The assay was performed as described in (Erel, 2005). The final results were shown in units of mmol H_2O_2 Eq/mg. the method for estimating antioxidant capacity as published by Benzie & Strain, 1999. Briefly, in summary, a working solution of FRAP (ferric reducing antioxidant power) solution was prepared by combining, buffer acetate with TPTZ solution in HCl. After the addition and combine FeCl3, a mixture of 10 μL of tissue homogenate and 290 μL of the special working solution were mixed and incubated for 10 min at room temperature. At 532 nm, the optical density of the samples was measured. Ascorbic acid was utilized as standard. The unit of measurement for TAC was μmole vitamin C Eq/mg protein.

OSI was calculated as follows: OSI = [(TOS, μmol H_2O_2 Eq/mg protein)/ (TAS, μmol Trolox Eq/mg protein)].

2.17. Estimation of serum enzyme activity

The serum levels of AST (sensitivity is 1.1 IU/L, *E*-BC-K236-M), ALT (sensitivity is 0.01 U/L E-BC-F038), and ALP (sensitivity is 0.2 King unit/100 mL, E-BC-K091-S) stand for aspartate amino transferase, alanine amino transferase, and alkaline phosphatase were measured using commercially available kit (Man Co, Iran) as recommended by the manufacturer.

2.18. RNA isolation and cDNA synthesis

A Tissue RNA Isolation kit (ParsTous, Iran) was utilized for RNA extract from liver tissues as recommended by the manufacturer. By using an Eppendorf μCuvette G1.0 microvolume measuring cell (Eppendorf, Germany) The purity of RNA at 260/280 OD ratio and the RNA integrity were evaluated. Moreover, for cDNA synthesis, high-purity RNA with an OD of 260/280 ratio above 1.8 was used. Also, by using 1 μg of RNA by Easy™ cDNA Synthesis Kit (ParsTous, Iran), the cDNA was synthesized and a random hexamer according to the manufacturer's instructions.

2.19. Real-time quantitative polymerase chain reaction (qRT-PCR)

The qRT-PCR primers were designed using the Primer3 software version 4.1.1 based on the sequences of **BAX**, bcl-2, NLRP3, NFκβ and GAPDH genes available in NCBI Genebank (Table 1). In this study, the GAPDH gene served as a reference gene for data analysis. The SYBR® Green Real Time PCR Master mix (ParsTous, Iran) was used to perform qRT-PCR on the StepOnePlus™ Real-Time PCR detection System (Applied Biosystems, USA). The PCR protocol consisted of 5 min of denaturation at 94 °C followed by 45 cycles of 94 °C for 15 s and 60 °C for 30 s. Two control reactions including a negative control without cDNA and a control containing RNA instead of DNA were considered. The relative expression of the genes compared to the calibrator gene was analyzed using the comparative $2^{-\Delta\Delta Ct}$ method. Evaluation of the amplification efficiency of the target genes compared to the reference gene (GAPDH) was done by preparing different dilutions of cDNA and drawing the efficiency graph as described previously (Tabandeh, Jozaie, Ghotbedin, & Gorani, 2022).

Table 1
Primers characteristics that used in the current study for qRT-PCR analysis.

Gname	Sequence	Size (bp)	Gene Bank accession No
GAPDH	F: AGTTCAACGGCACAGTCAAG R: TACTCAGCACCAGCATCACC	119	**XM_017593963.1**
BAX	F: AGGATGCGTCCACCAAGAAGCT R: TCCGTGTCCACGTCAGCAATCA	102	**NM_007527.4**
BCL2	F: CCTGTGGATGACTGAGTACCTG R: GCCAGGAGAAATCAAACAGAGG	122	**NM_009741.5**
NLRP3	F: TCACAACTCGCCCAAGGAGGAA R: AAGAGACCACGGCAGAAGCTAG	146	**NM_145827.4**
NFKB-p65	F: GCTGCCAAAGAAGGACACGACA R: GGCAGGCTATTGCTCATCACAG	130	**NM_008689.3**

2.20. Histopathological analysis

Fresh liver samples were fixed in 10 % formalin buffer. Tissue microscopic sections were prepared in the conventional method. After fixing, different stages of tissue passage including dehydration with increasing concentrations of alcohol ethanol, clarification with Xylene, and impregnation with paraffin were performed using a histokinet machine (Leica, Germany). Then, after leaving the Histokinet device, the samples were molded with paraffin and with a rotary hand microtome (Leica RM 2125, Leica Microsystems Nussloch GmbH, Germany), slices with a thickness of 5–6 μm were prepared. Sections were stained with hematoxylin and eosin (H&E). The slides were evaluated by an optical microscope (Olympus Optical Co., Japan) equipped with a digital camera (Microbin5, Microteb, Iran). Stained sections of control and treated rats were examined for alterations in the architecture, portal triads, hepatocytes, sinusoids, and histopathological changes.

2.21. Statistical analysis

The analysis was shown by using GraphPad Prism 10 software (GraphPad Software, Inc. San Diego, CA). Results were expressed using mean ± standard deviation (SD). Additionally, we used the Shapiro-Wilk or Levene's tests to see whether the data was normal or if the error variances were equal. The statistical analysis for all parameters included doing one-way ANOVA of variance and Tukey multiple-comparison post hoc tests. The observed comparison differences between distinct experimental groups were presented as follows: $*p < 0.05$, $**p < 0.01$, $*** p < 0.001$, $**** p < 0.000$.

All experiments were repeated for triplication, and the results were expressed as mean- ± standard deviation. ANOVA procedure and Duncan's multiple range method were used to evaluate the significant differences between treatments ($p < 0.05$).

3. Results

3.1. Characteristics of herbal extracts

Total phenol and flavonoid contents of Green tea, *Moringa oleifera* extracts and their combinations are shown in Table 2. The concentrations of TPC in Green tea, *Moringa oleifera* extracts and their combinations were 152 μg/g, 99.7 μg/g, and 89.4 μg/g, respectively. The

Table 2
Total phenol and flavonoid content in green tea, *Moringa oleifera*, and their mixture.

Formulations	Total Phenol Content (TPC)	Total Flavonoid Content (TFC)
Green Tea + *Moringa oleifera*	**89.4 mg/g**	**190 mg/g**
Green Tea	**152 mg/g**	**302 mg/g**
Moringa oleifera	**99.7 mg/g**	**140 mg/g**

concentrations of TFC in Green tea and *Moringa oleifera* extracts and their combinations were 302 μg/g, 140 μg/g, and 190 μg/g, respectively.

The antioxidant potentials of the combination of green tea and *Moringa oleifera* extracts which were assessed using various methods including DPPH, reducing power, and Iron chelating are shown in Table 3. The results indicated a significant difference in free radical scavenging activity with increasing concentration for green tea, *Moringa oleifera* and their combination. At a concentration of 200 μg/mL, green tea exhibited a DPPH inhibition of 82.2 %, which increased to 83.8 % at 400 μg/mL. *Moringa oleifera* showed a DPPH inhibition of 80.2 % at 200 μg/mL, raised to 81.5 % at 400 μg/mL. The combination of both extracts demonstrated superior activity, with 84.45 % inhibition at 200 μg/mL and 84.7 % at 400 μg/mL. In comparison, at 200 μg/mL, BHT inhibited DPPH by 88.65 % and at 400 μg/mL, it demonstrated a 90.5 % inhibition. These findings demonstrate that the mixed sample is effective for RSO, showing that it is on par with BHT at a concentration of 200 mg/mL but marginally less effective at 400 mg/mL. Although they were not as powerful as BHT, the leaves of green tea and the *Moringa oleifera* also showed significant antioxidant activity. This shows that of the therapies evaluated, BHT is still the most powerful antioxidant, even though the natural extracts work.

Iron-chelating activity of green tea was 42 % at 200 μg/mL and 43.6 % at 400 μg/mL. At 200 μg/mL, *Moringa oleifera* exhibited 30 % iron chelating activity, which increased to 36 % at 400 μg/mL. When green tea and *Moringa oleifera* were combined, their iron chelating activity was found to be lower, measuring 22 % at a concentration of 200 μg/mL and 41 % at a concentration of 400 μg/mL. EDTA exhibited markedly superior iron chelating abilities, with a 97.4 % activity at a concentration of 200 μg/mL and a 98 % activity at a concentration of 400 μg/mL, when compared to other substances. The results showed that while the separate samples had significant iron chelating properties, their combination was less effective. However, all the natural extracts that were studied show significantly less effectiveness when compared to EDTA, which remains the most potent chelating chemical among the biological substances. This demonstrates the lower effectiveness of natural extracts in reducing iron compared to EDTA.

Additionally, at a concentration of 200 μg/mL, green tea exhibited a reducing power of 0.89 nm, which increased to 1.37 nm at 400 μg/mL. *Moringa oleifera* showed a reducing power of 0.5 nm at 200 μg/mL, raised to 0.8 nm at 400 μg/mL. The combination of green tea and *Moringa oleifera* demonstrated the lowest reducing power, with values of 0.508 nm at 200 μg/mL and 0.779 nm at 400 μg/mL. Comparatively, Tocopherol showed the higher reducing power than herbal extracts, with values of 0.8 nm at 200 μg/mL and 1.3 nm at 400 μg/mL.

Table 3
Free radical scavenging by DPPH assay, reducing power by FRAP assay and Metal chelating ability for green tea, *Moringa oleifera* and their mixture.

DPPH %			
	G + MO	**G**	**MO**
200 mg/g	**84.45**	**82.2**	**80.2**
400 mg/g	**84.7**	**83.8**	**81.5**
Iron Chelating %			
	G + MO	**G**	**MO**
200 mg/g	**22**	**42**	**30**
400 mg/g	**41**	**43.6**	**36**
Reducing Power WA			
	G + MO	**G**	**MO**
200 mg/g	**0.508**	**0.893**	**0. 5**
400 mg/g	**0.779**	**1.378**	**0.8**

3.2. HPLC analysis

HPLC chromatograms of green tea, *Moringa oleifera*, and their combination were in Fig.S1B, C, and D. Detailed analysis concentration of the active compounds in green tea, *Moringa oleifera*, and their combination were showed in FigS2. as following: The results indicate that combination of green tea and *Moringa oleifera* of Gallic acid has high significant elevating in the combination (71.6 ± 0.6 mg/Kg $p < 0.0001$), compare with green tea (54 ± 1 mg/Kg) and *Moringa oleifera* (33.6 ± 0.6 mg/Kg). Moreover, P-coumaric acid in the combination has significant increase (47.3 ± 0.2 mg/Kg $p < 0.0001$) compare with grean tea and *Moringa oleifera* (22.6 ± 1.6 mg/Kg), (32.6 ± 0.1 mg/Kg), respectively. In *Moringa oleifera*, Ferulic acid, Rutin, and Apigenin were detected at concentrations of (24.9 ± 0.1 mg/Kg), (41.5 ± 0.32 mg/Kg), and (32.9 ± 2 mg/Kg) respectively, whereas they were absent in green tea, however, *P*-value of the herbal combination were ($p < 0.0001$), ($p < 0.0001$), and ($p < 0.0001$), respectively. In contrast, Caffeic acid, Chlorogenic acid and Tannic acid were all absent in *Moringa oleifera*. Moreover, in green tea were (60.5 ± 0.2 mg/Kg), (25.4 ± 0.2 mg/Kg) and (39.8 ± 0.1 mg/Kg), respectively, whereas both of Caffeic acid, Chlorogenic acid in herbal combinations showed decreasing significant effect ($p < 0.0001$) compared with green tea, however, there is no significant effect with Tannic acid compared with green tea.

3.3. Effect of herb extracts on body weight and tumor size in DOX treated mice

Our results revealed that administration of DOX in healthy mice resulted in body weight reduction compared to untreated animals ($P < 0.001$). Similar result was observed for cancerous mice following DOX treatment (BCD Group) compared to untreated cancerous mice (BC group) ($P < 0.01$). In mice with cancer and chemotherapy, 1 % herb mixture treatment (Group BCDLH) could increase the body weight compared to untreated cancerous mice (BCD group) ($p < 0.01$), while 2 % herb mixture treatment had no such effect on body weight change in DOX treated cancerous mice (BCDHH) compared to untreated mice (Fig. 1-A). (See Fig. 2.)

The results of tumor size are shown in Fig. 1-B. Our results indicated that treatment of cancerous mice with DOX (BCD group) resulted in reducing significantly of tumor size compared to untreated mice (DC group) ($P < 0.001$). There were no statistically significant changes in tumor size between the BCD group and those treated with 1 % (BCDLH group) or 2 % (BCDHH group) herb extract.

3.4. Effect of herb extracts on hepatic activities of antioxidant enzymes in DOX treated mice

The activities of antioxidant enzymes including CAT, GPX, and SOD (catalase, glutathione, and peroxidase) are shown in Fig. 3. A, B, and C. In healthy mice (Group H), the activities of CAT, GPX and SOD were 80.92 ± 6.50, 75.87 ± 9.72, and 64.82 ± 8.38, respectively. Our results showed that, mice with cancer (Group BC) exhibited significant decrease in GPX activity (61.66 ± 13.01, $p < 0.0001$), while cancer induction had no significant effect on the activities of SOD (43.42 ± 10.39) and CAT (64.11 ± 9.07) in comparison with healthy mice. Upon administration of DOX to healthy mice (Group HD), CAT ($p < 0.0001$), GPX ($p < 0.001$), and SOD ($p < 0.01$) activities were significantly decreased compare to healthy mice. Treatment of mice with cancer had no significant effect on the activities of GPX and SOD, while it results in a significant reduction of CAT activity ($p < 0.001$) compared to cancer mice without DOX

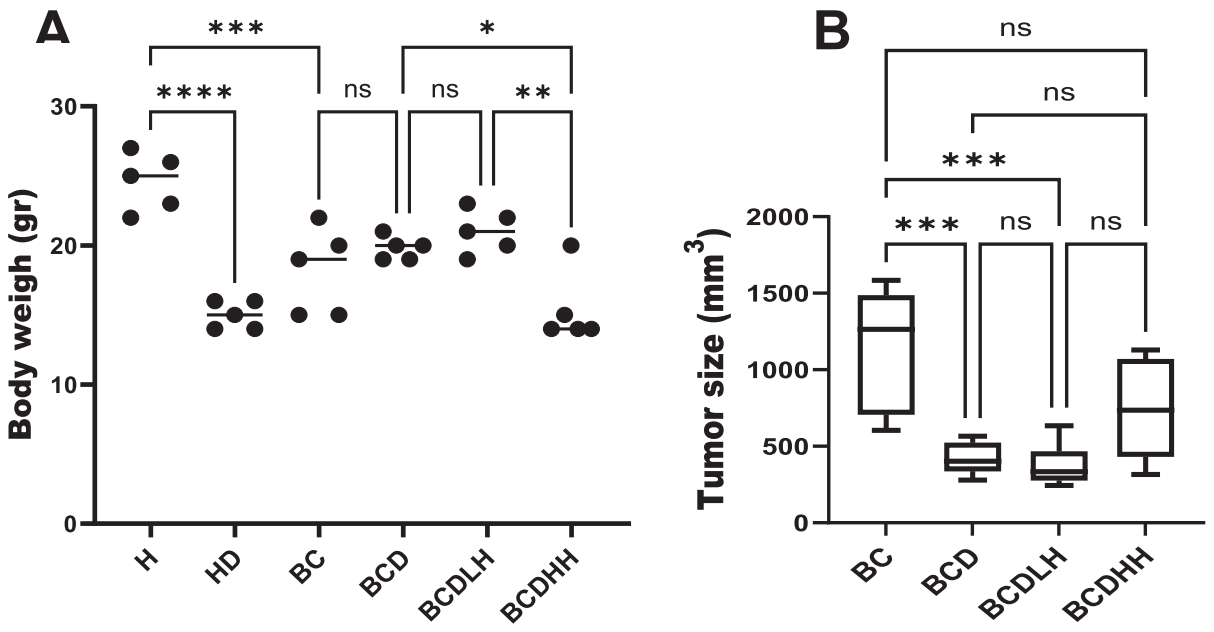

Fig. 1. The body weight and tumor size in different experimental groups. Results are means ± SD. *, ** and *** represent the significant difference between different experimental groups at $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively. H; healthy group, HD; healthy group treated with DOX, BC; mice with breast cancer, BCD; DOX treated mice with cancer, BCDLH; cancer mice treated with DOX and 1 % herbal extract, BCDHH; cancer mice treated with DOX and 2 % herbal extract.

Fig. 2. Breast cancer tumor size before starting the experiment.

treatment. In mice with cancer and chemotherapy, 1 % herb mixture treatment (Group BCDLH) had no significant effects on the activities of liver antioxidant enzymes compared to un-treated animals. In Group BCDHH, where mice received 2 % herbal extract alongside cancer and chemotherapy, the activities of CAT ($p < 0.05$), GPX ($p < 0.01$), and SOD ($p < 0.05$) were significantly increased compared to untreated mice (BCDHD group).

3.5. *Effect of herbs extracts on hepatic oxidative stress index in DOX treated mice*

Our finding indicated that in healthy mice (Group H), the TOS, TAC and OSI values were 0.88 ± 0.29, 0.793 ± 0.19, and 0.487 ± 0.104, respectively. Mice with cancer (Group BC) exhibited decreased level of TAC and no significant change in the levels of TOS and OSI compared to healthy mice. Upon administration of chemotherapy to healthy mice (Group HD), TOS and OSI levels were significantly increased (TOS: 7.11, ± 0.48; OSI 24.808 ± 13.07), while TAC level was significantly decreased (0.33 ± 0.13) compared to untreated mice. Mice with cancer and chemotherapy (Group BCD) showed a significant elevated levels of TOS and OSI levels (TOS: 7.71, ± 1.27; OSI: 26.66 ± 7.308) and a significant reduced level of TAC (0.296 ± 0.04) compared to untreated mice with cancer. Mice with cancer and chemotherapy, along with 1 % and 2 % herbal treatment (Group BCDLH) and treatment (Group BCDLH), exhibited significant reduction in TOS ($p < 0.001$) and OSI ($p < 0.05$), however, there was exhibited significant elevation of TAC only in (Group BCDLH) ($p < 0.05$) levels compared to Group BCD. There was no significant difference observed in the mitigating effects of the herb

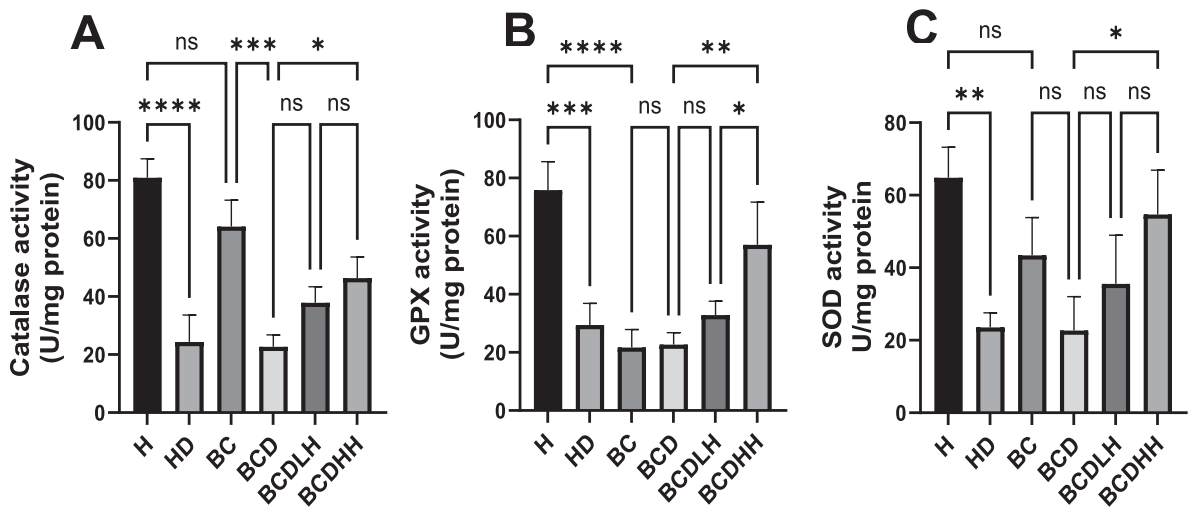

Fig. 3. Effect of green tea and *Moringa oleifera* mixture on the activities of hepatic catalase (A), GPX (B) and SOD (C) in liver of doxorubicin (DOX) treated mice with cancer. Results are means ± SD. *, **, *** and **** represent the significant difference between different experiment groups at $p < 0.05$, $p < 0.01$, $p < 0.001$ and $p < 0.0001$, respectively. H; healthy group, HD; healthy group treated with DOX, BC; mice with breast cancer, BCD; DOX treated mice with cancer, BCDLH; cancer mice treated with DOX and 1 % herbal extract, BCDHH; cancer mice treated with DOX and 2 % herbal extract. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

mixture against oxidative stress in the liver of DOX treated mice with cancer between the 1 % and 2 % concentrations, Fig. 4 A, B, and C.

3.6. The effect of herb mixture on hepatic proinflammatory cytokines production in DOX treated mice

Our results indicated that in healthy mice (Group H), the hepatic IL-1β and TNF-α level were 15.46 ± 4.81 pg/mg proteins, 35.40 ± 6.70 pg/mg proteins. Mice with cancer (Group BC) exhibited no significant change in IL-1β and TNF-α level 46.57 ± 10.93 pg/mg protein and TNF-α 56.57 ± 9.30 pg/mg protein concentrations compared to healthy mice. Upon administration of DOX to healthy mice (Group HD), IL1β and TNF-α levels were significantly increased (160.31 ± 33.92 pg/mg protein; $p < 0.001$, 147.23 ± 17.13; pg/mg protein; $p < 0.001$) compared to untreated animals. Mice with cancer and chemotherapy (Group BCD) showed elevated levels of IL1β (215.26 ± 40.35 pg/mg protein; $p < 0.001$) and TNF-α (188.07 ± 23.65 pg/mg protein; $p < 0.001$) levels compared to untreated mice with cancer. Our results indicated that mice with cancer and chemotherapy, along with 1 % herbal treatment (Group BCDLH), only exhibited a significant reduction in IL1β (127.39 ± 38.23 pg/mg protein, $P < 0.05$), while TNF-α level (141.1 ± 27.32 pg/mg protein) showed no significant change in BCD group following 1 % herb extract treatment. Furthermore, 2 % herba extract (Group BCDHH) shows significant reduction in both IL1β (81.93 ± 21.27 pg/mg protein, $P < 0.001$) and TNF-α levels (103.6 ± 18.93 pg/mg protein, $P < 0.01$) compared to BCD group, Fig. 5, A and B.

3.7. The effect of herb mixture on the activities of enzymes associated with liver damage in DOX treated mice

As shown in Fig. 6, (A, B, and C) the serum activates of AST, ALT, and ALP in (Group H) were 109.7 ± 12.06 U/L, 34.67 ± 7.095 U/L, and 145.7 ± 16.65 U/L, respectively. There was no significant difference in the serum activates of AST, ALT, and ALP between healthy group and mice with cancer. When DOX was administrated to healthy mice (HD group), notable increase in all serum activates of AST (217.3 ± 25.11 U/

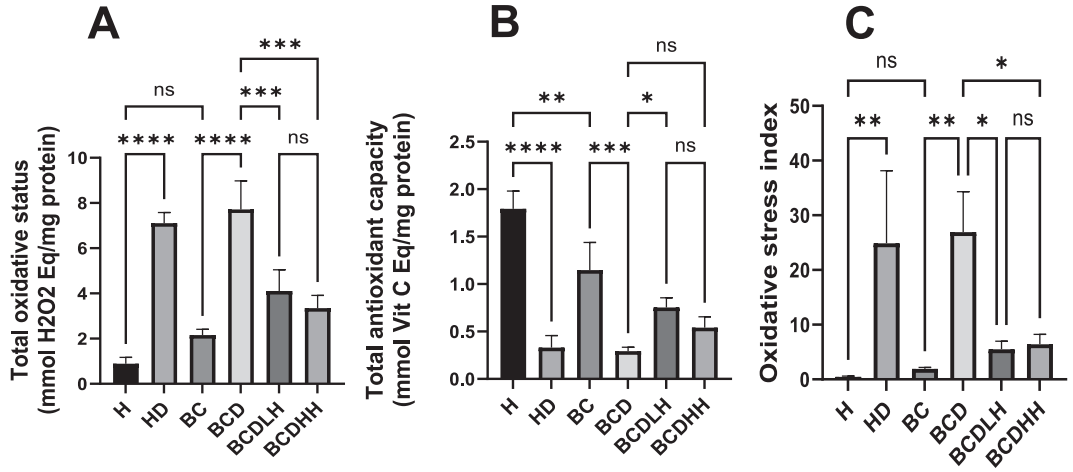

Fig. 4. Effect of green tea and *Moringa oleifera* mixture on hepatic levels of total oxidative status (TOS) (A), total antioxidant capacity (TAC) (B) and oxidative stress index (OSI) (C) in liver of doxorubicin (DOX) treated mice with cancer. Results are means ± SD. *, **, *** and **** represent the significant difference between different experiment groups at $p < 0.05$, $p < 0.01$, $p < 0.001$ and $p < 0.0001$, respectively. H; healthy group, HD; healthy group treated with DOX, BC; mice with breast cancer, BCD; DOX treated mice with cancer, BCDLH; cancer mice treated with DOX and 1 % herbal extract, BCDHH; cancer mice treated with DOX and 2 % herbal extract. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

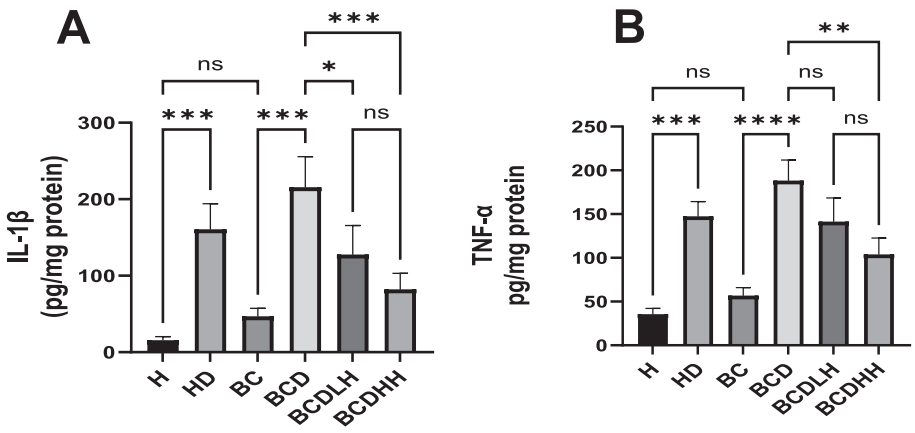

Fig. 5. Effect of green tea and *Moringa oleifera* mixture on hepatic levels IL1β (A) and TNF-α (B) in liver of doxorubicin (DOX) treated mice with cancer. Results are means ± SD. *, **, *** and **** represent the significant difference between different experiment groups at $p < 0.05$, $p < 0.01$, $p < 0.001$ and $p < 0.0001$, respectively. H; healthy group, HD; healthy group treated with DOX, BC; mice with breast cancer, BCD; DOX treated mice with cancer, BCDLH; cancer mice treated with DOX and 1 % herbal extract, BCDHH; cancer mice treated with DOX and 2 % herbal extract. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

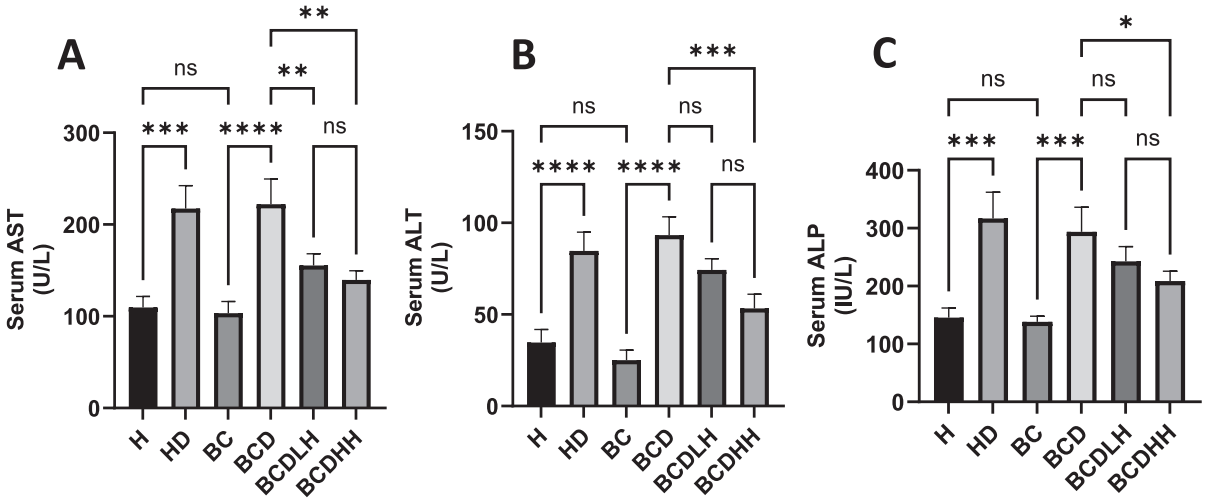

Fig. 6. Serum levels of AST (A), ALT (B) and ALP (C) IN doxorubicin (DOX) treated mice with cancer. Results are means ± SD. *, **, *** and **** represent the significant difference between different experimental groups at $p < 0.05$, $p < 0.01$, $p < 0.001$ and $p < 0.0001$, respectively. H; healthy group, HD; healthy group treated with DOX, BC; mice with breast cancer, BCD; DOX treated mice with cancer, BCDLH; cancer mice treated with DOX and 1 % herbal extract, BCDHH; cancer mice treated with DOX and 2 % herbal extract.

L, $P < 0.001$), ALT (84.67 ± 10.26 U/L, $P < 0.0001$), and ALP (316.7 ± 45.56 U/L, $P < 0.001$) were found in comparison to the healthy mice. Similarly, cancerous mice under DOX (Group BCD) demonstrated a significant increase in AST (222 ± 27.62 U/L, $P < 0.0001$), 93.33 ± 10.02 U/L ($P < 0.0001$), and 293.7 ± 42.57 U/L ($P < 0.001$), compared to untreated cancer group. The study also found that mice in BCDLH group treated by 1 % herb extract showed only significant decrease in AST serum activity (155.3 ± 12.74 U/L, $P < 0.01$), while treatment of mice in BCDHH group with 2 % herb extract resulted in decreasing AST (139.7 ± 10.02 U/L, $P < 0.01$), ALT (53.33 ± 7.767 U/L, $P < 0.001$) and ALP (208.7 ± 16.86 U/L, $P < 0.05$) enzyme activities compared to BCD group.

3.8. The effect of herb mixture on the expression levels of BAX and BCL2 genes

Fig. 7 illustrates the expression level of **BAX** and **BCL2** genes in different experimental groups. Our results indicated that there were no significant differences between the expression levels of BAX and BCL2 genes in liver of healthy and cancerous mice. Following the DOX administration to healthy (Group HD) and cancerous mice (BCD), there was a significant increasing in Bax expression ($P < 0.0001$for HD group; $P < 0.0001$ for BCD group) and a significant decreasing the BCL2 expression ($P < 0.001$ for HD group; $P < 0.05$ for BCD group) in comparison with untreated animals.

Our findings indicated that cancerous mice under DOX and 1 % herb extract (Group BCDLH), showed a significant reduction in BAX expression ($P < 0.0001$) and a significant elevation of BCL2 gene in comparison to BCD group without herb treatment. Similar results were also observed for the expression of BAX ($p < 0.01$) and BCL2 ($P < 0.05$) genes in the liver of BCDHH group compared to BCD group without herb extract treatment.

3.9. The effect of herb mixture on the expression levels of NLRP3 and NFKβ

The hepatic expression of NLRP3 and NFKβ in different experimental groups is shown in Fig. 8. Animals in BC groups that had breast cancer showed no significant difference in NLRP3 and NFKβ expression with healthy mice. In healthy mice (Group HD) and mice with cancer,

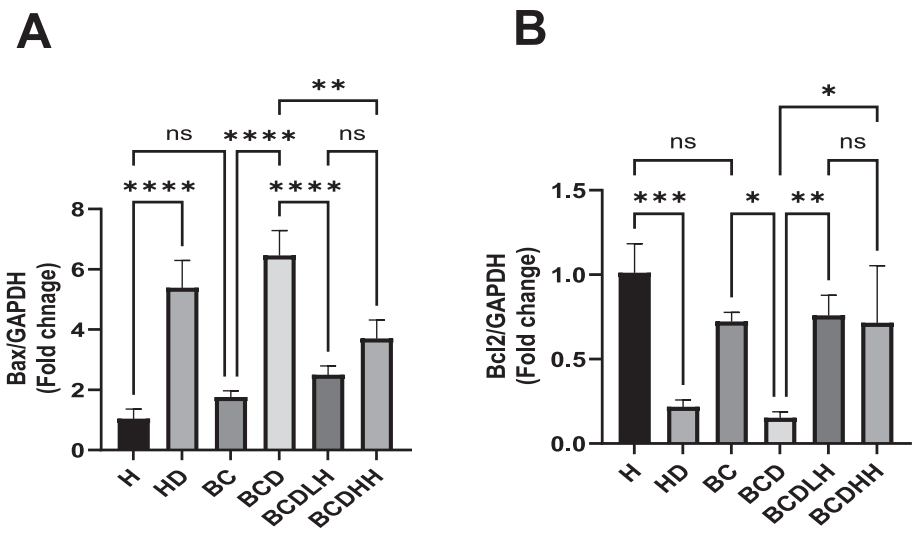

Fig. 7. Effect of green tea and *Moringa oleifera* mixture on expression levels of BAX (A) and BCL2 (B) genes in liver of doxorubicin (DOX) treated mice with cancer. GAPDH was used as a housekeeping gene. Relative quantification was performed according to the comparative $2^{-\Delta\Delta Ct}$ method. Each reaction was run in triplicate. Results are means ± SD. *, **, *** and **** represent the significant difference between different experiment groups at $p < 0.05$, $p < 0.01$, $p < 0.001$ and $p < 0.0001$, respectively. H; healthy group, HD; healthy group treated with DOX, BC; mice with breast cancer, BCD; DOX treated mice with cancer, BCDLH; cancer mice treated with DOX and 1 % herbal extract, BCDHH; cancer mice treated with DOX and 2 % herbal extract. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

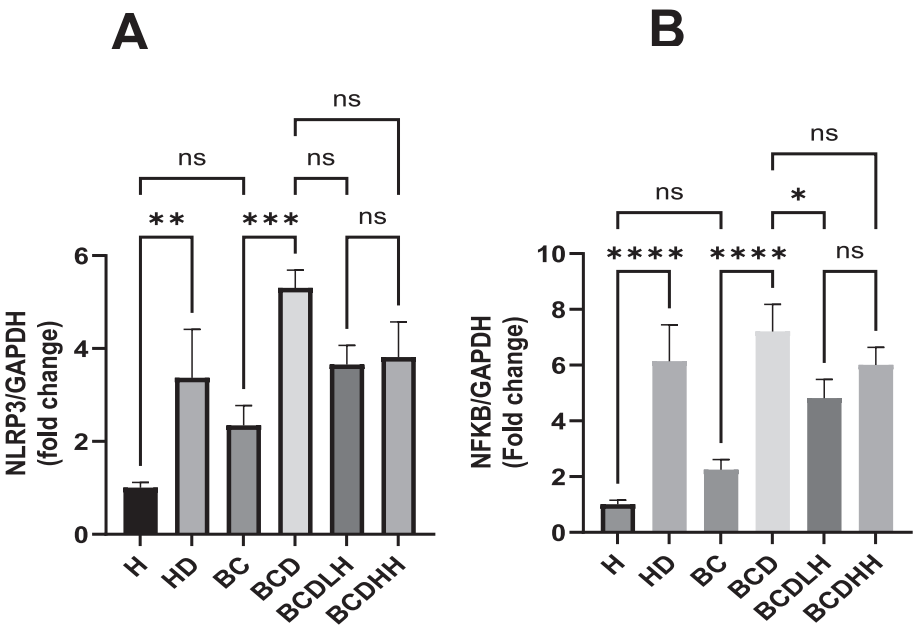

Fig. 8. Effect of green tea and *Moringa oleifera* mixture on expression levels of NLRP3 (A) and NFκβ (B) genes in liver of doxorubicin (DOX) treated mice with cancer. GAPDH was used as a housekeeping gene. Relative quantification was performed according to the comparative $2^{-\Delta\Delta Ct}$ method. Each reaction was run in triplicate. Results are means ± SD. *, **, *** and **** represent the significant difference between different experiment groups at $p < 0.05$, $p < 0.01$, $p < 0.001$ and $p < 0.0001$, respectively. H; healthy group, HD; healthy group treated with DOX, BC; mice with breast cancer, BCD; DOX treated mice with cancer, BCDLH; cancer mice treated with DOX and 1 % herbal extract, BCDHH; cancer mice treated with DOX and 2 % herbal extract. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

administration of DOX resulted in the elevation of the expression of NLRP3 ($P < 0.01$ for HD group; $P < 0.001$ for BCD group) and NFKβ ($P < 0.0001$ for HD group; $P < 0.0001$ for BCD group) genes in comparison with untreated animals. Treatment of cancerous mice with only 1 % herb extract resulted in significant reduction of NFKβ compared to BCD group. Herb extract at both doses had no significant effects on the expression of NLRP3 compared to BCD group.

3.10. Histopathological findings

The histopathological results of the liver tissue showed a normal structure of the liver cells in the control group, with no special complications observed (Fig. 9 A&B). DOX administration resulted in degeneration of liver cells, focal necrosis, diffuse acute inflammatory reaction, and congestion of blood vessels (Fig. 9 C&D). In the group treated with a low dose of the herb mixture (BCDLH), liver damage was improved, but a small number of inflammatory cells and swelling of

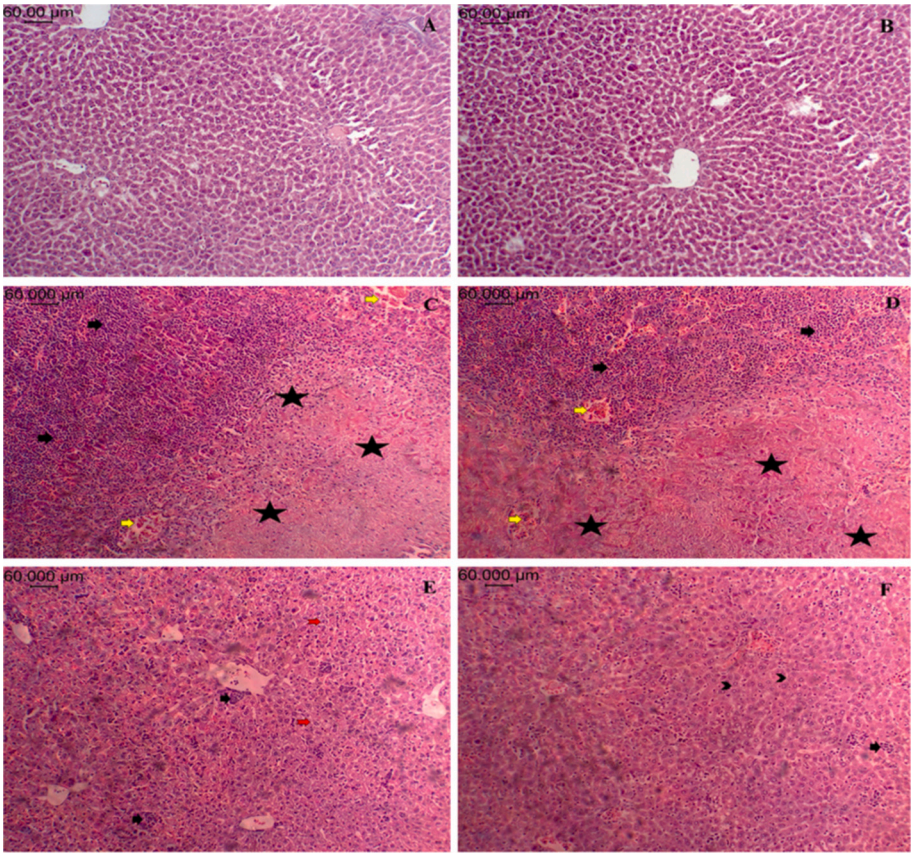

Fig. 9. Histopathological examination of rat liver (H&E staining magnification 10×) in different experimental groups. Control group (H) and BC group (A, B): the normal structure of the liver cells in the control group and no special complication is seen. HD (C) and BCD (D) groups: Focal necrosis (star sign) with diffuse acute inflammatory reaction (Black arrow) and congestion of blood vessels (yellow arrow). BCDLH group (E): Cell swelling of hepatocytes (Red arrow) and mild inflammatory reaction (Black arrow). BCDHH group (F): Most of the hepatocytes have a normal structure (arrowhead) and no special lesion was seen except for a mild inflammatory reaction (Black arrow). Control group (H): Tumor group (BC): Control + DOX group (HD): Tumor + DOX Group (BCD): Tumor+ DOX+ low dose of herb mixture (BCDLH): Tumor + DOX + low dose of herb mixture (BCDHH). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

hepatocytes were also observed (Fig. 9 E). The liver damage induced by DOX was significantly inhibited by treatment with a high dose of the herb mixture (BCDHH). Most of the hepatocytes showed a normal structure, with no special lesions seen except for a mild inflammatory reaction, making the liver morphology near to normal (Fig. 9 F).

4. Discussion

Doxorubicin is a mighty chemotherapeutic agent broadly used in the remedy of diverse cancers, particularly, breast cancer. Despite its efficacy in tumor increase inhibition, DOX has numerous facet consequences, particularly its potential to cause hepatotoxicity or damage the liver (Damodar, Smitha, Gopinath, Vijayakumar, & Rao, 2014). Given the limitations and toxicities related to the traditional use of DOX, there was a developing interest in the use of natural products to mitigate doxorubicin-induced hepatotoxicity. These natural compounds provide a safer substitute due to their many therapeutic benefits, such as antioxidant, anti-inflammatory, and anti-apoptotic effects. Several prior investigations studies have investigated the hepatoprotective effects of green tea or *Moringa oleifera* extracts against DOX-induced liver damage.

When several natural extracts are combined, their antioxidant, anti-inflammatory, and anti-apoptotic properties may work synergistically to increase the protective benefits against chemotherapy-induced hepatotoxicity. In this examination, we investigated the protective effects of green tea and *Moringa oleifera* extracts and their combination towards DOX treatment about hepatotoxicity in a murine model of breast cancer. The findings indicate that both extracts of green tea and *Moringa oleifera* mitigate DOX-induced liver damage by working as antioxidants, anti-inflammatory agents. Our findings indicated that the high total phenolic and flavonoid contents of and green tea extracts are associated with their antioxidant activity. *Moringa oleifera* demonstrated reduced chelation activity at levels of (30 % and 36 %), and the combination exhibited even less effectiveness at (22 % and 41 %) in comparison to EDTA, a potent chelator with activities of 97.4 % and 98 % on the same concentrations. This finding suggests that even as our herb extracts possess strong antioxidant characteristics, their effectiveness in iron chelation is significantly decreased than synthetic chelators such as EDTA. The reduced power of the extracts was evaluated, with green tea exhibiting values of 0.89 nm at 200 µg/mL and 1.37 nm at 400 µg/mL, and *Moringa oleifera* displaying 0.5 nm and 0.8 nm at the same concentrations. The combination exhibited the lowest level of reducing power, with values of 0.508 nm at 200 µg/mL and 0.779 nm at 400 µg/mL, compared to Tocopherol, which confirmed higher reducing power (0.8 nm and 1.3 nm). High-performance liquid chromatography (HPLC) evaluation confirmed the presence of several bioactive compounds in each extract, along with gallic acid, chlorogenic acid, and caffeic acid. Those compounds are known for their antioxidant and anti-inflammatory properties, which in addition aid the determined protecting results of the extracts. These outcomes are consistent with preceding research highlighting the rich antioxidant profiles of green tea and *Moringa oleifera* plants (Senanayake, 2013; Musial, Kuban-Jankowska, & Gorska-Ponikowska, 2020; Pękal, Dróżdż, Biesaga, & Pyrzynska, 2012; Lorenzo & Munekata, 2016; Peñalver, Martínez-Zamora, Lorenzo, Ros, & Nieto, 2022; Ntshambiwa, Seifu, & Mokhawa,

2023; Sreelatha & Padma, 2009). The decreasing tumor size in the DOX-treated mice group is due to the characteristic effectiveness of DOX as an anti-tumor. The combination of the herbal extract showed no significant effect on tumor size in mice with DOX-treated, this suggests that despite the herbal combination reducing the toxicity adverse effects caused by DOX, they do not interfere with DOX therapeutic side effects. This is crucial as it indicates that the herbal combination has the potential to be used in conjunction with chemotherapy to mitigate the side effects without interfering with the effect of DOX in cancer treating.

The oxidative stress induced by DOX treatment, as demonstrated by our findings, is characterized by a significant increase in the oxidative stress index (OSI) and a significant decrease in the activities of important hepatic antioxidant enzymes like catalase, glutathione peroxidase, and superoxide dismutase. The 2 % herbal combinations notably restored the activities of those enzymes, suggesting that the extracts can enhance the liver's antioxidant ability and defend against oxidative damage due to DOX. The administration of the extracts, especially at the 2 %, significantly reduced TOS and OSI levels while increasing TAC. This suggests that the extracts can mitigate oxidative stress by improving the liver's antioxidant potential, thereby reducing the damage caused because of ROS. The extracts contain several antioxidant compounds, including flavonoids (catechins and quercetin) and polyphenols (gallic, chlorogenic, and caffeineic acids) which have been identified through HPLC analysis.

These substances are known to directly neutralize reactive oxygen species (ROS) and possess potent free radical scavenging properties. For instance, gallic acid and tannic acid have the ability to give free radicals hydrogen atoms, stopping the chain reactions that cause oxidative damage. Additionally, research has shown that chlorogenic acid and caffeic acid may increase the production and effectiveness of natural antioxidant enzymes such as catalase and SOD. This enhances and facilitates the enzymatic breakdown of hydrogen peroxide and superoxide anions into less detrimental substances, hence reducing oxidative stress (Na & Surh, 2008; Modesto et al., 2021; Lang et al., 2024; Patintingan et al., 2023). Based on these results, we conclude that the synergistic activity of these bioactive compounds in combination with herbs serves to restore the antioxidant balance in the liver in a dose-dependent way, reducing the oxidative stress brought by DOX.

Our results showed that DOX treatment led to significant increases in hepatic levels of pro-inflammatory cytokines IL-1β and TNF-α, which are indicative of inflammation and play a role in DOX-induced hepatotoxicity. The herbal extracts, especially at the 2 % concentration, significantly reduced these cytokine levels, demonstrating their anti-inflammatory potential. IL-1β is a potent pro-inflammatory cytokine that contributes to liver inflammation and damage. It activates signaling pathways that lead to the expression of various inflammatory mediators and enzymes, such as nitric oxide synthase and cyclooxygenase, which can exacerbate tissue injury (Molina-Holgado, Ortiz, Molina-Holgado, & Guaza, 2000). In the liver, IL-1β promotes the recruitment of inflammatory cells, enhances the production of other pro-inflammatory cytokines, and induces hepatocyte apoptosis. This cytokine also plays a role in the pathogenesis of liver fibrosis by stimulating hepatic stellate cells to produce extracellular matrix components, leading to scarring and impaired liver function (Barbier et al., 2019). TNF-α is another critical cytokine involved in liver inflammation and damage. It binds to its receptors on hepatocytes and other liver cells, triggering a cascade of intracellular events that can result in cell death through apoptosis or necrosis. TNF-α is known to increase the production of ROS, which further exacerbates oxidative stress and cellular damage. It also disrupts the balance of anti-inflammatory and pro-inflammatory cytokines leads to fibrosis and cirrhosis (Kastl et al., 2014). Moreover, TNF-α can compromise the integrity of the liver's blood vessels, leading to increased vascular permeability and subsequent tissue damage (Urschel & Cicha, 2015). The observed anti-inflammatory potential of green tea and *Moringa oleifera* extract combination is likely due to the presence of bioactive compounds such as flavonoids, phenolic acids, and other antioxidants, which are known to inhibit the expression and activity of pro-inflammatory cytokines (Singh, Yau, Leung, El-Nezami, & Lee, 2020). To confirm this opinion, previous reports have shown that compounds like quercetin and catechins, found in green tea, down regulate the expression of pro-inflammatory genes by inhibiting key signaling pathways such as NF-κB (Park et al., 2012). Similarly, bioactive constituents such as *Moringa oleifera* and other polyphenols in *Moringa oleifera* can suppress the production of TNF-α and IL-1β, thereby reducing inflammation (Hamza, 2010; Abd-Elnaby et al., 2022a, 2022b). These compounds achieve this by blocking the activation of macrophages and other inflammatory cells, decreasing the production of cytokines and chemokines that perpetuate the inflammatory response. These findings highlight the protective role of our herb combination against DOX induced liver damage by attenuating inflammatory cytokine production. However; future studies should consider employing Western Blot (WB) analysis to verify the levels of key inflammatory markers and gene expressions associated with metabolic pathways. This approach would provide further mechanistic insights into the protective effects of the green tea and *Moringa oleifera* combination against chemotherapy-induced liver toxicity.

We observed the increased serum liver enzymes (AST, ALT, and ALP) in DOX-treated mice, indicating liver damage. The herbal extracts, particularly at the 2 % concentration, significantly reduced these enzyme levels, indicating their hepatoprotective effects. Histopathological analysis further confirmed the protective effects of the herbal extracts. The liver tissue of DOX-treated mice showed significant damage, including degeneration of liver cells, focal necrosis, diffuse acute inflammatory reaction, and congestion of blood vessels. Treatment with the herbal extracts, especially at the higher dose, significantly ameliorated these histopathological changes, resulting in liver morphology that was nearly normal. AST, ALT, and ALP are enzymes that play vital roles in liver function. Under normal conditions, these enzymes are predominantly found within liver cells. However, when liver cells are damaged, these enzymes leak into the bloodstream, resulting in elevated serum levels (Kleiner et al., 2014). Degeneration of liver cells concomitant with focal necrosis indicates that hepatocytes have undergone severe stress, leading to a loss of cell structure and function and leakage of liver enzymes such as AST, ALT, and ALP (Krishna, 2017). The observed decrease in serum enzyme levels, plus histopathological changes in DOX treated groups receiving both green tea and *Moringa oleifera* extracts confirm the hepatoprotective effect of this herbal formulation in managing DOX integrated liver toxicity.

The expression levels of apoptosis-related genes (BAX and BCL2) and inflammation-related genes (NLRP3 and NFKB) were significantly altered by DOX treatment. DOX increased the expression of the pro-apoptotic gene BAX and reduced the expression of the anti-apoptotic gene BCL2, leading to enhanced apoptosis. The herbal extracts modulated these gene expressions, decreasing BAX and increasing BCL2 levels, which suggests they help to reduce apoptosis and promote cell survival. The extracts also significantly reduced the expression of the pro-inflammatory genes NLRP3 and NFKB, which are involved in the inflammatory response. This indicates that the extracts can modulate both apoptotic and inflammatory pathways, contributing to their overall hepatoprotective effects. Previous reports have shown that green tea is contain a plethora of bioactive compounds, including polyphenols such as epigallocatechin gallate (EGCG), epigallocatechin (EGC), epicatechin gallate (ECG), and epicatechin (EC). EGCG, in particular, has been shown to inhibit NF-κB activation and NLRP3 inflammasome assembly, thereby suppressing the expression of pro-inflammatory cytokines and mitigating inflammation (Lakshmi, Reddy, Kodidhela, & Varadacharyulu, 2020). Additionally, EGCG can modulate the expression of BAX and BCL2, promoting cell survival and attenuating apoptosis (Mostafa-Hedeab, Hassan, & Halawa, 2022; Tak et al., 2016). Similarly, *Moringa oleifera* is rich in bioactive compounds such as flavonoids, phenolic acids, and glucosinolates, which possess potent antioxidant and anti-inflammatory properties (Abdel Fattah, Sobhy, Reda, & Abdelrazek,

2020). Quercetin, a flavonoid abundant in *Moringa oleifera*, has been reported to inhibit the expression of pro-inflammatory genes via suppression of NF-κB signaling. Moreover, quercetin has been shown to regulate apoptotic pathways by modulating the expression of BAX and BCL2, thereby preventing cell death (Zhang et al., 2020).

The synergistic action of these bioactive compounds present in green tea and *Moringa oleifera* extracts could account for their observed effects on apoptosis-related and inflammation-related gene expressions. By targeting multiple signaling pathways involved in apoptosis and inflammation, the combined administration of green tea and *Moringa oleifera* extracts exerts a potent hepatoprotective effect against DOX-induced liver damage. Furthermore, the antioxidant properties of these phytochemicals, as indicated by their HPLC profiles, contribute to the overall hepatoprotective mechanism. By scavenging reactive oxygen species (ROS) and reducing oxidative stress, the bioactive compounds in green tea and *Moringa oleifera* extracts help preserve hepatocellular integrity and function, thereby mitigating liver injury induced by DOX chemotherapy.

Further research needs to be conducted to analyze the impact of long-term administration of green tea and *Moringa oleifera* extracts, how these extracts are combined with other chemotherapeutic drugs, and their efficiency in different cancer types. A better understanding of the potential roles these agents play in other organ toxicity induced by chemotherapy, as well as the identification of molecular targets responsible for antioxidant and anti-inflammatory functions and related signaling pathways, may reveal more extensive applications of these agents.

In this study, we focused on the transcriptional regulation of NF-κB and its downstream targets, NLRP3, by DOX and green tea and *Moringa oleifera* olivera combination. We acknowledge that measuring phosphorylated NF-κB (p-NF-κB) as a direct measure of pathway activation is important. While we would have liked to analyze protein level parameters such as western blot, the protein level analysis is impossible in our facility. Yet the observed changes in NF-κB gene expression in conjunction with the major modifications in inflammatory markers, oxidative stress parameters (TOS, TAS, CAT, GPX), and liver enzymes (AST, ALT, ALP) strongly suggest that NF-κB signaling is functionally important in this model. Additional future studies will endeavor to incorporate analysis of protein level proceeds, such as quantification of phosphorylation of NF-κB, to shed more light on its state of activation from the protein level, and to validate the findings further.

5. Conclusion

In conclusion, green tea and *Moringa oleifera* combination extracts prove that notable protective effect with Doxorubicin-induced hepatotoxicity, according to their groups of antioxidant, anti-inflammatory, and anti-apoptotic properties. Through the coordinated inhibition of several mechanisms that contribute to damaging the liver, including oxidative stress, inflammation, and apoptosis. The synergistic impact of green tea and *Moringa oleifera* holds great promising potential as an adjunct therapy to mitigate the hepatotoxicity caused by chemotherapy agents.

Ethics statement

None declared.

CRediT authorship contribution statement

Abdulrahman H. Laftah: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Conceptualization. **Nawfal Alhelfi:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Data curation, Conceptualization. **Sadeq K. Al Salait:** Writing – review & editing, Writing – original draft, Visualization, Formal analysis, Conceptualization. **Ammar B. Altemimi:** Methodology, Formal analysis. **Mohammad Reza Tabandeh:** Writing – review & editing, Writing – original draft, Methodology, Data curation, Conceptualization. **Efstathia Tsakali:** Writing – review & editing, Writing – original draft. **Jan F.M. Van Impe:** Writing – review & editing, Writing – original draft. **Ahmed A. Abd El-Maksoud:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Software, Conceptualization. **Tarek Gamal Abedelmaksoud:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Software, Resources, Formal analysis, Data curation, Conceptualization.

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.

Acknowledgments

All authors would like to thank Chamran University of Ahvaz, Shahid Chamran University of Ahvaz Golestan Blvd., Ahvaz, Khuzestan, Iran for helping to carry out a part of the research project including cancer induction, treatment of patient samples, sampling, and some molecular and biochemical tests in the biochemical laboratory of the Faculty of Veterinary Medicine of this university under Dr. Mohammad Reza Tabandeh from May 22, 2023, to February 20, 2024. The authors also extend their gratitude to the European Commission for support within the framework of the Erasmus+ FOOD4S Programme (Erasmus Mundus Joint Master Degree in Food Systems Engineering, Technology, and Business 619864-EPP-1-2020-1-BE-EPPKA1-JMD-MOB). Furthermore, we would like to thank the Faculty of Agriculture at Cairo University for providing support for some experiments conducted in their laboratory.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jff.2024.106626.

Data availability

Data will be made available on request.

References

Abdel Fattah, M. E., Sobhy, H. M., Reda, A., & Abdelrazek, H. M. (2020). Hepatoprotective effect of *Moringa oleifera* leaves aquatic extract against lead acetate–induced liver injury in male Wistar rats. *Environmental Science and Pollution Research, 27*, 43028–43043.

Abd-Elnaby, Y., ElSayed, I., AbdEldaim, M., Badr, E., Abdelhafez, M., & Elmadbouh, I. (2022b). Anti-inflammatory and antioxidant effect of *Moringa oleifera* against bisphenol-a-induced hepatotoxicity. *Egyptian Liver Journal, 12*(1). https://doi.org/10.1186/s43066-022-00219-7

Abd-Elnaby, Y. A., ElSayed, I. E., AbdEldaim, M. A., Badr, E. A., Abdelhafez, M. M., & Elmadbouh, I. (2022a). Anti-inflammatory and antioxidant effect of Moringa oleifera against bisphenol-A-induced hepatotoxicity. *Egyptian Liver Journal, 12*(1), 57.

Alansari, B. M. (2020). Development of antioxidant activity of potato starch edible films incorporate with rosemary Rosmarinus officinalis L. oil and used it in packaging beef Pattis. *Basrah journal of. Agricultural Sciences, 33*(2), 67–79.

Al-Temimi, W. K., Al-Garory, N. H., & Khalaf, A. A. (2020). Diagnose the bioactive compounds in flaxseed extract and its oil and use their mixture as an antioxidant. *Basrah Journal of Agricultural Sciences, 33*(1), 172–188.

Aly, O., Abouelfadl, D., Shaker, O., Hegazy, G., Fayez, A., & Zaki, H. (2020). Hepatoprotective effect of *Moringa oleifera* extract on tnf-α and tgf-β expression in acetaminophen-induced liver fibrosis in rats. *Egyptian Journal of Medical Human Genetics, 21*(1). https://doi.org/10.1186/s43042-020-00106-z

Amiri, R., Tabandeh, M. R., & Hosseini, S. A. (2021). Novel cardioprotective effect of L-carnitine on obese diabetic mice: Regulation of chemerin and CMKLRI expression in heart and adipose tissues. *Arquivos Brasileiros de Cardiologia, 117*, 715–725.

Anyene, I., Ergas, I. J., Kwan, M. L., Roh, J. M., Ambrosone, C. B., Kushi, L. H., & Feliciano, E. M. C. (2021). Plant-based dietary patterns and breast cancer recurrence and survival in the pathways study. *Nutrients, 13*(10), 3374. https://doi.org/10.3390/nu13103374

Barbier, L., Ferhat, M., Salamé, E., Robin, A., Herbelin, A., Gombert, J. M., & Barbarin, A. (2019). Interleukin-1 family cytokines: Keystones in liver inflammatory diseases. *Frontiers in Immunology, 10*, 2014.

Benzie, I. F., & Strain, J. J. (1999). *[2] ferric reducing/antioxidant power assay: Direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. In methods in enzymology (Vol. 299, pp. 15-27). Academic press*.

Cheon, M., Chung, M. S., & Park, Y. (2021). Association between dietary intake of flavonoids and cancer recurrence among breast cancer survivors. *Nutrients, 13*(9), 3049. https://doi.org/10.3390/nu13093049

Damodar, G., Smitha, T., Gopinath, S., Vijayakumar, S., & Rao, Y. A. (2014). An evaluation of hepatotoxicity in breast cancer patients receiving injection doxorubicin. *Annals of Medical and Health Sciences Research, 4*(1), 74–79.

Darweish, M., GabAllh, M., El-mashad, A. E., Moustafa, S., & Amin, A. S. (2021). Ameliorative effect of *Moringa oleifera* and rosemary ethanolic extracts on thioacetamide-induced liver fibrosis in rats. *Kafrelsheikh Veterinary Medical Journal, 19*(1), 34–40. https://doi.org/10.21608/kvmj.2021.85579.1021

Erel, O. (2005). A new automated colorimetric method for measuring total oxidant status. *Clinical Biochemistry, 38*(12), 1103–1111.

Gulcin, İ., & Alwasel, S. H. (2022). Metal ions, metal chelators and metal chelating assay as antioxidant method. *Processes, 10*(1), 132.

Hamza, A. A. (2010). Ameliorative effects of *Moringa oleifera* lam seed extract on liver fibrosis in rats. *Food and Chemical Toxicology, 48*(1), 345–355.

Hussain, M. A., Abogresha, N. M., Kader, G. A., Hassan, R., Abdelaziz, E. Z., & Greish, S. M. (2021). Antioxidant and anti-inflammatory effects of crocin ameliorate doxorubicin-induced nephrotoxicity in rats. *Oxidative Medicine and Cellular Longevity, 2021*, 1–12. https://doi.org/10.1155/2021/8841726

Jin, X., Wei, Y., Liu, Y., Lu, X., Ding, F., Wang, J., & Yang, S. (2019). Resveratrol promotes sensitization to doxorubicin by inhibiting epithelial-mesenchymal transition and modulating SIRT1/β-catenin signaling pathway in breast cancer. *Cancer Medicine, 8*(3), 1246–1257. https://doi.org/10.1002/cam4.1993

Kastl, L., Sauer, S. W., Ruppert, T., Beissbarth, T., Becker, M. S., Süss, D., & Gülow, K. (2014). TNF-α mediates mitochondrial uncoupling and enhances ROS-dependent cell migration via NF-κB activation in liver cells. *FEBS Letters, 588*(1), 175–183.

Kleiner, D. E., Chalasani, N. P., Lee, W. M., Fontana, R. J., Bonkovsky, H. L., Watkins, P. B., … (DILIN).. (2014). Hepatic histological findings in suspected drug-induced liver injury: Systematic evaluation and clinical associations. *Hepatology, 59*(2), 661–670.

Krishna, M. (2017). Patterns of necrosis in liver disease. *Clinical liver disease, 10*(2), 53–56.

Krstonošić, M. A., Hogervorst, J. C., Mikulić, M., & Gojković-Bukarica, L. (2020). Development of HPLC method for determination of phenolic compounds on a core shell column by direct injection of wine samples. *Acta Chromatographica, 32*(2), 134–138.

Kumar, K. P., Reddy, V. R., Prakash, M. G., & Kumar, K. P. (2018). In vitro estimation of total phenolics and DPPH radical scavenging activity of Withania somnifera extract. *Pharma Innov J, 7*(3), 588–590.

Lakshmi, S. P., Reddy, A. T., Kodidhela, L. D., & Varadacharyulu, N. C. (2020). The tea catechin epigallocatechin gallate inhibits NF-κB-mediated transcriptional activation by covalent modification. *Archives of Biochemistry and Biophysics, 695*, Article 108620.

Lang, Y., Gao, N., Zang, Z., Meng, X., Lin, Y., Yang, S., & Li, B. (2024). Classification and antioxidant assays of polyphenols: A review. *Journal of Future Foods, 4*(3), 193–204.

Lehuédé, C., Li, X., Dauvillier, S., Vaysse, C., Franchet, C., Clement, E., & Muller, C. (2019). Adipocytes promote breast cancer resistance to chemotherapy, a process amplified by obesity: Role of the major vault protein (mvp). *Breast Cancer Research, 21*(1). https://doi.org/10.1186/s13058-018-1088-6

Lorenzo, J. M., & Munekata, P. E. S. (2016). Phenolic compounds of green tea: Health benefits and technological application in food. *Asian Pacific Journal of Tropical Biomedicine, 6*(8), 709–719.

Modesto, P. N., Polegato, B. F., Dos Santos, P. P., Grassi, L. D., Molina, L. C., Bazan, S. G., & Azevedo, P. S. (2021). Green tea (Camellia sinensis) extract increased topoisomerase IIβ, improved antioxidant defense, and attenuated cardiac remodeling in an acute doxorubicin toxicity model. *Oxidative Medicine and Cellular Longevity, 2021*(1), 8898919.

Molina-Holgado, E., Ortiz, S., Molina-Holgado, F., & Guaza, C. (2000). Induction of COX-2 and PGE2 biosynthesis by IL-1β is mediated by PKC and mitogen-activated protein kinases in murine astrocytes. *British Journal of Pharmacology, 131*(1), 152–159.

Mostafa-Hedeab, G., Hassan, M. E., & Halawa, T. F. (2022). Epigallocatechin gallate ameliorates tetrahydrochloride-induced liver toxicity in rats via inhibition of TGFβ/p-ERK/p-Smad1/2 signaling, antioxidant, anti-inflammatory activity. *Saudi Pharmaceutical Journal, 30*(9), 1293–1300.

Musial, C., Kuban-Jankowska, A., & Gorska-Ponikowska, M. (2020). Beneficial properties of green tea catechins. *International Journal of Molecular Sciences, 21*(5), 1744.

Na, H. K., & Surh, Y. J. (2008). Modulation of Nrf2-mediated antioxidant and detoxifying enzyme induction by the green tea polyphenol EGCG. *Food and Chemical Toxicology, 46*(4), 1271–1278.

Ntshambiwa, K. T., Seifu, E., & Mokhawa, G. (2023). Nutritional composition, bioactive components and antioxidant activity of *Moringa oleifera* stenopetala and *Moringa oleifera* leaves grown in Gaborone, Botswana. *Food Production, Processing and Nutrition, 5*(1), 7.

Obayuwana, E., Imafidon, E. O., Odiase, D. E., Alih, O. J., Nweke, S. M., Enoghase, R. J., & Okpako, R. A. (2022). Effects of aqueous extract of *Moringa oleifera* on phenylhydrazine-induced liver toxicity in wistar rats. *Journal of Applied Sciences and Environmental Management, 26*(5), 949–954.

Ohgitani, E., Shin-Ya, M., Ichitani, M., Kobayashi, M., Takihara, T., Kawamoto, M., & Mazda, O. (2021). Significant inactivation of sars-cov-2 in vitro by a green tea catechin, a catechin-derivative, and black tea galloylated theaflavins. *Molecules, 26*(12), 3572. https://doi.org/10.3390/molecules26123572

Özgen, Z. E., Erdinç, M., Kelle, İ., Erdinç, L., & Nergız, Y. (2022). Protective effects of necrostatin-1 on doxorubicin-induced cardiotoxicity in rat heart. Human & experimental. *Toxicology, 41*, Article 096032712110660. https://doi.org/10.1177/09603271211066066

Pandey, B., & Rajbhandari, M. (2014). Estimation of total phenolic and flavonoid contents in some medicinal plants and their antioxidant activities. *Nepal Journal of Science and Technology, 15*(1), 53–60.

Park, H. J., Lee, J. Y., Chung, M. Y., Park, Y. K., Bower, A. M., Koo, S. I., & Bruno, R. S. (2012). Green tea extract suppresses NFκB activation and inflammatory responses in diet-induced obese rats with nonalcoholic Steatohepatitis3. *The Journal of Nutrition, 142*(1), 57–63.

Patintingan, C. G., Louisa, M., Juniantito, V., Arozal, W., Hanifah, S., Wanandi, S. I., & Thandavarayan, R. (2023). *Moringa oleifera* leaves extract ameliorates doxorubicin-induced cardiotoxicity via its mitochondrial biogenesis modulatory activity in rats. *Journal of Experimental Pharmacology*, 307–319.

Pękal, A., Dróżdż, P., Biesaga, M., & Pyrzynska, K. (2012). Screening of the antioxidant properties and polyphenol composition of aromatised green tea infusions. *Journal of the Science of Food and Agriculture, 92*(11), 2244–2249.

Peñalver, R., Martínez-Zamora, L., Lorenzo, J. M., Ros, G., & Nieto, G. (2022). Nutritional and antioxidant properties of *Moringa oleifera* leaves in functional foods. *Foods, 11*(8), 1107.

Phuyal, N., Jha, P. K., Raturi, P. P., & Rajbhandary, S. (2020). Total phenolic, flavonoid contents, and antioxidant activities of fruit, seed, and bark extracts of Zanthoxylum armatum DC. *The Scientific World Journal, 2020*(1), 8780704.

Rajaratinam, H., Rasudin, N. S., Safuan, S., Abdullah, N. A., Mokhtar, N. F., & Fuad, W. E. M. (2022). Passage number of 4T1 cells influences the development of tumour and the progression of metastasis in 4T1 orthotopic mice. *The Malaysian Journal of Medical Sciences: MJMS, 29*(3), 30.

Renu, K., Abilash, V. G. P. B. T. P., & Arunachalam, S. (2018). Molecular mechanism of doxorubicin-induced cardiomyopathy–an update. *European Journal of Pharmacology, 818*, 241–253.

Sauter, B. V., Martinet, O., Zhang, W. J., Mandeli, J., & Woo, S. L. (2000). Adenovirus-mediated gene transfer of endostatin in vivo results in high level of transgene expression and inhibition of tumor growth and metastases. *Proceedings of the National Academy of Sciences, 97*(9), 4802–4807.

Senanayake, S. N. (2013). Green tea extract: Chemistry, antioxidant properties and food applications–a review. *Journal of Functional Foods, 5*(4), 1529–1541.

Shareef, S., Ibrahim, I., Alzahrani, A., Al-Medhtiy, M., & Abdulla, M. (2022). Hepatoprotective effects of methanolic extract of green tea against thioacetamide-induced liver injury in Sprague dawley rats. *Saudi Journal of Biological Sciences, 29*(1), 564–573. https://doi.org/10.1016/j.sjbs.2021.09.023

Singh, A., Yau, Y. F., Leung, K. S., El-Nezami, H., & Lee, J. C. Y. (2020). Interaction of polyphenols as antioxidant and anti-inflammatory compounds in brain–liver–gut axis. *Antioxidants, 9*(8), 669.

Sojoodi, M., Wei, L., Erstad, D., Yamada, S., Fujii, T., Hirschfield, H., & Fuchs, B. (2020). Epigallocatechin gallate induces hepatic stellate cell senescence and attenuates development of hepatocellular carcinoma. *Cancer Prevention Research, 13*(6), 497–508. https://doi.org/10.1158/1940-6207.capr-19-0383

Sreelatha, S., & Padma, P. R. (2009). Antioxidant activity and total phenolic content of *Moringa oleifera* leaves in two stages of maturity. *Plant Foods for Human Nutrition, 64*, 303–311.

Tabandeh, M. R., Jozaie, S., Ghotbedin, Z., & Gorani, S. (2022). Dimethyl itaconic acid improves viability and steroidogenesis and suppresses cytokine production in LPS-treated bovine ovarian granulosa cells by regulating TLR4/nfkβ, NLRP3, JNK signaling pathways. *Research in Veterinary Science, 152*, 89–98.

Tak, E., Park, G. C., Kim, S. H., Jun, D. Y., Lee, J., Hwang, S., & Lee, S. G. (2016). Epigallocatechin-3-gallate protects against hepatic ischaemia–reperfusion injury by reducing oxidative stress and apoptotic cell death. *Journal of International Medical Research, 44*(6), 1248–1262.

Teibo, J., Bello, S., Olagunju, A., Olorunfemi, F., Ajao, O., & Fabunmi, O. (2020). Functional foods and bioactive compounds: Roles in the prevention, treatment and management of eurodegenerative diseases. *GSC Biological and Pharmaceutical Sciences, 11*(2), 297–313. https://doi.org/10.30574/gscbps.2020.11.2.0143

Tian, Z., Yang, Y., Yang, Y., Zhang, F., Li, P., Wang, J., & Wang, X. (2020). High cumulative doxorubicin dose for advanced soft tissue sarcoma. *BMC Cancer, 20*(1). https://doi.org/10.1186/s12885-020-07663-x

Tormo, E., Ballester, S., Adam-Artigues, A., Burgues, O., Alonso, E., Bermejo, B., & Eroles, P. (2019). The mirna-449 family mediates doxorubicin resistance in triple-negative breast cancer by regulating cell cycle factors. *Scientific Reports, 9*(1). https://doi.org/10.1038/s41598-019-41472-y

Urschel, K., & Cicha, I. (2015). TNF-α in the cardiovascular system: From physiology to therapy. *International Journal of Interferon, Cytokine and Mediator Research*, 9–25.

Védékoi, J., Selestin, S. D., Abdoulaye, H., Justin, K., Djamilah, Z., & Pierre, K. (2019). Investigation of antioxidant activity of the ethanol extract of the resin exudates of trunk bark of Boswellia dalzielii hutch (Burseraceae). *Journal of Materials and Environmental Science, 10*, 1413–1419.

Wu, Z., Sun, L., Chen, R., Wen, S., Li, Q., Lai, X., & Sun, S. (2022). Chinese tea alleviates ccl4-induced liver injury through the nf-κbornrf2signaling pathway in c57bl-6j mice. *Nutrients, 14*(5), 972. https://doi.org/10.3390/nu14050972

Xu, J., Yan, B., Zhang, L., Zhou, L., Zhang, J., Yu, W., & Shan, L. (2020). <p>theabrownin induces apoptosis and tumor inhibition of hepatocellular

carcinoma huh7 cells through ask1-jnk-c-jun pathway</p&gt. *Oncotargets and Therapy, 13*, 8977–8987. https://doi.org/10.2147/ott.s254693

Zeiss, C. J., Gatti, D. M., Toro-Salazar, O., Davis, C., Lutz, C. M., Spinale, F., … Churchill, G. A. (2019). *Doxorubicin-induced cardiotoxicity in collaborative cross (CC) mice recapitulates individual cardiotoxicity in humans. G3 (Bethesda, Md.), 9(8), 2637–2646*. https://doi.org/10.1534/g3.119.400232

Zhang, Y., Qu, X., Gao, H., Zhai, J., Tao, L., Sun, J., & Zhang, J. (2020). Quercetin attenuates NLRP3 inflammasome activation and apoptosis to protect INH-induced liver injury via regulating SIRT1 pathway. *International Immunopharmacology, 85*, Article 106634.