

Oleuropein attenuated docetaxel-induced liver and kidney toxicity in rats by modulating oxidative stress, gene expressions and histopathological damage

Metin Deniz Karakoc^{1*}, Ozlem Ozmen², Munevver Nazlıcan Zengin¹ and Osman Ciftci¹

¹Pamukkale University, Faculty of Medicine, Department of Medical Pharmacology, Denizli, Türkiye

²Burdur Mehmet Akif Ersoy University, Faculty of Veterinary Medicine, Department of Veterinary Pathology, Burdur, Türkiye

Abstract: Docetaxel (Dtx) is a frequently used antineoplastic agent despite its dose-limiting toxic effects. Our objective was to assess the effects of oleuropein (Ole), a natural polyphenol, on Dtx-induced toxicity. Thirty-two male rats were randomly assigned to four groups for a four-week treatment: Control (sham), Dtx (5 mg/kg weekly, i.p.), Ole (30 mg/kg daily, p.o.) and Dtx+Ole. Biochemical and gene expression analyses were performed on liver, kidney and blood samples. Additionally, histological and immunohistochemical evaluations were conducted on the liver and kidneys. Ole reduced the Dtx-induced oxidative stress index in tissues. In contrast to Dtx, it decreased caspase-3 and Bax gene expressions while increasing Bcl-2 expression. Furthermore, Ole improved the ALT, AST, urea and creatinine levels, which were impaired by Dtx administration. It also reduced serum IL-6, IL-1 β and TNF- α levels, which had been elevated due to Dtx. Histopathological and immunohistochemical examinations revealed that Ole administration mitigated Dtx-related damage in both tissues. These findings suggest that Ole might offer protection against Dtx-induced liver and kidney toxicity in rats.

Keywords: Docetaxel, oleuropein, oxidative stress, hepatotoxicity, nephrotoxicity.

Submitted on 06-06-2024 – Revised on 25-04-2025– Accepted on 25-04-2025

INTRODUCTION

Docetaxel (Dtx) is a commonly used intravenous antineoplastic agent for treating various solid tumors. It can be used alone or in combination with other antineoplastic agents for cancer chemotherapy (Cimbro *et al.*, 2024; Kida *et al.*, 2024; Ajdari *et al.*, 2024). Dtx hinders tumor cell growth by preventing the assembly of mitotic spindles, leading to G2/M cell cycle arrest. This process ultimately leads to apoptosis. Additionally, it promotes apoptosis by inducing the phosphorylation of B-cell lymphoma 2 (Bcl-2) (Yu *et al.*, 2020; Qian *et al.*, 2022). Dtx is predominantly metabolized in the liver via CYP450 (CYP) 3A4 to several metabolites that are all considered to be therapeutically less effective. On the other hand, severe dose-limiting toxicities, including hepatotoxicity, neutropenia, neuropathy, anaemia and nephrotoxicity, may occur even at its therapeutic dose range (Cimbro *et al.*, 2024; Kida *et al.*, 2024; Beheshtizadeh *et al.*, 2024). These toxic effects may lead to a dose reduction, delay in the chemotherapy schedule, or early termination of the drug treatment. Therefore, there is a critical need to develop complementary approaches to reduce Dtx-induced toxicity in order to enhance the success of chemotherapy and improve patients' quality of life.

In recent years, numerous studies have concentrated on utilizing plant-derived polyphenolic compounds to alleviate chemotherapy-induced toxicity. Oleuropein (Ole) is the primary polyphenolic substance in the olive tree

(*Olea europaea* L.). Olive fruits and extra virgin olive oil are important components of the Mediterranean diet. This non-toxic secoiridoid exhibits numerous pharmacological effects, such as anti-inflammatory, antioxidant and anticancer activities, by modulating various mechanisms (Ahmad Farooqi *et al.*, 2017). There are several studies on the health benefits of Ole and its protective effects on the organotoxicity induced by xenobiotics (Mahmoudi *et al.*, 2015; Yu *et al.*, 2016; Potočnjak *et al.*, 2016; Geyikoglu *et al.*, 2017; Koc *et al.*, 2019; Jemai *et al.*, 2020; Karakoç and Sekkin, 2021). Furthermore, a study reported that Ole demonstrated a synergistic antitumor effect with anthracycline-based cytotoxic chemotherapy in BALB/c mice with breast tumors (Elamin *et al.*, 2019). On the other hand, the potential protective efficiency of this polyphenol against Dtx-induced toxicity remains to be elucidated. Therefore, our study aimed to investigate the effects of Ole on Dtx-induced hepatic and renal damage.

MATERIALS AND METHODS

Chemicals

Dtx (Doxel Ready[®]) was obtained from Gensenta İlaç San. A.Ş. (Istanbul, Türkiye). Ole (CAS: 32619-42-4; purity >98%) was purchased from Santa Cruz Biotechnology Inc. (California, USA). All other chemicals employed in the study were of analytical grade and were used as received without further purification.

Animals and ethics

The Local Ethics Committee of Pamukkale University approved the experimental protocol (protocol number:

*Corresponding author: e-mail: mdkarakoc@pau.edu.tr

PAUHDEK-2023/05). Thirty-two healthy adult male Sprague-Dawley rats, 3 months old and weighing 220-250 g, were used for the experiment. Rats were sourced from the Pamukkale University Experimental Research Center (Denizli, Türkiye) and were kept under standard housing and feeding conditions throughout the study. Water was given *ad libitum*.

Experimental design and drug treatment.

The rats were randomly assigned to four groups, each containing eight animals; 1: Control, 2: Dtx, 3: Ole, 4: Dtx+Ole. The body weights of the rats were monitored daily. Ole was suspended in distilled water (DW) before each oral administration. The control group rats were administered 0.2 ml of 0.9% NaCl solution intraperitoneally (i.p.) once a week and they received 1 ml of DW by oral gavage (p.o.) per day for 4 weeks (approximately equal drug volumes to those in other groups). Dtx group animals were administered 5 mg/kg of Dtx (i.p.) once a week and 1 ml of DW (p.o.) per day for 4 weeks. Ole group were administered 30 mg/kg of Ole (p.o.) per day and 0.2 ml of 0.9% NaCl solution (i.p.) once a week for 4 weeks. The rats in the Dtx+Ole group were administered 5 mg/kg Dtx (i.p.) once a week and 30 mg/kg Ole (p.o.) per day for 4 weeks. The Dtx dosage was based on previous research (Kim *et al.*, 2018). Dtx is administered at doses ranging from 60 to 100 mg/m² every 21 days in monotherapy. Therefore, the weekly dose preferred in rats falls within the range of human dosing. On the other hand, there is no standard dose for Ole. Hence, the minimum daily oral dose reported to exhibit an exact pharmacological effect in previous studies has been preferred (Janahmadi *et al.*, 2015; Sherif *et al.*, 2016). All animals survived during the experiment and were then sacrificed under anesthesia (50 mg/kg ketamine, i.p. and 5 mg/kg xylazine, i.p.) for sample collection. Blood samples were taken by cardiac puncture and serum was separated for biochemical analysis. The livers and kidneys were promptly excised for reverse transcription quantitative real-time polymerase chain reaction (RT-qPCR), ELISA and histopathological-immunohistochemical analysis.

Preparation of tissue homogenates

Left kidney and liver samples were homogenized in a phosphate buffer (pH 7.4) at 2000 rpm for 1 minute, then centrifuged at 12,000 rpm and 4°C for 10 minutes. The supernatants were collected and immediately analyzed.

Gene expression analysis

Throughout the process from total RNA isolation to gene expression analysis, Trizol reagent (HibriGen Biotechnology, Türkiye), complementary DNA (cDNA) synthesis kit and 2X qPCR MasterMix kits (A.B.T. Laboratories, Türkiye) were used according to the manufacturers' instructions. RNA purity was measured using a Nano Drop ND-2000C spectrophotometer (Thermo Fisher Scientific Co., USA). mRNA expression was

determined using StepOnePlus® RT-qPCR (Applied Biosystems, Massachusetts, USA). The conditions for RT-qPCR were as follows: initial denaturation at 95°C for 10 min, followed by 40 cycles of denaturation at 95°C for 30 s and annealing at 60°C for 60 s. β -Actin served as the reference housekeeping gene for analyzing the mRNA expressions of Bcl-2, Bcl-2 associated X protein (Bax) and caspase-3 (Casp3). Sequences of primers are presented in Table 1. Experiments were conducted in triplicate. In analyzing RT-qPCR data, quantification was carried out using the $\Delta\Delta$ CT method available on the Gene Globe platform (<https://geneglobe.qiagen.com/us/analyze>).

Biochemical analysis

Serum obtained by centrifuging whole blood was used to determine certain biochemical parameters associated with liver and kidney damage, such as urea, uric acid, creatinine, AST, ALT and GGT. Analysis was performed within two hours using a Roche® Cobas c501 auto analyzer (Roche Diagnostics, Switzerland).

Liver CYP3A1, the total oxidant status (TOS) and total antioxidant status (TAS) of liver and kidney tissues and serum levels of TNF- α , IL-1 β and IL-6 were measured using commercial ELISA kits [BT LAB (E1828Ra), China; Elabscience Laboratories (E-BC-K802-M and E-BC-K801-M), USA; Thermo Fisher Scientific Co., (ERA56RB, BMS630 and ERA31RB), USA, respectively] following the manufacturers' instructions. The oxidative stress index (OSI) was determined by TOS/TAS formula. The results were presented in arbitrary units (Erel, 2005).

Histopathological analysis

Liver and right kidney tissues were fixed in 10% neutral formalin. Then, the samples were subjected to an automatic tissue processor (Leica ASP300S, Germany), embedded in paraffin and 5- μ m sections were cut using a rotary microtome (Leica RM2155, Germany). The resulting sections were stained with hematoxylin-eosin (H-E) and examined under a light microscope. Rat liver tissues were stained with H-E to determine the presence and degree of steatosis, inflammation, necrosis and fibrosis, which are histological changes. The lesions were combined from previous studies and graded according to the criteria listed in Table 2 (Veteläinen *et al.*, 2006; Thoolen *et al.*, 2010; Frazier *et al.*, 2012).

Immunohistochemical analysis

Three series of slices from each block of the tissues were cut and drawn on poly-L-lysine coated slides for immunohistochemical examination. According to the manufacturer's instructions, the sections were stained for the expression of Casp3 (using Anti-Caspase-3 p12 antibody [EPR16888] (ab179517), Bax (using Anti-Bax antibody [E63] (ab32503) and Bcl-2 (using Anti-Bcl-2 antibody [ab196495]). Immunohistochemistry was performed on tissue sections using a streptavidin-alkaline

phosphatase conjugate and a biotinylated secondary antibody, following a 60-minute incubation with primary antibodies diluted 1:100. The EXPOSE Mouse and Rabbit Specific HRP/DAB Detection IHC Kit (Catalog no: ab80436, Abcam Ltd., UK) was used for detection, with diaminobenzidine (DAB) as the chromogen. Antigen dilution solution served as a negative control in place of primary antibodies. Each blinded sample was examined by a trained pathologist from a different university.

Each antibody was tested on separate tissue sections for immunohistochemical analysis. At 40x magnification, the percentage of positively stained cells was assessed at ten distinct locations on each slide across all groups. The ImageJ program (Bethesda, MD, version 1.48) was used to tally the results. Images were first cropped, split into color channels and any artifacts were removed. Cells were selected and counted using the software's tools, with only those showing a clear brown stain being considered positive. Morphometric analyses were conducted using the Olympus CX41 microscope and Axios camera and microphotography was performed with the Cell Sens Life Science Imaging Software (Olympus Co., Japan).

STATISTICAL ANALYSIS

Statistical analysis was conducted with SPSS 29.0 (SPSS Inc., Chicago, IL, USA). Data are presented as mean $\pm$ standard error (mean $\pm$ S.E.). The Shapiro-Wilk test assessed data normality, while Levene's test evaluated variance homogeneity. One-way ANOVA was used to compare group differences, with significance determined through the post hoc Tukey test. Paired samples t-tests analyzed changes in body weight between initial and final measurements. A significance level of $p < 0.05$ was established.

RESULTS

Body weights

At the start of the study, no statistically significant differences in weight were observed between the experimental groups ($p > 0.05$). However, at the end of the study, significant weight gains were observed in the control, Ole and Dtx+Ole groups compared to the initial weights ($p = 0.003$, $p = 0.001$ and $p = 0.027$, respectively). On the other hand, there was no significant difference in weight between the beginning and the end in the Dtx group ($p > 0.05$). The distribution of body weight measurements is provided in Table 3.

Gene expressions

Dtx caused a significant increase in the expression of pro-apoptotic genes in hepatic and renal tissues, while leading to a decrease in anti-apoptotic gene expression. In the liver tissue, the expressions of Bax and Casp3 increased approximately equally following Dtx application.

However, in the kidney tissue, the expression of Casp3 increased more than that of Bax. The Ole treatment, however, reversed this effect. Ole was found to increase Bcl-2 expression while decreasing Bax and Casp3 expression. Table 4 presents the results of fold changes for the examined genes in the experimental groups, comparing them with the control group.

Serum biochemical parameters

Biochemical analyses revealed no significant differences in GGT and uric acid parameters among the experimental groups ($p > 0.05$). On the other hand, it was determined that serum levels of TNF- α , IL-1 β , IL-6, ALT, AST, urea and creatinine significantly increased in the Dtx group compared to the control group ($p = 0.015$ for creatinine levels and $p < 0.001$ for the others). Ole co-administration mitigated all inflammation-related parameters. No significant differences were observed in these parameters between the control group and the Ole-treated groups ($p > 0.05$).

Administering Dtx led to a significant increase in serum levels of all cytokines compared to the control group ($p < 0.001$ for each parameter). Ole administration resulted in a significant decrease in cytokine levels in Dtx-treated rats. The differences between the Dtx+Ole and Dtx groups were found to be statistically significant ($p < 0.001$, $p = 0.002$ and $p < 0.001$, respectively). Additionally, it was found that there were no significant differences in TNF- α , IL-1 β and IL-6 levels between the Control, Ole and Dtx+Ole groups ($p > 0.05$). The results of the serum biochemical parameters are detailed in Table 5.

CYP3A1 levels

The Dtx treatment caused a significant increase in CYP3A1 expression in the liver tissues ($p = 0.01$). On the other hand, Ole treatment had no notable effect on the expression of this enzyme ($p > 0.05$). The mean CYP3A1 enzyme levels of the groups are presented in Fig. 1.

OSI

The mean renal and hepatic TOS and TAS levels of experimental groups are presented in Table 6. The results show that Dtx significantly increased the OSI in both the liver and kidney, but Ole successfully restored the Dtx-induced OSI to normal levels. The distribution of experimental groups in terms of OSI in tissues is presented in Fig. 2.

Histopathology and immunohistochemical findings

The Control and Ole groups showed normal liver tissue architecture during the histopathological investigation. The Dtx group had noticeable pathological changes including hyperemia, hepatocyte destruction, steatosis, minor inflammation, small necrotic regions and slight fibrosis, which was mostly restricted to the centrilobular vein. The Dtx-related findings were mitigated by the Ole treatment in the Dtx+Ole group. Histopathological findings of livers between the groups are presented in Fig. 3.

Immunohistochemical findings revealed negative to very slight Casp3 and Bax expressions in the Control and Ole groups. Dtx application slightly or moderately increased the expressions in hepatocytes. Ole treatment decreased the Casp3 and Bax expressions in the Dtx+Ole group. Casp3, Bax and Bcl-2 expressions of experimental groups in hepatic tissues are presented in Fig. 4, 5 and 6, respectively. The results of immunohistochemical scores of the liver in experimental groups are presented in Table 7. The findings from this study indicate that Dtx induces liver damage, while Ole exhibits healing effects on Dtx-induced lesions.

The kidney sections from the Dtx group that underwent histological examination revealed considerable hyperemia, small to moderate hemorrhages, tubular epithelial cell degeneration, protein droplets in the proximal tubules, proteinaceous materials in the tubular lumens and infiltration of inflammatory cells. Ole therapy reduced the pathological results in the Dtx+Ole group. The Control and Ole groups had normal tissue histology. Histopathological findings of renal tissues between the groups are presented in Fig.7.

During the immunohistochemical examination, higher expressions of Casp3 and Bax were found in the tubular epithelial cells in the Dtx group. Following Ole therapy, the expressions decreased in the Dtx+Ole group. Only very mild or unfavorable expressions were observed in the Ole and Control groups. Conversely, Bcl-2 expressions were considerably higher in these groups compared to the Dtx group. It was also determined that Ole administration significantly elevated the Bcl-2 expressions in Dtx treatment. The immunohistochemical findings demonstrated that Dtx damages the kidneys, while Ole therapy improved the problematic findings. Casp3, Bax, and Bcl-2 expressions of experimental groups in renal tissues are presented in Fig. 8, 9 and 10, respectively. Results of immunohistochemical scores of liver in experimental groups are presented in Table 8.

DISCUSSION

Dtx is a widely used anticancer drug that has been in clinical practice for over two decades. However, it can cause serious cellular toxicity, including hepatotoxicity and nephrotoxicity (Baş and Nazıroğlu, 2019; Beheshtizadeh *et al.*, 2024). Previous studies have reported that these drug-related toxic effects are due to an imbalance in oxidative stress and DNA mutations (Tang *et al.*, 2017; Cimbro *et al.*, 2024; Saito *et al.*, 2024). Thus, we evaluated the impact of Ole on dose-limiting toxicities from molecular, biochemical, and histopathological perspectives. To the best of our knowledge, our study is the first to evaluate the effects of Ole on Dtx-induced toxicity, representing toxicity caused by a taxane derivative. The findings of this study may contribute significantly to the development of safer and more effective strategies for managing side effects in cancer treatment.

Neutropenia, asthenia, nausea, vomiting, diarrhea and/or constipation are common side effects of Dtx (Hadrich *et al.*, 2016; Stoicescu and Cherecheanu, 2023). Over time, weight loss may occur due to these side effects. The results of our study showed that Ole administration prevented the weight loss caused by Dtx treatment and provided a slight weight gain in rats, although this gain was not statistically significant. The study demonstrates that Ole has the potential to prevent severe weight loss induced by Dtx. This result is consistent with our previous study, in which we evaluated the effects of Ole against the toxicity of an epirubicin and cyclophosphamide combination (Karakoç and Sekkin, 2021). In another study, it was reported that Ole (50 mg/kg/day, for 8 weeks, orally) prevented excessive weight gain in rats fed a fat-rich diet (Hadrich *et al.*, 2016). In a mouse model that received a high-fat diet, Ole supplementation was reported to reduce final body weight and minimize weight gain by increasing total body oxygen consumption, thermogenesis and plasma catecholamine secretion (Park *et al.*, 2011). Our results and previous studies indicate that Ole prevents excessive weight loss caused by antineoplastic drugs ($p < 0.05$) and has benefits in maintaining normal weight.

Oxidative stress may increase as a result of either excessive free radical production or diminished antioxidant capacity. The disruption of crucial biomolecules and cells due to this imbalance could have far-reaching effects on the entire organism (Jomova *et al.*, 2023; Hassan *et al.*, 2024). The mammalian body contains various oxidant and antioxidant molecules. Instead of measuring these molecules individually, the study measured TOS and TAS to determine the OSI (Erel, 2004; Erel, 2005). In the kidney tissues of the Dtx group, TOS levels were significantly elevated, while TAS levels were notably reduced compared to all other groups. As a result, the OSI in the kidney tissues of the Dtx group was significantly higher than in the other groups. Our study demonstrated that the administration of Ole effectively reduced the Dtx-induced OSI, bringing it to levels similar to those of the control group ($p < 0.05$). Dtx increases lipid peroxidation, reactive oxygen species (ROS), apoptosis and caspase enzyme activities in mice (Baş and Nazıroğlu, 2019). Previous studies have reported that Dtx induces oxidative stress in the liver by increasing certain oxidant parameters and decreasing the levels of antioxidant parameters (Tang *et al.*, 2017; Guo *et al.*, 2021). On the other hand, some studies have reported that Ole provides protective effects in the liver and kidney against toxicities induced by various substances by reducing the levels of oxidant parameters and increasing antioxidant parameters (Mahmoudi *et al.*, 2015; Karakoç and Sekkin, 2021). In another study evaluating the effects of Ole on kidney damage induced by cisplatin, it was reported that Ole treatment resulted in a decrease in OSI (Geyikoğlu *et al.*, 2017). Our results align with previous studies highlighting the oxidative stress-reducing effects of Ole in hepatic and renal tissues. The current study

demonstrates that Ole effectively reduces oxidative stress induced by Dtx in both tissues, with a more pronounced protective effect observed in the kidneys ($p < 0.05$).

Bcl-2 and Bax proteins are primarily located on the outer mitochondrial membrane. Bcl-2 acts as an anti-apoptotic protein, while Bax functions as a pro-apoptotic protein (Qian *et al.*, 2022). When activated, Bax translocates to the inner mitochondrial membrane, increasing its permeability and leading to the release of cytochrome c. These proteins then contribute to the formation of the apoptosome complex, initiating a cascade of cellular events that culminate in apoptosis (Zhang *et al.*, 2019; Perini *et al.*, 2021; Pravdic *et al.*, 2023). The apoptosome complex triggers the activation of caspase enzymes, which ultimately carry out the process of cell death. Casp3 serves as a central executioner in the apoptotic process. Its activation and subsequent proteolytic activity are crucial for the efficient execution of apoptosis (Han *et al.*, 2023; Tweedell *et al.*, 2024). Our study found that Dtx increased the expression of the Bax and Casp3 while decreasing the Bcl-2 in renal and hepatic tissues. However, Ole application counteracted this effect and suppressed apoptosis in the tissues. Our immunohistochemical findings regarding the expression of these three genes corroborate the RT-qPCR results. Various *in-vitro* and animal studies conducted with Ole and Dtx have previously reported that they affect the above-mentioned gene expression in a manner similar to our findings (Lopes *et al.*, 2014; Mohammadian *et al.*, 2016; Yu *et al.*, 2016; Dinda *et al.*, 2019; Xu *et al.*, 2022). In this context, the results of our study align with those of previous research. The reduction of oxidative stress through Ole in the liver and kidneys has led to a decrease in apoptotic gene expression, while triggering an increase in anti-apoptotic gene expression, thereby reducing cellular damage.

Increased levels of ROS are closely associated with chronic inflammation, making it a key factor in oxidative stress-induced cellular changes. The production of ROS has been linked to inflammation triggered by cytokines like IL-6 and TNF- α (Tu and Li, 2023; Nong *et al.*, 2024). In our study, we observed a significant increase in the levels of pro-inflammatory serum cytokines following Dtx treatment ($p < 0.05$). However, Ole administration significantly reduced inflammation by lowering the levels of pro-inflammatory cytokines to levels similar to those observed in the control group. In this study, Dtx was found to cause a significant increase in serum ALT and AST levels, important biomarkers for liver damage ($p < 0.05$), while Ole prevented the rise in these parameters. Additionally, no significant differences in ALT and AST levels were observed among the Control, Ole and Dtx+Ole groups. In contrast, GGT levels did not differ significantly among the experimental groups. An elevation in GGT is typically associated with damage to the bile ducts and liver cells near the bile ducts. Our results suggest that liver damage due to

Dtx toxicity was more pronounced in regions other than the bile ducts and their vicinity, but Ole effectively mitigated this damage. Histopathological examination of the liver further confirmed that Ole alleviated Dtx-induced damage in parameters such as steatosis, inflammation, necrosis and fibrosis, supporting our biochemical findings. Furthermore, previous studies using different toxicity inducers in rats and mice have demonstrated that Ole possesses hepatoprotective effects, consistent with our findings (Mahmoudi *et al.*, 2015; Jemai *et al.*, 2020; Karakoç and Sekkin, 2021).

The elevation of urea and creatinine levels are non-specific biochemical markers commonly associated with kidney toxicity (Gupta *et al.*, 2024; Pradeep *et al.*, 2024). In our study, we observed a significant increase in serum urea and creatinine levels due to Dtx treatment ($p < 0.05$). However, Ole administration significantly reduced these levels. The histopathological and immunohistochemical analyses of renal tissues further supported our biochemical findings. These results indicate that Ole has a renoprotective effect against Dtx-induced toxicity. Previous studies have demonstrated that Ole can ameliorate or prevent nephrotoxicity induced by various antineoplastic agents and other xenobiotics (Mahmoudi *et al.*, 2015; Potočnjak *et al.*, 2016; Geyikoğlu *et al.*, 2017; Koç *et al.*, 2019; Karakoç and Sekkin, 2021). In this context, the results of our study are consistent with the results of the mentioned studies.

The metabolism of Dtx is similar in both rats and humans (Kapelemera *et al.*, 2022). The CYP3A subfamily plays a crucial role in hepatic Dtx metabolism. The primary isoform responsible for this process in rats is CYP3A1, which exhibits significant nucleotide homology with its human counterpart, CYP3A4 (Kappler *et al.*, 2021; Rendic and Guengerich, 2024). There is limited information in the literature regarding the effects of Ole on CYP enzymes. It has been reported in previous studies that Ole inhibits CYP3A *in vitro* (Malliou *et al.*, 2021; Gervasi and Pojero, 2024). When a xenobiotic inhibits a CYP450 enzyme, the enzyme level in the body does not immediately decrease or increase. Enzyme activity may temporarily decline. However, the body attempts to compensate for this loss and over the long term, the enzyme amount can increase through mechanisms involving the upregulation of CYP450 enzyme gene expression and synthesis (Zhao *et al.*, 2021; Song *et al.*, 2021). After a period of time that cannot be considered as short as one month, any differences that may occur in enzyme levels could offer insights into the potential inhibition or activation that might be associated with Ole. In our study, to the best of our knowledge, this is the first investigation of the *in vivo* effects of Ole on CYP3A enzyme levels. At the end of the study, there was no significant difference in CYP3A1 levels between the Control, Dtx and Dtx+Ole groups ($p > 0.05$).

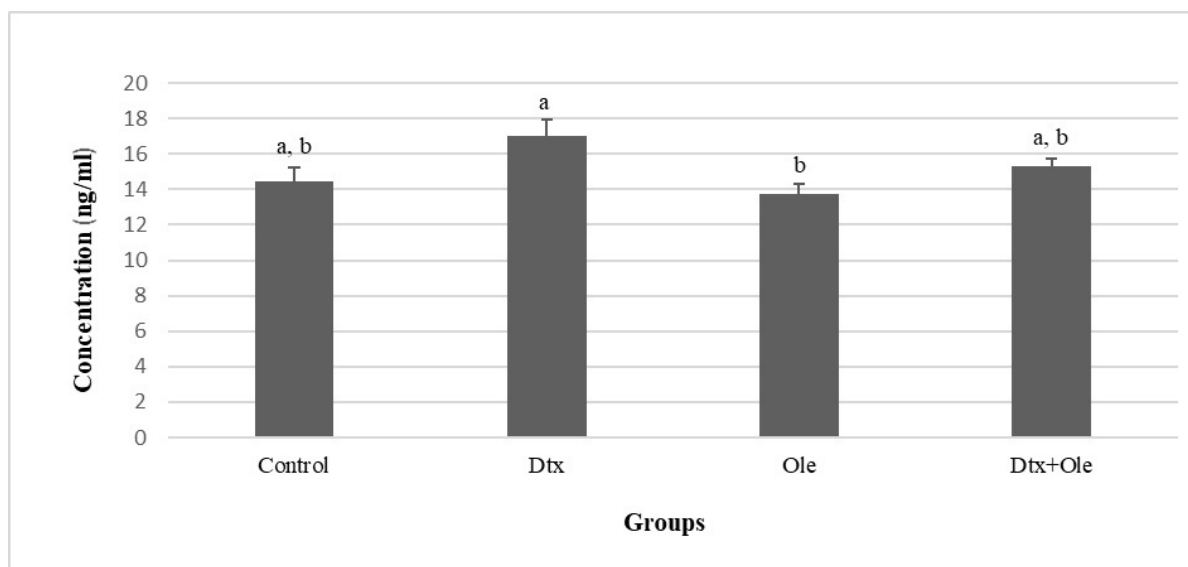


Fig. 1: Mean CYP3A1 enzyme levels of the liver tissues in experimental groups. ^{a, b} Different letters at the top of the columns indicates statistically significant difference ($p < 0.05$) with each other; Results are presented as mean $\pm$ S.E.; Dtx: Docetaxel, Ole: Oleuropein

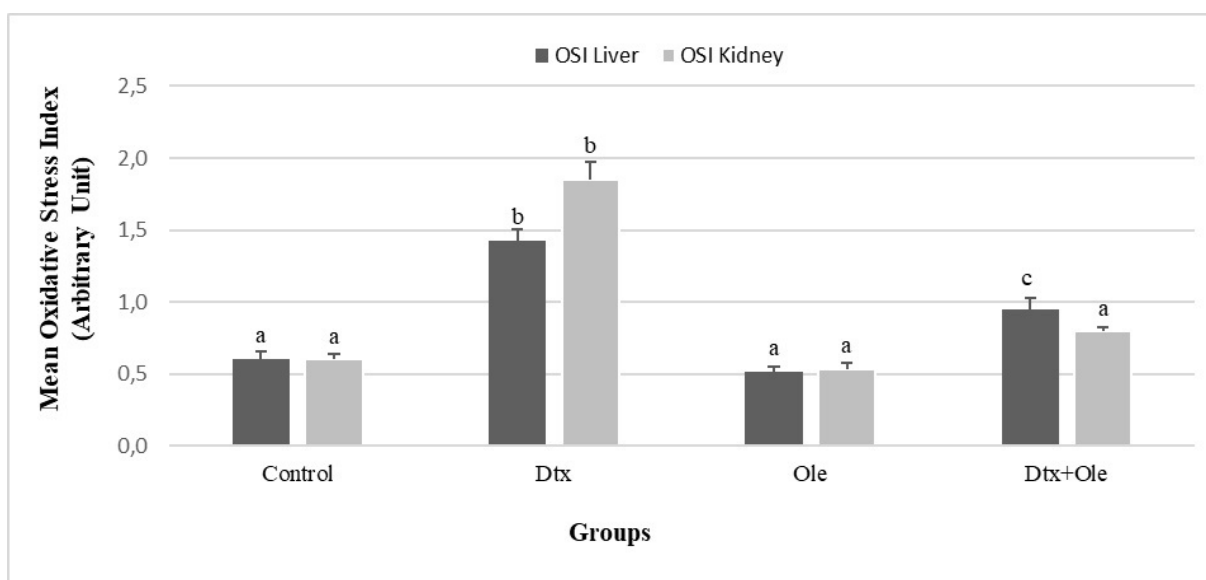


Fig. 2: Mean Oxidative Stress Index values of experimental groups. ^{a, b, c} Different letters at the top of the columns indicates statistically significant difference ($p < 0.05$) with each other; Result of each organ was evaluated on its own; Results are presented as mean $\pm$ S.E.; Dtx: Docetaxel; Ole: Oleuropein

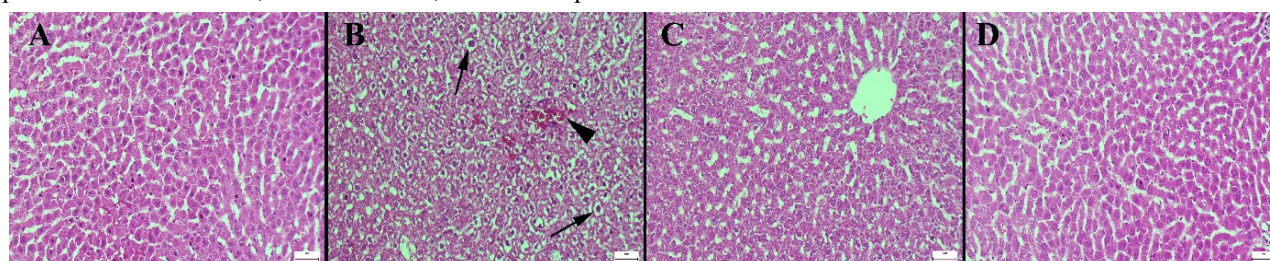


Fig. 3: Histopathological findings of livers between the groups. (A) Normal liver appearance in control group, (B) Hyperemia (arrow head) and steatosis in the hepatocytes (arrows) in Dtx group (C) Decreased liver damage in Dtx+Ole group, (D) Normal tissue architecture in Ole group, Dtx: Docetaxel; Ole: Oleuropein, H-E, scale bars=50µm.

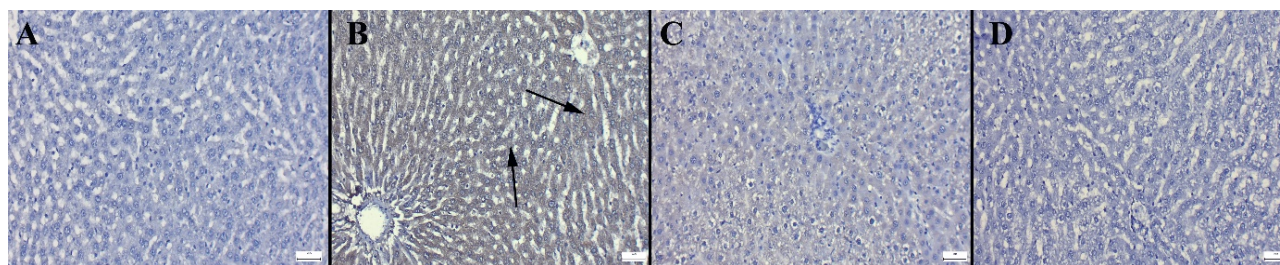


Fig. 4: Caspase-3 expressions between the groups in liver tissues. (A) Negative expression in control group. (B) Marked increase in expressions (arrows) in Dtx group. (C) Decreased expressions in Dtx+Ole group. (D) No expressions in Ole group, Dtx: Docetaxel; Ole: Oleuropein, Streptavidin biotin peroxidase method, scale bars=50µm.

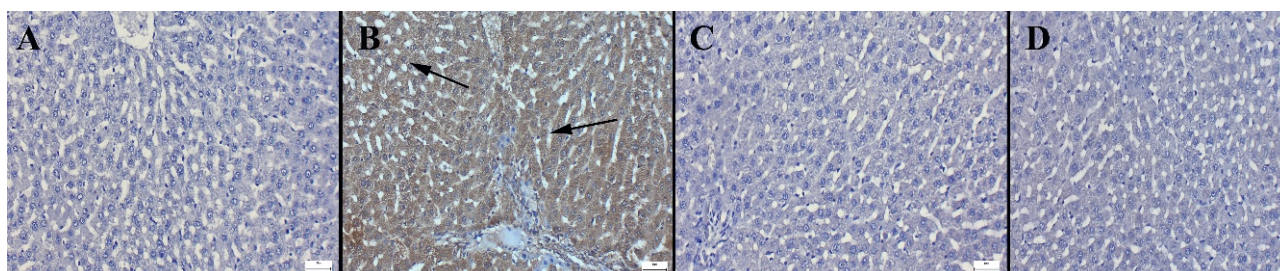


Fig. 5: Bax expressions between the groups in liver tissues. (A) No expression in control group. (B) Marked increase in expressions (arrows) in Dtx group. (C) Decreased expressions in Dtx+Ole group. (D) Negative expressions in Ole group, Bax: Bcl-2-associated X protein, Dtx: Docetaxel; Ole: Oleuropein, Streptavidin biotin peroxidase method, scale bars=50µm.

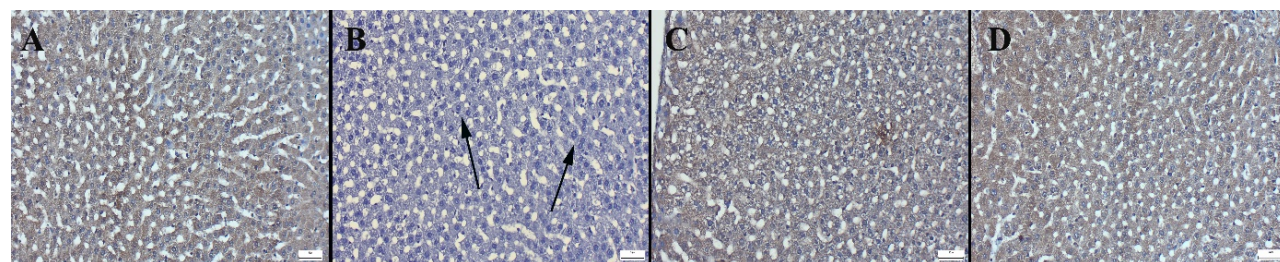


Fig. 6: Bcl-2 expressions between the groups in liver tissues. (A) Marked expression in control group. (B) Decrease in expressions (arrows) in Dtx group. (C) Increased expressions in Dtx+Ole group. (D) Marked expressions in Ole group, Bcl-2: B-cell lymphoma gene-2, Dtx: Docetaxel; Ole: Oleuropein, Streptavidin biotin peroxidase method, scale bars=50µm.

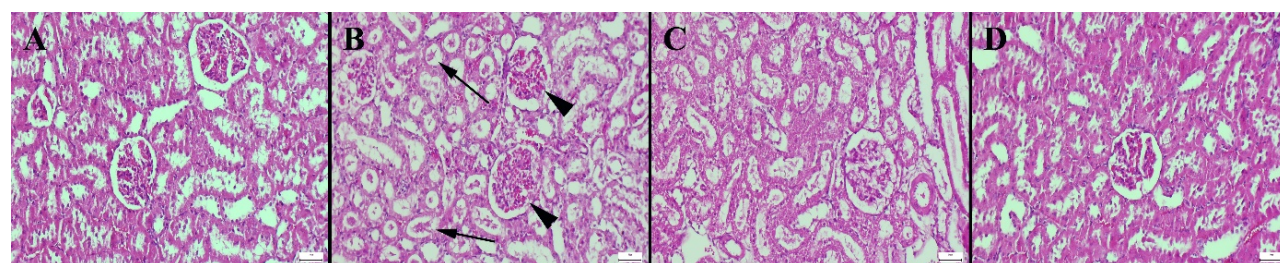


Fig. 7: Microscopical appearances among the groups in renal tissues. (A) Normal tissue histoarchitecture in Control group. (B) Marked hyperemia in glomerulus (arrow head) and proteinous materials (arrows) in the tubular lumens (arrows) in the Dtx group. (C) Decreased pathological findings in Dtx+Ole group. (D) Normal kidney histology in Ole group, Dtx: Docetaxel; Ole: Oleuropein, H-E, scale bars=50µm.

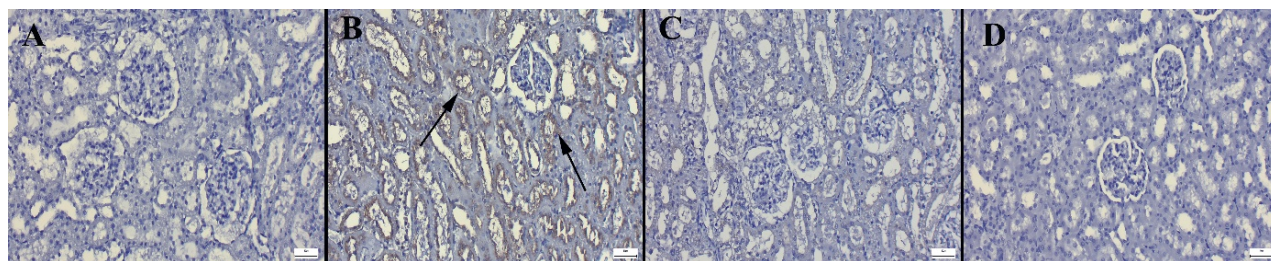


Fig. 8: Caspase-3 immunohistochemical findings between the groups in renal tissues. (A) Negative expression in Control group, (B) Marked increase in expressions in tubular epithelial cells (arrows) in Dtx group, (C) Decreased expressions (arrow) in Dtx+Ole group, (D) No expression in Ole group, Dtx: Docetaxel; Ole: Oleuropein, Streptavidin biotin peroxidase method, scale bars=50µm.

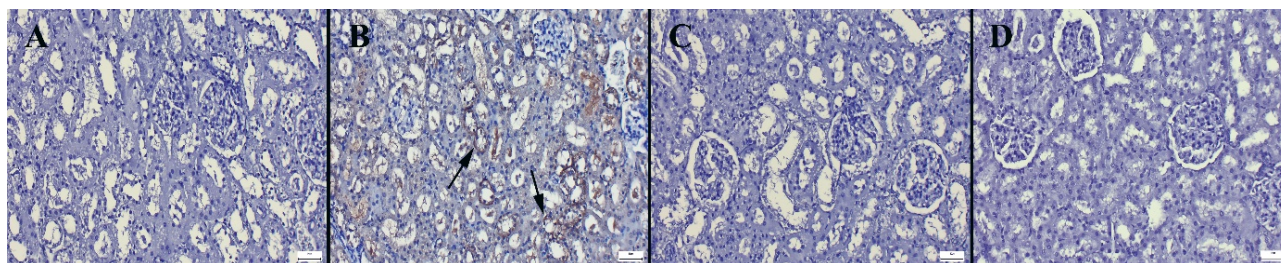


Fig. 9: Bax immunohistochemical findings between the groups in renal tissues. (A) Negative expression in Control group, (B) Marked increase in expressions in tubular epithelial cells (arrows) in Dtx group, (C) Decreased expressions (arrow) in Dtx+Ole group, (D) No expression in Ole group, Dtx: Docetaxel; Ole: Oleuropein, Bax: Bcl-2-associated X protein, Streptavidin biotin peroxidase method, scale bars=50µm.

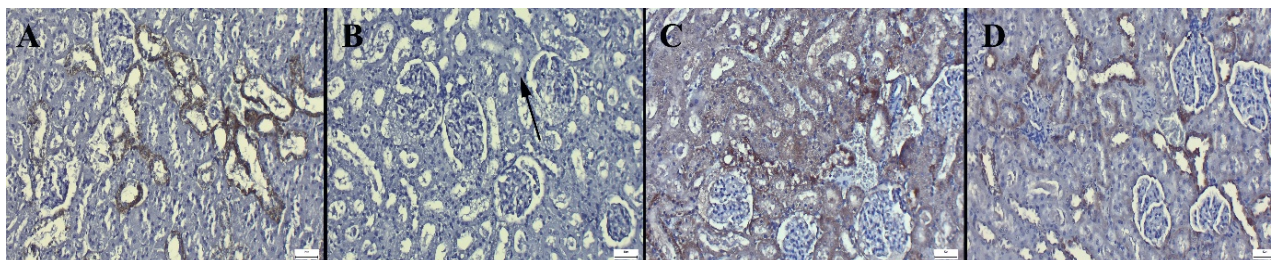


Fig. 10: Bcl-2 immunohistochemical findings between the groups in renal tissues. (A) Marked expression in Control group, (B) Decrease in expressions in tubular epithelial cells (arrows) in Dtx group, (C) Increased expressions (arrow) in Dtx+Ole group, (D) Marked expression in Ole group, Dtx: Docetaxel; Ole: Oleuropein, Bcl-2: B-cell lymphoma gene-2. Streptavidin biotin peroxidase method, scale bars=50µm.

Table 1: Primer sequences for RT-qPCR

Gene	Sequence of Primers	NCBI Reference
β-actin	F: 5'- GACGATATCGCTGCGCTCG-3'	NM_031144.3
	R: 5'- CAATGCCGTGTTCAATGGGG-3'	
Bcl-2	F: 5'- CATGTGTGTGGAGAGCGTCA-3'	NM_016993.2
	R: 5'- ACTCAGTCATCCACAGAGCG -3'	
Bax	F: 5'- CAACATGGAGCTGCAGAGGA-3'	NM_017059.2
	R: 5'- GGAAAGGAGGCCATCCCAG-3'	
Caspase -3	F: 5'- CTTTGCGCCATGCTGAAACT-3'	NM_001436900.1
	R: 5'- CAAATTCCGTGGCCACCTTC-3'	

Bcl-2: B-cell lymphoma 2. Bax: Bcl 2-associated x protein. RT-qPCR: Reverse Transcription quantitative real-time Polymerase Chain Reaction. F: Forward. R: Reverse. NCBI: National Center for Biotechnology Information

Table 2: Histopathology score of hepatic lesions

Histological criteria	Description	Severity	Distribution	Score
Steatosis	A few hepatocytes have a slightly "foamy" appearance.	Absent	<10%	0
	Some hepatocytes have one or more smaller vacuoles.	Mild	10–30%	1
	Hepatocytes are partly or entirely occupied by numerous tiny lipid vacuoles.	Marked	31–60%	2
	Hepatocytes with a large, well-defined single rounded vacuole present in all cells, with their nuclei displaced to the periphery of the cytoplasm.	Severe	>60%	3
Inflammation	No inflammatory reaction.	None		0
	Scattered predominantly neutrophil and a few lymphocytes.	Moderate	Scattered	1
	Focal or multifocal mainly mononuclear cell infiltrations.	Marked	Foci	2
	Diffuse infiltrating of mononuclear cells.	Severe	Diffuse	3
Necrosis	No necrotic cell	Absent	0%	0
	Hepatocytes that typically retain normal morphological borders but appear as single or multiple foci of pale staining.	Mild	<10%	1
	Focal necrotic areas, limited in the periaciner area.	Marked	10–50%	2
	Prominent and large necrotic areas extending to the subcapsular region.	Severe	>50%	3
Fibrosis	Normal hepatic architecture maintained.	Absent		0
	Formation of connective tissue in the portal areas of the liver above the normal level.	Mild	Periportal	1
	Periaciner and peribiliary fibrosis.	Marked	Periaciner	2
	Significant fibrosis that can encircle hepatic lobules and form connections between neighboring portal regions.	Severe	Diffuse	3

Table 3: Distribution of the initial and final body weights of the groups

Groups	Initial b.w.	Final b.w.	p*
Control	232.75±3.48	305.00±14.87 ^a	0.003*
Dtx	235.50±1.57	228.80±21.20 ^b	0.767
Ole	233.38±3.46	306.38±12.93 ^a	0.001*
Dtx+Ole	228.38±0.87	243.50±4.92 ^b	0.027*
p**	0.193	<0.001**	

^{a, b} Superscript letters in the final b.w. column indicates statistically significance (p<0.01) with each other. * Statistically significant p values of intra-group paired sample t tests. ** Statistically significant p values of one way-ANOVA. Dtx: Docetaxel. Ole: Oleuropein. b.w.: body weights. Data expressed as mean ± S.E.

Table 4: Fold changes of the genes in experimental groups.

Organ/ Gene	Groups*					
	Docetaxel		Oleuropein		Docetaxel+Oleuropein	
	Fold Change	p value	Fold Change	p value	Fold Change	p value
Liver						
Bcl-2	0.78	0.137747	1.62	0.010628	3.09	0.016178
Bax	4.54	0.000077	0.54	0.515522	0.51	0.514099
Casp3	4.06	0.418446	0.85	0.028653	1.81	0.472452
Kidney						
Bcl-2	0.78	0.137878	4.29	0.000007	5.41	0.008136
Bax	2.63	0.002728	0.38	0.867902	0.51	0.514099
Casp3	8.78	0.104808	0.66	0.022156	1.08	0.218570

* The results of fold changes compared with the control group. The expression level of the reference group (control group) is considered as 1-fold. Bcl-2: B-cell lymphoma 2. Bax: Bcl 2-associated x protein. Casp3: Caspase-3

Table 5: Results of serum biochemical parameters

Parameters	Groups			
	Control	Oleuropein	Docetaxel	Docetaxel+ Oleuropein
ALT (U/L)	82.31±4.39 ^a	74.25±3.06 ^a	117.61±6.85 ^b	84.90±3.52 ^a
AST (U/L)	189.69±10.25 ^a	168.10±9.97 ^a	261.04±10.09 ^b	201.30±15.35 ^a
GGT (U/L)	1.95±0.15 ^a	2.03±0.12 ^a	2.10±0.18 ^a	2.06±0.12 ^a
Creatinine (mg/dl)	0.31±0.02 ^a	0.30±0.01 ^a	0.37±0.18 ^b	0.28±0.01 ^a
Urea (mg/dl)	44.51±0.68 ^a	43.94±0.92 ^a	54.13±1.81 ^b	45.13±2.09 ^a
Uric Acid (mg/dl)	1.39±0.15 ^a	1.18±0.05 ^a	1.50±0.11 ^a	1.38±0.10 ^a
IL-1 β (pg/ml)	174.90±3.81 ^a	173.43±3.06 ^a	202.53±4.97 ^b	182.10±1.79 ^a
IL-6 (pg/ml)	73.63±0.92 ^a	75.39±1.318 ^a	92.53±2.48 ^b	80.08±2.29 ^a
TNF- α (pg/ml)	83.58±1.35 ^a	86.56±1.36 ^a	99.96±1.52 ^b	87.78±1.28 ^a

^{a,b} Statistically significant differences ($p < 0.05$) are indicated by different superscript letters within the same row, with each other. ALT: Alanine Aminotransferase. AST: Aspartate Aminotransferase. GGT: Gamma-glutamyl transferase. IL-1 β : Interleukin-1 Beta. IL-6: Interleukin 6. TNF- α : Tumor necrosis factor-alpha. Data expressed as mean $\pm$ S.E.

Table 6: The mean renal and hepatic TOS and TAS levels of experimental groups

Tissue- Parameter	Groups				p value
	Control	Dtx	Ole	Dtx + Ole	
Liver					
TAS	14.51±0.89 ^a	12.51±0.66 ^a	14.71±0.62 ^a	13.42±0.76 ^a	>0.05
TOS	8.54±0.54 ^a	17.52±0.39 ^b	7.51±0.59 ^a	12.30±0.56 ^c	<0.001
Kidney					
TAS	18.77±1.33 ^a	11.50±0.46 ^{b,c}	19.75±1.30 ^{a,d}	14.91±0.82 ^{a,c,d}	<0.001
TOS	10.94±0.38 ^a	20.89±0.70 ^b	11.73±0.64 ^a	11.73±0.64 ^a	<0.001

TAS: Total antioxidant status (nmol Trolox eq/mg protein). TOS: Total oxidant status (nmol H₂O₂ eq/mg protein). Dtx: Docetaxel. Ole: Oleuropein. ^{a, b, c, d} Different superscript letters in the same row indicates statistically significant difference ($p < 0.05$) with each other. Data expressed as mean $\pm$ S.E.

Table 7: Results of histopathologic and immunohistochemical scores of liver in experimental groups.

Groups	Control	Dtx	Dtx+Ole	Ole	p value
Histopathology scores					
Steatosis	0.12±0.12 ^a	1.50±0.19 ^b	0.37±0.18 ^a	0.12±0.12 ^a	<0.001
Inflammation	0.00±0.00 ^a	0.75±0.16 ^b	0.37±0.18 ^a	0.00±0.00 ^a	<0.001
Necrosis	0.00±0.00 ^a	1.50±0.26 ^b	0.75±0.16 ^c	0.00±0.00 ^a	<0.001
Fibrosis	0.00±0.00 ^a	0.62±0.18 ^b	0.25±0.16 ^a	0.00±0.00 ^a	<0.001
Immunohistochemical positive cell percentage					
Casp3	0.87±0.39 ^a	23.00±2.39 ^b	4.25±1.52 ^a	0.37±0.26 ^a	<0.001
Bax	2.12±0.22 ^a	36.37±1.70 ^b	2.75±0.55 ^a	0.62±0.32 ^a	<0.001
Bcl-2	49.25±2.05 ^a	10.62±4.85 ^b	43.25±1.44 ^a	51.62±1.83 ^a	<0.001

^{a,b,c} Different superscript letters in the same row indicates statistically significant difference ($p < 0.001$) with each other. Dtx: Docetaxel. Ole: Oleuropein. Casp3: Caspase-3. Bax: Bcl-2-associated X protein. Bcl-2: B-cell lymphoma gene-2. Results are presented as mean $\pm$ S.E.

Table 8: Results of immunohistochemical scores of kidney tissues in experimental groups.

Gene	Groups				p value
	Control	Dtx	Ole	Dtx+Ole	
Casp3	1.00±0.26 ^a	15.62±0.94 ^b	0.25±0.16 ^a	1.87±0.35 ^a	<0.001
Bax	1.62±0.32 ^a	12.12±0.51 ^b	0.25±0.16 ^a	2.50±0.26 ^c	<0.001
Bcl-2	33.12±1.00 ^a	3.75±0.52 ^b	39.00±1.05 ^c	21.50±0.53 ^d	<0.001

^{a,b,c,d} Different superscript letters in the same row indicates statistically significant difference ($p < 0.001$) with each other. Dtx: Docetaxel. Ole: Oleuropein. Casp3: Caspase-3. Bax: Bcl-2-associated X protein. Bcl-2: B-cell lymphoma gene-2. Data expressed as mean $\pm$ S.E.

This suggests that no significant changes in enzyme expression or synthesis occurred by the end of the study period. The effectiveness of Ole in preventing Dtx-induced toxicity was examined for the first time, yielding important findings. However, the study only focused on changes in CYP3A1 levels and enzyme activity assessment through the pharmacokinetic profile of a known substrate of rat CYP3A enzymes was not performed. While we examined the expression of certain genes involved in apoptosis, the expression of genes in other apoptosis-related pathways (e.g., the PI3K-Akt-mTOR signaling pathway) was not investigated. These limitations should be considered in interpreting our study.

CONCLUSION

The study revealed that repeated doses of Dtx (5 mg/kg/week; i.p.) trigger oxidative stress in rat liver and kidney tissues, leading to histopathological damage. Ole (30 mg/kg/day; p.o.) effectively ameliorated Dtx-induced toxicity in these tissues. Our results demonstrate the protective efficacy of Ole against Dtx-induced toxicity, as evidenced by molecular, biochemical and histopathological findings. This protective effect is attributed to its antioxidant activity. Ole, a non-toxic dietary component, holds potential as a protective agent against liver and kidney toxicity in cancer patients receiving Dtx. Therefore, further studies are essential to elucidate the mechanisms underlying this protective effect and to assess the potential clinical benefits of Ole in mitigating Dtx-induced toxicity.

Funding

This work was supported by grants from Pamukkale University Scientific Research Project Unit (Project Number: 2023BSP005).

Data availability statement

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Author's contributions

Metin Deniz KARAKOÇ designed the project and experimental groups. Metin Deniz KARAKOÇ, Özlem ÖZMEN, Münevver Nazlıcan ZENGİN, and Osman ÇİFTÇİ performed the experiments and collected the data. Metin Deniz KARAKOÇ, Özlem ÖZMEN and Osman ÇİFTÇİ carried out the statistical evaluation and visualized the data. Metin Deniz KARAKOÇ and Özlem ÖZMEN wrote the manuscript. All authors approved the final version of the manuscript and agree to be accountable for all aspects of the work.

Conflict of interest

All Authors declare no competing interests.

REFERENCES

- Ahmad Farooqi A, Fayyaz S, Silva AS, Sureda A, Nabavi SF, Mocan A, Nabavi SM and Bishayee A (2017). Oleuropein and cancer chemoprevention: The link is hot. *Molecules*, **22**(5): 705.
- Ajdari M, Ranjbar A, Karimian K, Karimi M, Heli H and Sattarahmady N (2024). Characterization and evaluation of nano-niosomes encapsulating docetaxel against human breast, pancreatic and pulmonary adenocarcinoma cancer cell lines. *J. Biomed. Phys. Eng.*, **14**(2): 159-168.
- Baş E and Nazıroğlu M (2019). Treatment with melatonin and selenium attenuates docetaxel-induced apoptosis and oxidative injury in kidney and testes of mice. *Andrologia*, **51**(8): e13320.
- Beheshtizadeh N, Amiri Z, Tabatabaei SZ, Seraji AA, Gharibshahian M, Nadi A, Saeinasab M, Sefat F and Kolahi Azar H (2024). Boosting antitumor efficacy using docetaxel-loaded nanoplateforms: From cancer therapy to regenerative medicine approaches. *J. Transl. Med.*, **22**(1): 520.
- Cimbro E, Dessi M, Ziranu P, Madeddu C, Atzori F, Lai E, Pretta A, Mariani S, Donisi C, Spanu D, Pozzari M, Murgia S, Saba G, Codipietro C, Palmas E, Sanna G, Semonella F, Sardo S, Finco G and Scartozzi M (2024). Early taxane exposure and neurotoxicity in breast cancer patients. *Support Care Cancer*, **32**(10): 709.
- Dinda B, Dinda M, Kulsi G, Chakraborty A and Dinda S (2019). Therapeutic potentials of plant iridoids in Alzheimer's and Parkinson's diseases: A review. *Eur. J. Med. Chem.*, **169**: 185-199.
- Elamin MH, Elmahi AB, Daghestani MH, Al-Olayan EM, Al-Ajmi RA, Alkhuriji AF, Hamed SS and Elkhadragy MF (2019). Synergistic anti-breast-cancer effects of combined treatment with oleuropein and doxorubicin in vivo. *Altern Ther Health Med.*, **25**(3): 17-24.
- Erel O (2004). A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. *Clin. Biochem.*, **37**(4): 277-285.
- Erel O (2005). A new automated colorimetric method for measuring total oxidant status. *Clin Biochem.*, **38**(12): 1103-1111.
- Frazier KS, Seely JC, Hard GC, Betton G, Burnett R, Nakatsuji S, Nishikawa A, Durchfeld-Meyer B and Bube A (2012). Proliferative and nonproliferative lesions of the rat and mouse urinary system. *Toxicol. Pathol.*, **40**(Suppl): 14S-86S.
- Gervasi F and Pojero F (2024). Use of oleuropein and hydroxytyrosol for cancer prevention and treatment: Considerations about how bioavailability and metabolism impact their adoption in clinical routine. *Biomedicines*, **12**(3): 502.
- Geyikoglu F, Emir M, Colak S, Koc K, Turkez H, Bakir M, Hosseinigouzdagani M, Cerig S, Keles ON and Ozek NS (2017). Effect of oleuropein against chemotherapy

- drug-induced histological changes, oxidative stress and DNA damages in rat kidney injury. *J. Food. Drug. Anal.*, **25**(2): 447-459.
- Guo Z, Zeng X and Zheng Y (2021). Zafirlukast ameliorates Docetaxel-induced activation of NOD-like receptor protein 3 (NLRP3) inflammasome, mediated by sirtuin1 (SIRT1) in hepatocytes. *Bioengineered*, **12**(2): 11030-11040.
- Gupta A, Sontakke T, Acharya S and Kumar S (2024). A comprehensive review of biomarkers for chronic kidney disease in older individuals: Current perspectives and future directions. *Cureus*, **16**(9): e70262.
- Hadrich F, Mahmoudi A, Bouallagui Z, Feki I, Isoda H, Feve B and Sayadi S (2016). Evaluation of hypocholesterolemic effect of oleuropein in cholesterol-fed rats. *Chem. Biol. Interact.*, **252**: 54-60.
- Han JH, Tweedell RE and Kanneganti TD (2023). Evaluation of caspase activation to assess innate immune cell death. *J. Vis. Exp.*, **191**: 10.3791/64308.
- Hassan HA, Ahmed HS and Hassan DF (2024). Free radicals and oxidative stress: Mechanisms and therapeutic targets. *Hum Antibodies*, **32**(4): 151-167.
- Janahmadi Z, Nekooeian AA, Moaref AR and Emamghoreishi M (2015). Oleuropein offers cardioprotection in rats with acute myocardial infarction. *Cardiovasc. Toxicol.*, **15**(1): 61-68.
- Jemai H, Mahmoudi A, Feryeni A, Fki I, Bouallagui Z, Choura S, Chamkha M and Sayadi S (2020). Hepatoprotective effect of oleuropein-rich extract from olive leaves against cadmium-induced toxicity in mice. *Bio. Med. Res. Int.*, **2020**: 4398924.
- Jomova K, Raptova R, Alomar SY, Alwasel SH, Nepovimova E, Kuca K and Valko M (2023). Reactive oxygen species, toxicity, oxidative stress and antioxidants: Chronic diseases and aging. *Arch. Toxicol.*, **97**(10): 2499-2574.
- Karakoç MD and Sekkin S (2021). Effects of oleuropein on epirubicin and cyclophosphamide combination treatment in rats. *Turk. J. Pharm. Sci.*, **18**(4): 420-429.
- Kapelemera AM, Uang YS, Wang LH, Wu TY, Lee FY, Tai L, Wang CC and Lee CJ (2022). Pharmacokinetic herb-drug interactions of Xiang-Sha-Liu-Jun-Zi-Tang and paclitaxel in male Sprague Dawley rats and its influence on enzyme kinetics in human liver microsomes. *Front Pharmacol.*, **13**: 858007.
- Kappler P, Morgan MA, Ivanyi P, Brunotte SJ, Ganser A and Reuter CWM (2021). Prognostic role of docetaxel-induced suppression of free testosterone serum levels in metastatic prostate cancer patients. *Sci. Rep.*, **11**(1): 16457.
- Kida K, Yamada A, Shimada K, Narui K, Sugae S, Shimizu D, Doi T, Oba M, Endo I and Ishikawa T (2024). A prospective comparison study utilizing patient-reported outcomes of taxane-related peripheral neuropathy between nab-paclitaxel and standard paclitaxel in patients with breast cancer. *Breast Cancer*, **31**(3): 409-416.
- Kim ST, Kyung EJ, Suh JS, Lee HS, Lee JH, Chae SI, Park ES, Chung YH, Bae J, Lee TJ, Lee WM, Sohn UD and Jeong JH (2018). Phosphatidylcholine attenuated docetaxel-induced peripheral neurotoxicity in rats. *Drug Chem. Toxicol.*, **41**(4): 476-485.
- Koc K, Cerig S, Ozek NS, Aysin F, Yildirim S, Cakmak O, Hosseinigouzdagani M and Geyikoglu F (2019). The efficacy of oleuropein against non-steroidal anti-inflammatory drug induced toxicity in rat kidney. *Environ. Toxicol.*, **34**(1): 67-72.
- Lopes F, Smith Randerson RA and Spears N (2014). Docetaxel induces moderate ovarian toxicity in mice, primarily affecting granulosa cells of early growing follicles. *Mol. Hum. Reprod.*, **20**(10): 948-959.
- Mahmoudi A, Ghorbel H, Bouallegui Z, Marrekchi R, Isoda H and Sayadi S (2015). Oleuropein and hydroxytyrosol protect from bisphenol A effects in livers and kidneys of lactating mother rats and their pups. *Exp. Toxicol. Pathol.*, **67**(7-8): 413-425.
- Malliou Fandriopoulou CE, Gonzalez FJ, Kofinas A, Skaltsounis AL and Konstandi M (2021). Oleuropein-induced acceleration of cytochrome P450-catalyzed drug metabolism: Central role for nuclear receptor peroxisome proliferator-activated receptor α . *Drug Metab Dispos.*, **49**(9): 833-843.
- Mohammadian J, Sabzichi M, Molavi O, Shanehbandi D and Samadi N (2016). Combined treatment with statin and docetaxel alters the Bax/Bcl-2 gene expression ratio in human prostate cancer cells. *Asian Pac. J. Cancer Prev.*, **17**(11): 5031-5035.
- Nong Y, Lu J, Yu D and Wei X (2024). Neohesperidin dihydrochalcone alleviates lipopolysaccharide-induced vascular endothelium dysfunction by regulating antioxidant capacity. *Immun. Inflamm. Dis.*, **12**(12): e70107.
- Park S, Choi Y, Um SJ, Yoon SK and Park T (2011). Oleuropein attenuates hepatic steatosis induced by high-fat diet in mice. *J. Hepatol.*, **54**(5): 984-993.
- Perini GF, Feres CCP, Teixeira LLC and Hamerschlag N (2021). BCL-2 inhibition as treatment for chronic lymphocytic leukemia. *Curr. Treat Options Oncol.*, **22**(8): 66.
- Potočnjak I, Škoda M, Pernjak-Pugel E, Peršić MP and Domitrović R (2016). Oral administration of oleuropein attenuates cisplatin-induced acute renal injury in mice through inhibition of ERK signaling. *Mol. Nutr. Food Res.*, **60**(3): 530-541.
- Pradeep U, Chiwhane A, Acharya S, Daiya V, Kasat PR, Sachani P, Mapari SA and Bedi GN (2024). A comprehensive review of advanced biomarkers for chronic kidney disease in older adults: Current insights and future directions. *Cureus*, **16**(9): e70413.
- Pravdic Z, Vukovic NS, Gasic V, Marjanovic I, Karan-Djurasevic T, Pavlovic S and Tosic N (2023). The influence of BCL2, BAX and ABCB1 gene expression on prognosis of adult de novo acute myeloid leukemia

- with normal karyotype patients. *Radiol. Oncol.*, **57**(2): 239-248.
- Qian S, Wei Z, Yang W, Huang J, Yang Y and Wang J (2022). The role of BCL-2 family proteins in regulating apoptosis and cancer therapy. *Front Oncol.*, **12**: 985363.
- Rendic SP and Guengerich FP (2024). Formation of potentially toxic metabolites of drugs in reactions catalyzed by human drug-metabolizing enzymes. *Arch Toxicol.*, **98**(6): 1581-1628.
- Saito Y, Takekuma Y, Takahashi M, Oshino T and Sugawara M (2024). Evaluation of the impact of systemic dexamethasone dosage on docetaxel-induced hand-foot syndrome in patients with breast cancer. *Sci Rep.*, **14**(1): 14083.
- Sherif IO, Nakshabandi ZM, Mohamed MA and Sarhan OM (2016). Uroprotective effect of oleuropein in a rat model of hemorrhagic cystitis. *Int. J. Biochem. Cell Biol.*, **74**: 12-17.
- Song Y, Li C, Liu G, Liu R, Chen Y, Li W, Cao Z, Zhao B, Lu C and Liu Y (2021). Drug-metabolizing cytochrome P450 enzymes have multifarious influences on treatment outcomes. *Clin. Pharmacokinet.*, **60**(5): 585-601.
- Stoicescu EA and Cherecheanu AP (2023). Meibomian gland changes in breast cancer patients treated with docetaxel-partial results. *Rom. J. Ophthalmol.*, **67**(2): 111-116.
- Tang N, Liu J, Chen B, Zhang Y, Yu M, Cai Z and Chen H (2017). Effects of gap junction intercellular communication on the docetaxel-induced cytotoxicity in rat hepatocytes. *Mol. Med. Rep.*, **15**(5): 2689-2694.
- Thoolen B, Maronpot RR, Harada T, Nyska A, Rousseaux C, Nolte T, Malarkey DE, Kaufmann W, Küttler K, Deschl U, Nakae D, Gregson R, Vinlove MP, Brix AE, Singh B, Belpoggi F and Ward JM (2010). Proliferative and nonproliferative lesions of the rat and mouse hepatobiliary system. *Toxicol Pathol.*, **38**(7 Suppl): 5S-81S.
- Tweedell RE, Hibler T and Kanneganti TD (2024). Defining PANoptosis: Biochemical and mechanistic evaluation of innate immune cell death activation. *Curr. Protoc.*, **4**(7): e1112.
- Veteläinen RL, Bennink RJ, de Bruin K, van Vliet A and van Gulik TM (2006). Hepatobiliary function assessed by ^{99m}Tc-mebrofenin cholescintigraphy in the evaluation of severity of steatosis in a rat model. *Eur. J. Nucl. Med. Mol. Imaging.*, **33**(10): 1107-1114.
- Tu H and Li YL (2023). Inflammation balance in skeletal muscle damage and repair. *Front Immunol.*, **14**: 1133355.
- Xu LZ, He KX, Ning JZ and Cheng F (2022). Oleuropein attenuates testicular ischemia-reperfusion by inhibiting apoptosis and inflammation. *Tissue Cell*, **78**: 101876.
- Yu H, Liu P, Tang H, Jing J, Lv X, Chen L, Jiang L, Xu J and Li J (2016). Oleuropein, a natural extract from plants, offers neuroprotection in focal cerebral ischemia/reperfusion injury in mice. *Eur. J. Pharmacol.*, **775**: 113-119.
- Yu H, Xu Z, Guo M, Wang W, Zhang W, Liang S, Xu Z, Ye J, Zhu G, Zhang C and Lin J (2020). FOXM1 modulates docetaxel resistance in prostate cancer by regulating KIF20A. *Cancer Cell Int.*, **20**(1), 545.
- Zhang Y, Yang X, Ge X and Zhang F (2019). Puerarin attenuates neurological deficits via Bcl-2/Bax/cleaved caspase-3 and Sirt3/SOD2 apoptotic pathways in subarachnoid hemorrhage mice. *Biomed. Pharmacother.*, **109**: 726-733.
- Zhao M, Ma J, Li M, Zhang Y, Jiang B, Zhao X, Huai C, Shen L, Zhang N, He L and Qin S (2021). Cytochrome P450 enzymes and drug metabolism in humans. *Int. J. Mol. Sci.*, **22**(23): 12808.