

# The role of glutathione in modulating organ injury in a bleomycin, etoposide and cisplatin-induced fibrosis model

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## ABSTRACT

**Aims:** The bleomycin, etoposide, and cisplatin (BEP) regimen is associated with organ fibrosis. In this study, the potential beneficial effect of the antioxidant glutathione (GSH) against BEP-induced fibrosis was investigated in a rat model.

**Methods:** This randomized controlled study was performed on male rats, which were divided into control, BEP, or BEP+GSH groups. BEP was administered for nine weeks, and the BEP+GSH group received concomitant GSH treatment. The primary endpoints were multi-organ fibrosis and tissue injury, evaluated in the liver, lung, kidney, and skin using histopathological scoring systems. Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), and urea levels were analyzed as secondary endpoints for functional organ impairment.

**Results:** Twenty-four 12-week-old male Sprague-Dawley rats were included in this study. Histopathological fibrosis scores were significantly increased in the BEP group in comparison with the control group (Ishak score  $4.00 \pm 0.00$  vs.  $0.50 \pm 0.53$ , Ashcroft score  $7.00 \pm 0.76$  vs.  $1.00 \pm 1.41$ , renal injury score  $5.50 \pm 0.53$  vs.  $0.00 \pm 0.00$ , dermal thickness  $241.82 \pm 24.83$  vs.  $203.90 \pm 26.83$   $\mu\text{m}$ ;  $p < 0.0001$ ,  $p < 0.0001$ ,  $p < 0.0001$ ,  $p < 0.05$ , respectively). Conversely, GSH co-administration significantly reduced tissue injury and fibrosis in the liver ( $1.88 \pm 0.83$ ;  $p < 0.0001$ ), lungs ( $4.38 \pm 0.92$ ;  $p < 0.0001$ ), kidneys ( $3.7 \pm 0.71$ ;  $p < 0.0001$ ), and skin ( $193.64 \pm 23.70$   $\mu\text{m}$ ;  $p < 0.01$ ). AST levels exhibited a slight decrease in the BEP+GSH group relative to both the control ( $105.76 \pm 14.71$  U/L vs.  $144.79 \pm 25.81$  U/L;  $p < 0.01$ ) and BEP groups ( $142.61 \pm 8.31$  U/L;  $p < 0.01$ ), yet remained within the reference range in all groups. No significant differences were observed in ALT ( $31.09 \pm 3.93$  U/L,  $29.49 \pm 4.57$  U/L, and  $33.08 \pm 4.46$  U/L, respectively) and urea levels ( $13.11 \pm 2.41$  mg/dL,  $12.96 \pm 1.60$  mg/dL, and  $13.20 \pm 1.98$  mg/dL, respectively) in control, BEP, and BEP+GSH groups.

**Conclusions:** GSH supplementation may attenuate BEP-induced fibrosis and tissue injury in the liver, lung, kidney, and skin.

## Introduction

Oxidative stress (OS) represents an imbalance between the body's antioxidant defense systems and the production of reactive oxygen species (ROS). Glutathione (GSH), composed of L-γ-glutamyl-L-cysteinyl-glycine, represents the principal intracellular thiol-based antioxidant, essential for maintaining redox homeostasis and protecting cells against OS (1). In addition to its role in oxidative defense, GSH and its components contribute to tissue and cell protection under physiological and pathological conditions by participating in various physiological processes such as xenobiotic detoxification, redox signaling, cysteine metabolism, cell proliferation, DNA synthesis, immune modulation, prostaglandin metabolism, and fibrogenesis (1,2).

Fibrosis is defined as a pathological process characterized by the excessive proliferation, hardening, and scarring of tissue as a result of sustained injury. It involves the excessive deposition of extracellular matrix (ECM) components, primarily type I and III collagen (3). The progressive deposition of ECM disrupts normal tissue architecture and function, ultimately leading to organ dysfunction and failure in advanced cases (3,4). Fibrosis can affect many organs, including the lungs, liver, kidneys, skin, and heart, and is estimated to contribute to nearly 45% of deaths in Western countries (5).

Elevated OS plays a crucial role in the development and progression of tissue fibrosis through multiple mechanisms, including myofibroblast differentiation, fibroblast activation, ECM deposition, cellular senescence, and ferroptosis (6). A central mediator of these processes is the bidirectional crosstalk between OS and transforming growth factor-beta (TGF-β), which establishes a self-amplifying loop that leads to increased ROS production and further activation of TGF-β. This, in turn, amplifies downstream pro-fibrotic signaling cascades, including Smad, mitogen-activated protein kinase (MAPK), and NF-κB pathways (3,7). TGF-β has been reported to deplete intracellular GSH by suppressing its synthesis (8) and increasing its consumption (9), thereby creating a pro-oxidant environment that drives fibrogenesis and promotes tumorigenesis.

The administration of chemotherapeutic agents intensifies OS, which in turn activates pro-inflammatory and pro-fibrotic pathways, thereby accelerating the onset and progression of fibrosis across multiple organs, including the lung, skin, kidney, and liver (4,10,11). Among these therapies, the combination regimen of bleomycin, etoposide, and cisplatin (BEP), despite achieving high long-term survival rates in the treatment of ovarian and testicular cancers (12), is associated with severe adverse profiles. Of particular concern, BEP-associated pulmonary fibrosis is a common complication linked to considerable mortality (13). Furthermore, BEP chemotherapy has been associated with acute kidney injury and reductions in glomerular filtration rate, affecting approximately 25% of patients (4). Therefore, identifying preventive approaches to mitigate BEP-

induced organ toxicity and subsequent development of fibrosis remains a critical clinical priority.

Several animal studies have demonstrated that GSH supplementation can reduce organ fibrosis induced by experimental agents, such as CCL<sub>4</sub>, diethylnitrosamine, and glucose, by improving the antioxidant status and inhibiting fibrogenic signaling (14-16). Moreover, GSH has been shown to protect against cisplatin-induced nephrotoxicity without diminishing its antitumor activity (17). GSH may also exert hepatoprotective effects by reducing liver injury and limiting pathological changes associated with the progression from non-alcoholic fatty liver disease (NAFLD) to non-alcoholic steatohepatitis (NASH) (18). Nevertheless, the efficacy of GSH on multi-organ fibrosis and histopathological tissue damage associated with BEP therapy has not yet been investigated. In particular, whether GSH can attenuate BEP-induced fibrotic changes in the lung, skin, kidney, and liver remains unknown. This knowledge gap is significant, as BEP-related organ toxicity may limit adherence to treatment and contribute to long-term morbidity. Therefore, our study aims to investigate the potential benefits of GSH in mitigating multiple organ damage and fibrosis induced by the BEP regimen in an experimental rat model. For this aim, histopathological evaluations of the liver, lungs, kidneys, and skin tissues were performed, and serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and urea levels were analyzed.

## Methods

### Experimental design and ethical approval

This randomized controlled experimental animal study was conducted using adult Sprague-Dawley rats bred in the Maltepe University Experimental Animal Center. The sample size was determined in accordance with ethical principles, international guidelines, and the 3Rs principles, taking into account the number of rats used in previous experimental models involving antioxidants and the combined antineoplastic agents BEP (19). Cards with the names of the control, BEP, and BEP+GSH groups were prepared by laboratory staff independent of the study and placed in serially numbered, opaque, sealed envelopes. To randomly assign the rats to the three experimental groups, the envelopes were opened in sequence, and the rats were allocated accordingly. The animals were housed in groups of up to four per cage under controlled conditions (room temperature and 50-60% humidity) with a 12-hour light/dark cycle. Standard laboratory pellet feed and water were available to the rats *ad libitum*. All experimental methods were performed in accordance with the guidelines approved by the Maltepe University Experimental Animal Local Ethics Committee (approval no: 2024.02.03, approval date: February 29, 2024). The experimental protocols and analyses performed as part of the study were conducted between April 2024 and June 2025.

### Inclusion and exclusion criteria

The inclusion criteria were defined as male sex, an age of 12 weeks, Sprague-Dawley strain, and the absence of any systemic disease or physical abnormalities at the start of the experiment. The exclusion criteria were defined as death during the experimental period, the development of signs of infection or disease, failure to complete the experimental protocol, or the inability to obtain sufficient samples for biochemical or histological analyses.

### Treatment protocol

The rats were randomly assigned to three groups: Control, BEP, and BEP+GSH. The BEP treatment protocol was applied over the course of three 21-day cycles, as described previously (20). The treatment regimen comprised cisplatin at 0.17 mg/kg (Koçakfarma, İstanbul, Türkiye; CAS: 15663-27-1), etoposide at 0.83 mg/kg (Koçakfarma; CAS: 33419-42-0), and bleomycin at 0.083 mg/kg (Koçakfarma; CAS: 11056-06-7). Etoposide and cisplatin were injected intraperitoneally on days 1 to 5 of each cycle, while bleomycin was injected on days 2, 9, and 16. The control group was injected with equivalent volumes of 0.9% NaCl on the corresponding treatment days. In the BEP+GSH group, rats received the same BEP protocol along with intraperitoneal GSH injections (Laboratorio Farmaceutico, Imperia, Italy; CAS: 70-18-8) at a dose of 200 mg/kg, administered twice weekly for 9 weeks (20). When multiple drugs were injected on the same day, a 30-minute interval was allowed between consecutive injections.

### Histopathological evaluation

All rats were anesthetized by intraperitoneal injections of ketamine (100 mg/kg; Ketalar, Pfizer, İstanbul, Türkiye; CAS: 6740-88-1) and xylazine (10 mg/kg; Rompun, Bayer, Leverkusen, Germany; CAS: 23076-35-9). Under deep anesthesia, intracardiac blood samples were collected, and the animals were sacrificed. Subsequently, the liver, lungs, kidneys, and a portion of the skin were harvested for further analysis.

Tissues were fixed in 10% formaldehyde solution, routinely embedded in paraffin, and sectioned into 3-5 µm thick slices. The sections were subsequently stained with hematoxylin-eosin (H&E) (Thermo Fisher Scientific, Massachusetts, USA; CAS: 517-28-2, 6359-04-2) for routine histopathological evaluation under a light microscope (Carl Zeiss Microscopy GmbH, Jena, Germany). In addition, Masson's trichrome (MT) staining (MT Staining Kit, Beyotime, China; Lot No: 051424240702) was used in accordance with the recommended protocols to visualize type I collagen fibers and assess fibrotic changes. Histopathological scoring was performed on 10 randomly selected, non-overlapping fields per section across 3 tissue sections under 100× and 400× magnification for each rat (30 fields/rat). These scores were averaged for each rat to serve as the individual biological replicate (n=8) for statistical analysis. All histological evaluations were performed in a blinded manner.

The Ishak method is a validated histological scoring system widely used to semiquantitatively assess the degree of liver fibrosis (21). The stained liver sections were evaluated using the Ishak scoring method (0-6 scale): Score 0, no fibrosis in liver tissue; score 1, mild fibrous enlargement involving some portal areas; score 2, portal fibrosis with or without fibrous septa; score 3, portal fibrosis accompanied by occasional portal-to-portal bridging; score 4, extensive portal fibrosis with marked portal-to-portal and portal-to-central fibrous bridging; score 5, marked portal-to-portal and portal-to-central bridging with occasional nodules (incomplete cirrhotic changes); and score 6, probable or definite cirrhosis.

The Ashcroft scoring system is a standard assessment method used to histologically grade the severity of fibrotic changes in lung tissue (22). Lung sections were evaluated using the Ashcroft scoring system on a scale of 0-8: Score 0, normal lung tissue with no detectable pathological alterations; score 1, mild alveolar enlargement with slight thickening of alveolar septa ( $\leq 3$  times normal thickness); score 2, moderate thickening of alveolar septa ( $> 3$  times normal thickness) without disruption of the lung architecture; score 3, moderate alveolar septal thickening ( $> 3$  times normal thickness) with increased fibrotic tissue; score 4, mild lung structural damage with fibrotic areas involving less than 10% of the lung tissue; score 5, pulmonary structural damage with fibrosis affecting 10-50% of the lung tissue; score 6, extensive fibrosis involving more than 50% of the lung with obvious pulmonary structural damage; score 7, severe lung damage with widespread fibrosis and honeycomb-like changes; score 8, complete replacement of the field by fibrous tissue.

Kidney tissues were classified on a scale ranging from 0 to 10, based on the severity of injury (4): Score 0, no bleeding and normal histology; scores 1-2, minimal bleeding with isolated red blood cell extravasation and inflammatory cells in rare areas; scores 3-4, mild bleeding with focal interstitial or perivascular accumulation of red blood cells and inflammatory cells; scores 5-6, moderate bleeding with marked erythrocyte and inflammatory cell infiltration in multiple areas; scores 7-8, severe focal bleeding with extensive accumulation of red blood cells and inflammatory cells and tissue damage; and scores 9-10, very severe/diffuse hemorrhage with widespread bleeding and architectural disruption in the renal parenchyma.

Dermal thickness was measured in skin samples obtained from the same anatomical region (the left side of the abdominal skin where the injections were administered) in all animals (23).

### Biochemical parameters

Intracardiac blood was centrifuged at 1500× g for 15 minutes to obtain serum. Serum AST (BTlab®, Shanghai, China), ALT (BTlab®, Shanghai, China), and urea (Elabscience®, Wuhan, China) levels were analyzed using the ELISA method according to the manufacturers' instructions.

## Outcomes

The primary endpoints of this study were the histopathological assessments of fibrotic severity in the liver, lungs, kidneys, and skin following GSH treatment in BEP-induced organ damage. Histopathological scoring systems specific to each organ were used to assess multi-organ fibrosis and tissue damage in the liver (Ishak scoring method), lungs (Ashcroft scoring method), kidneys (renal injury score), and skin (dermal thickness measurement). Secondary endpoints included serum biochemical parameters reflecting organ function, including ALT, AST, and urea levels, across the experimental groups.

## Statistical Analysis

The statistical analyses were conducted using GraphPad Prism version 8.0 (GraphPad Software, San Diego, CA, USA). Descriptive statistics, including mean  $\pm$  standard deviation and minimum and maximum values, were used to characterize the data. Data normality was assessed using both the Kolmogorov-Smirnov and Shapiro-Wilk tests. All variables were normally distributed. One-way ANOVA was performed to compare the three groups, followed by Tukey's post-hoc test used for pairwise comparisons. A p-value of less than 0.05 was considered statistically significant.

## Results

This study included a total of 24 male Sprague-Dawley rats weighing 300-350 g and aged 12 weeks. All animals completed the experimental protocol, and no mortality or exclusions occurred during the study period. There were no significant

differences in baseline characteristics, including body weight, among the study groups.

### Effects of GSH on liver fibrosis

All groups were evaluated for liver fibrosis using the Ishak scoring method (Figure 1a). Histopathological evaluation was performed using H&E staining (Figure 1b) to assess overall tissue structure and MT staining (Figure 1c) to specifically confirm collagen deposition and visualize fibrosis. The mean Ishak scores were  $0.50 \pm 0.53$  in the control group,  $4.00 \pm 0.00$  in the BEP group, and  $1.88 \pm 0.83$  in the BEP+GSH group. In the BEP group, increased collagen deposition leading to portal tract expansion, along with pericellular fibrosis characterized by collagen fibrils encircling hepatocytes, was observed. As shown in Figure 1, liver fibrosis was significantly increased in the BEP group compared with the control group ( $p < 0.0001$ ). Co-administration of GSH significantly reduced fibrosis compared with the BEP group ( $p < 0.0001$ ). Fibrosis severity in the BEP+GSH group was significantly greater than that in the control group ( $p < 0.001$ ) (Figure 1).

### Effects of GSH on lung fibrosis

Lung fibrosis was evaluated in the control, BEP, and BEP+GSH groups using the Ashcroft scoring method (Figure 2a). H&E staining (Figure 2b) was used for routine histopathological evaluation, while MT (Figure 2c) staining was additionally employed to specifically confirm interstitial fibrosis. The mean Ashcroft scores were  $1.00 \pm 1.41$  in the control group,  $7.00 \pm 0.76$  in the BEP group, and  $4.38 \pm 0.92$  in the BEP+GSH group. A pronounced alveolar septal thickening, marked inflammatory

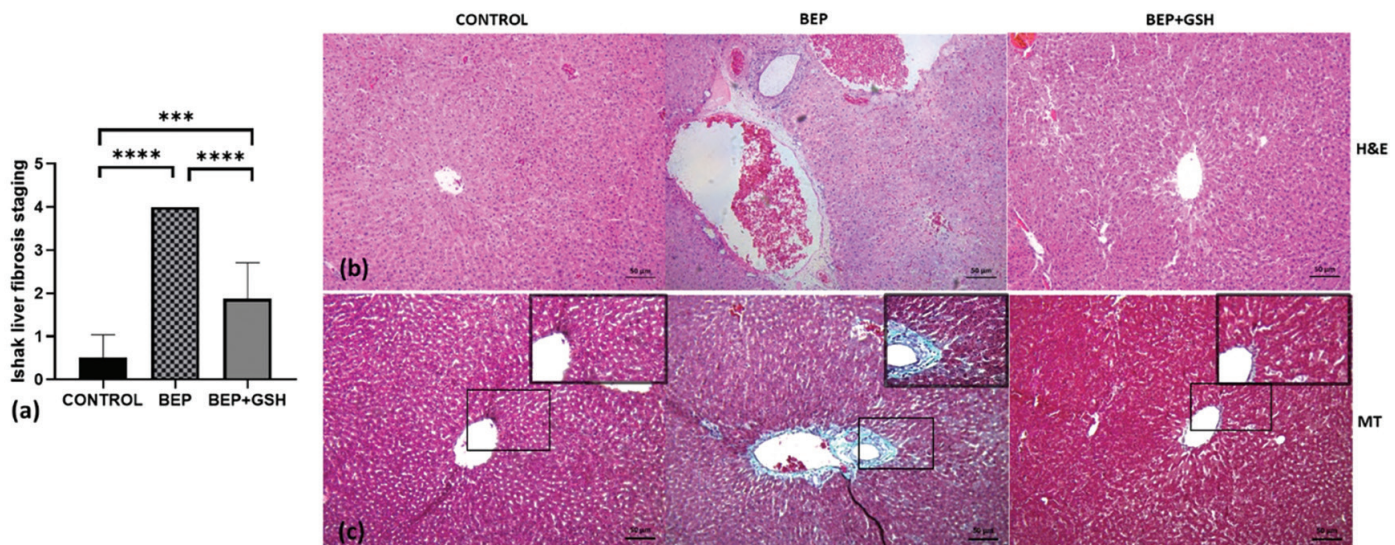


**Figure 1.** Effects of GSH treatment on BEP-induced liver injury and fibrosis. Changes in Ishak liver fibrosis scores in control, BEP, and BEP+GSH groups. (a) Data are presented as mean  $\pm$  SD ( $n=8$  rats per group). Statistical comparisons were performed by one-way ANOVA (\*\* $p < 0.001$ , \*\*\*\* $p < 0.0001$ ). Representative liver sections (3  $\mu$ m-thick) stained with (b) hematoxylin and eosin and (c) Masson's trichrome (MT). In the MT-stained sections, collagen deposition in fibrotic areas is shown in blue. In the BEP group, increased fibrosis was observed, and GSH treatment partially attenuated these changes. Representative images under (b, c) 100 $\times$  magnification

BEP: Bleomycin, etoposide, and cisplatin, GSH: Glutathione, SD: Standard deviation, H&E: Hematoxylin and eosin

cell infiltration, and increased interstitial lung fibrosis were observed in the BEP group compared to the control group ( $p<0.0001$ ). The co-administration of GSH significantly reduced fibrosis ( $p<0.0001$ ) (Figure 2a). However, the fibrosis score of the BEP+GSH group was significantly higher in comparison to the control group ( $p<0.0001$ ) (Figure 2).

### Effects of GSH on renal injury

Kidney tissues were evaluated based on the severity of injury (Figure 3a) using H&E-stained kidney sections (Figure 3b). MT staining (Figure 3c) was performed to demonstrate fibrosis. The mean kidney injury scores were  $0.00\pm0.00$  in the control group,  $5.50\pm0.53$  in the BEP group, and  $3.75\pm0.71$  in the BEP+GSH group. Compared with the control group, inflammatory cell infiltration and areas of hyperemia were observed in the BEP group, contributing to a significantly higher renal injury score ( $p<0.0001$ ). GSH co-administration significantly mitigated bleeding, hyperemia, and lymphocytic infiltration, resulting in a significantly lower renal injury score than that in the BEP group ( $p<0.0001$ ) (Figure 3).

### Effects of GSH on skin fibrosis

Dermal thickness was evaluated as shown in Figure 4a. H&E and MT staining revealed increased collagen fiber accumulation and reduced dermal white adipose tissue in the BEP group, as shown in Figures 4b and 4c. The mean dermal thicknesses were  $203.90\pm26.83$   $\mu\text{m}$  in the control group,  $241.82\pm24.83$   $\mu\text{m}$  in the BEP group, and  $193.64\pm23.70$   $\mu\text{m}$  in the BEP+GSH group. Consistently, dermal thickness was significantly higher in the BEP group compared to the control and BEP+GSH groups

( $p<0.05$  and  $p<0.01$ , respectively). No statistically significant difference was detected between the control and BEP+GSH groups ( $p>0.05$ ).

### Effects of GSH on AST, ALT, and urea levels

AST and ALT levels were analyzed as indicators of hepatocellular integrity and potential liver injury. The mean AST levels were  $144.79\pm25.81$  U/L in the control group,  $142.61\pm8.31$  U/L in the BEP group, and  $105.76\pm14.71$  U/L in the BEP+GSH group. No significant change in AST levels was observed between the BEP and control groups ( $p>0.05$ ). However, a significant decrease in AST levels was found in the BEP+GSH group compared to the control and BEP groups, although the values remained within the reference range ( $p<0.01$  for both comparisons) (Figure 5a). The mean ALT levels were  $31.09\pm3.93$  U/L,  $29.49\pm4.57$  U/L, and  $33.08\pm4.46$  U/L in the control, BEP, and BEP+GSH groups, respectively. No statistically significant difference was observed in ALT levels among the groups ( $p>0.05$  for all comparisons) (Figure 5b).

Furthermore, blood urea levels were measured to evaluate renal function. The mean urea levels were  $13.11\pm2.41$  mg/dL in the control group,  $12.96\pm1.60$  mg/dL in the BEP group, and  $13.20\pm1.98$  mg/dL in the BEP+GSH group. Urea levels were similar among the groups ( $p>0.05$ ) (Figure 5c).

## Discussion

In this study, the concurrent administration of GSH significantly reduced the marked histopathological damage and fibrosis in the liver, lungs, kidneys, and skin caused by the BEP chemotherapy regimen. In contrast, serum AST, ALT, and

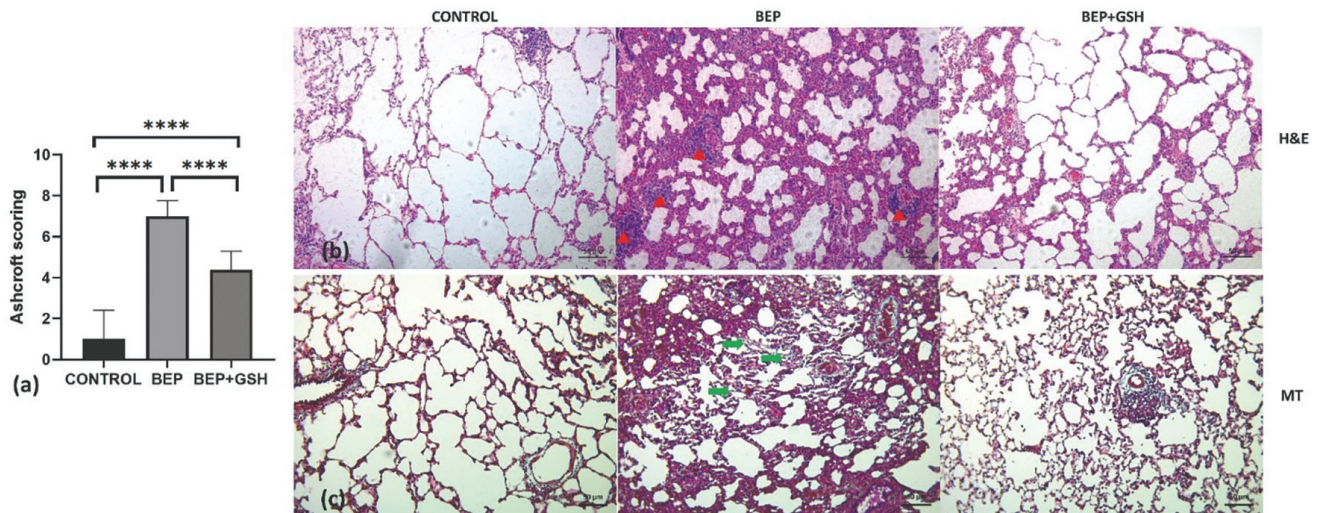


**Figure 2.** Effects of GSH treatment on BEP-induced lung damage and inflammation. Change in Ashcroft lung fibrosis scores in control, BEP, and BEP+GSH groups. (a) Data are presented as mean  $\pm$  SD ( $n=8$  rats per group). Statistical comparisons were performed by one-way ANOVA ( $****p<0.0001$ ). Representative lung sections (3  $\mu\text{m}$ -thick) stained with (b) hematoxylin and eosin (H&E) and (c) MT. In the H&E-stained sections, red triangles indicate inflammatory cell infiltration and alveolar edema. In the MT-stained sections, green arrows indicate fibrotic areas in the BEP group. In the BEP group, intense lymphocyte infiltration, alveolar edema, and an increase in fibrotic changes were observed, and GSH treatment resulted in a partial improvement in these changes. Representative images under (b, c) 100 $\times$  magnification

BEP: Bleomycin, etoposide, and cisplatin, GSH: Glutathione, SD: Standard deviation, MT: Masson's trichrom

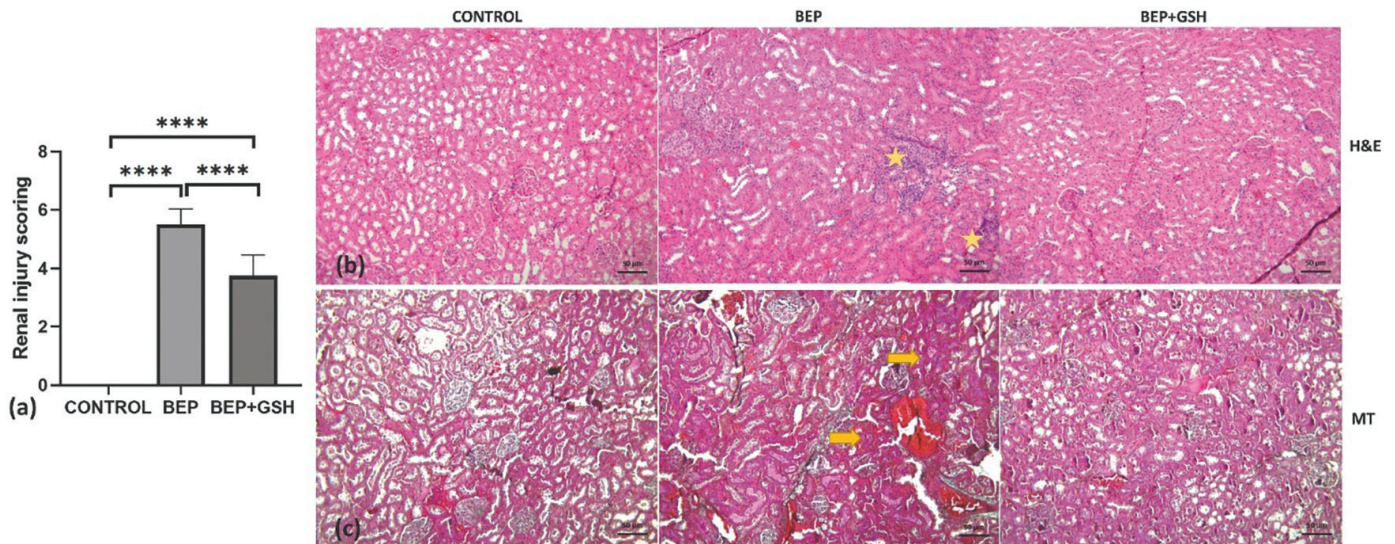


**Figure 3.** Effects of GSH treatment on BEP-induced renal damage and inflammation. Change in renal injury scores and histological analysis in control, BEP, and BEP+GSH groups. (a) Data are presented as mean  $\pm$  SD (n=8 rats per group). Statistical comparisons were performed by one-way ANOVA (\*\*p<0.01, \*\*\*p<0.001, \*\*\*\*p<0.0001). Representative renal sections (5  $\mu$ m -thick) stained with (b) H&E and (c) MT. In the H&E-stained sections, yellow stars indicate inflammatory cell infiltration. In the MT-stained sections yellow arrows indicate areas of hyperemia. In the BEP group, increased lymphocyte infiltration, hyperemia, and fibrotic changes were observed, and GSH treatment resulted in a partial improvement in these changes. Representative images under (b, c) 100 $\times$  magnification

BEP: Bleomycin, etoposide, and cisplatin, GSH: Glutathione, SD: Standard deviation, MT: Masson's trichrome, H&E: Hematoxylin and eosin, ANOVA: Analysis of variance

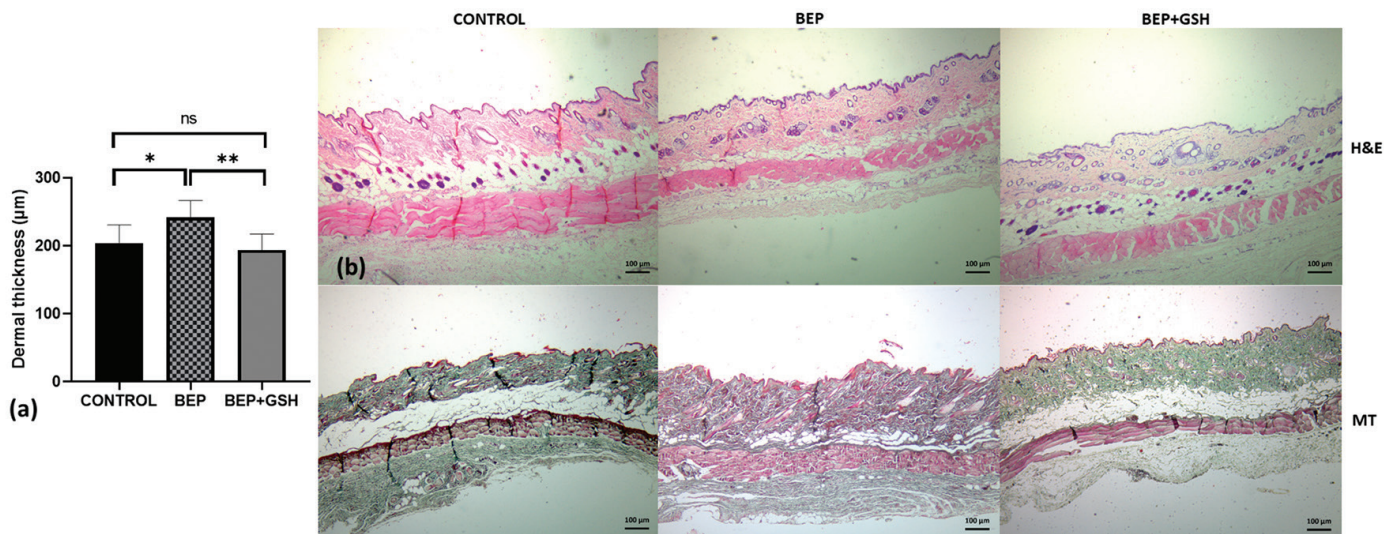


**Figure 4.** Effects of GSH treatment on BEP-induced skin damage and fibrosis. Changes in dermal thickness and histological analysis in control, BEP, and BEP+GSH groups. (a) Data are presented as mean  $\pm$  SD (n=8 rats per group). Statistical comparisons were performed by one-way ANOVA (\*p<0.05, \*\*p<0.01, ns: not significant). Representative cutaneous sections (5  $\mu$ m-thick) stained with (b) H&E and (c) MT. Dermal collagen fibrils in BEP-treated rats appear thicker than those in the control group, GSH treatment resulted in an improvement in these changes. Representative images under (b, c) at 40 $\times$  magnification

BEP: Bleomycin, etoposide, and cisplatin, GSH: Glutathione, SD: Standard deviation, MT: Masson's trichrome, H&E: Hematoxylin and eosin

urea levels did not change significantly between the groups. These findings suggest that GSH may play a beneficial role in suppressing oxidative damage and fibrogenesis caused by chemotherapeutic agents. The homogeneity of the experimental cohort likely reduced interindividual variability, supporting the reliability of the histopathological and biochemical

findings. Selecting 12-week-old male rats within a narrow weight range provided a stable physiological baseline and eliminated confounding factors, such as hormonal fluctuations or age-related metabolic differences. Furthermore, because no mortality occurred, the cohort maintained its complete representativeness.

Chemotherapeutic drugs cause significant damage to the histological architecture of many organs, such as the liver, lungs, kidneys, and skin (4,11,13,23). Bleomycin triggers ROS formation by chelating metal ions and interacting with intracellular water molecules, thereby causing DNA strand breaks. This DNA damage not only underlies its anticancer effect but also leads to histological alterations, such as cellular swelling and tissue injury, observable in affected organs (24). Etoposide, a topoisomerase II inhibitor, induces DNA breaks leading to cell death and aggravates tissue damage by generating ROS and activating MAPK signaling pathways, thereby promoting inflammation and fibrosis (25). The antineoplastic activity of cisplatin is mediated through its interaction with cellular DNA and subsequent induction of irreversible apoptosis (26).

GSH, a key intracellular tripeptide, plays a central role in neutralizing ROS and maintaining redox balance (27). Previous studies have reported that GSH supplementation may alleviate tissue damage and fibrotic changes induced by chemicals or chemotherapeutic agents through the suppression of fibrotic signaling pathways. It has also been reported to protect against cisplatin-induced nephrotoxicity without compromising the antitumor efficacy of the drug (17). Moreover, GSH may exert hepatoprotective effects by reducing liver injury and limiting pathological changes associated with the progression from NAFLD to NASH (18). Furthermore, in our previous study, GSH was shown to protect against BEP-induced reproductive system injury (20). However, to our knowledge, the potential effects of GSH on BEP-induced fibrosis and tissue damage in other organs have not yet been investigated. Thus, this study is the first to examine the potential beneficial effects of GSH on multiple organ fibrosis and histopathological damage caused by the BEP regimen.

In our study, GSH co-treatment with the BEP regimen substantially mitigated liver injury, as evidenced by reduced collagen deposition, limited portal tract expansion, and attenuated pericellular fibrosis around hepatocytes. Similar protective effects on liver fibrosis have been reported for other antioxidants, such as curcumin and quercetin (11,28). BEP-associated pulmonary fibrosis is a common complication associated with high mortality rates (13). Bleomycin, the key contributor to BEP-associated pulmonary fibrosis, induces toxicity through hydrolase deficiency, damage to type II alveolar cells, and recruitment of inflammatory cells into the lung parenchyma (24). In this study, GSH co-administration significantly reduced pulmonary fibrosis, inflammatory cell infiltration, and alveolar edema. The histological observations in our study suggest that GSH supplementation may attenuate BEP-induced organ fibrosis, possibly through its role in reducing OS. Previous studies have consistently demonstrated that modulation of OS through antioxidant supplementation can attenuate lung injury and fibrosis. For instance, Serrano-Mollar et al. (29) reported that N-acetylcysteine reduced bleomycin-induced pulmonary fibrosis in rats, while Zeng et al. (30) showed that bergenin alleviated fibrosis in both mice and rats. Similarly, Bakirhan and Parlar (31) evaluated glabridin and dexamethasone in bleomycin-induced pulmonary fibrosis and found increased GSH levels in bronchoalveolar lavage fluid, which was associated with improved lung function. Another finding of this study is that GSH co-administration markedly ameliorated renal damage, as evidenced by reduced inflammatory cell infiltration, hyperemia, and hemorrhage. Similarly, Bartani et al. (4) demonstrated that administration of another antioxidant, curcumin, to rats with BEP-induced renal injury reduced hemorrhage, hyperemia, and

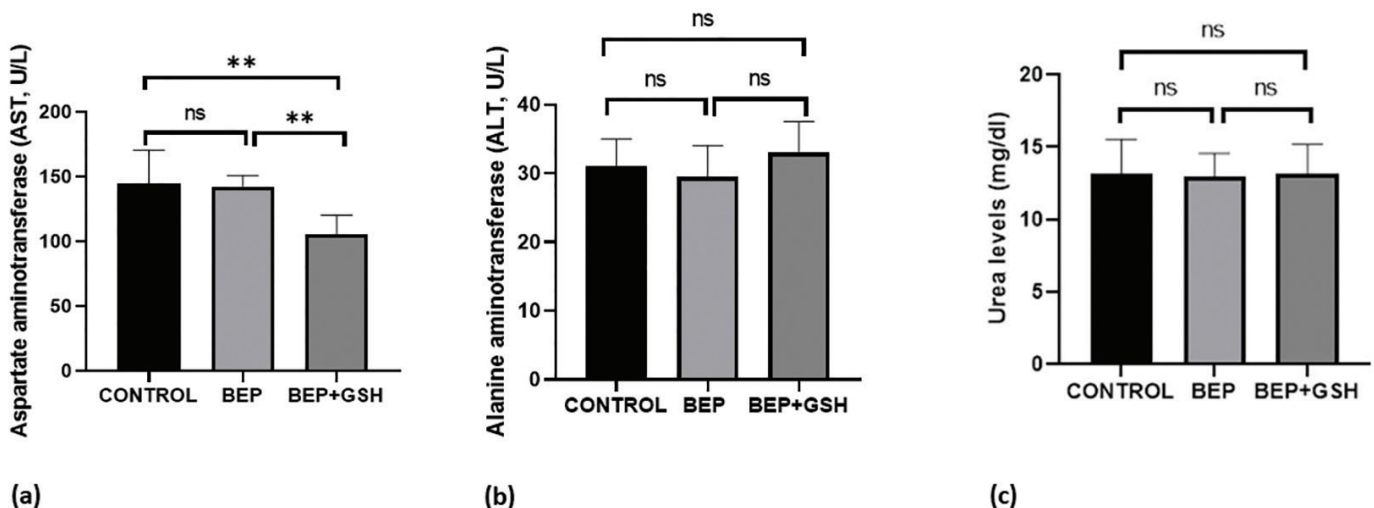


**Figure 5.** Serum (a) AST levels (U/L), (b) ALT levels (U/L), and (c) serum urea levels (mg/dL) in control, BEP, and BEP+GSH groups. Data are presented as mean  $\pm$  SD (n=8 rats in each group). Statistical comparisons were performed by one-way ANOVA. (\*\*p<0.01), ns: not significant. AST and ALT levels were within the reference ranges for all groups. While there was a slight decrease in AST values in the BEP+GSH group compared to the control and BEP groups, no change was detected in ALT values

BEP: Bleomycin, etoposide, and cisplatin, GSH: Glutathione, SD: Standard deviation, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase

lymphocytic infiltration. Zahran et al. (32) also demonstrated that the nanoform antioxidant N, N'-diphenyl-1,4-phenylenediamine (Nano-DPPD) mitigates cisplatin-induced renal injury. Gad et al. (33) reported that naringin, a flavonoid with well-known antioxidant properties, exerted a protective effect against doxorubicin-induced nephrotoxicity and reduced renal injury. Moreover, Zhao et al. (34) highlighted the therapeutic potential of natural compounds—such as glycosides, flavonoids, phenolic compounds, anthraquinones, and terpenoids—in the prevention of renal injury.

Quantitative analysis of skin histology revealed a significant increase in dermal thickness in the BEP group compared with the control group. Bleomycin is supported by multiple studies as the principal driver of BEP-associated cutaneous fibrosis, owing to low bleomycin-hydrolase activity in skin and evidence from bleomycin-induced dermal fibrosis models (35). In a recent study, Rosa et al. (23) reported that bleomycin treatment resulted in extensive replacement of dermal white adipose tissue with collagen-rich ECM. In our study, GSH co-administration effectively prevented the increase in skin thickness, maintaining it at levels similar to that of the control group. Similar to our findings, phytoestrogens daidzein and genistein, which are well known for their antioxidant, anti-inflammatory, and antiangiogenic properties, have been shown to reduce bleomycin-induced skin fibrosis (10,35).

ALT levels were similar across all groups, while AST levels were slightly lower in the BEP+GSH group relative to both the control and BEP groups. Nonetheless, both markers remained within the physiological reference ranges for rats in all groups (6-114 IU/L and 37-205 IU/L, respectively) (36). Serum AST and ALT levels primarily reflect hepatocellular injury and necroinflammatory activity rather than hepatic fibrosis. Therefore, the absence of significant changes in these enzymes does not exclude the presence of early fibrotic alterations. Adams et al. (37) reported that the comparison of the ALT levels and AST levels of patients with and without progressive fibrosis revealed no statistically significant alterations in the magnitude of change. Moreover, they found that changes in ALT and AST levels were not associated with alterations in fibrosis stage (37). Additionally, the study by Diaz et al. (38) showed that AST and ALT levels remained unchanged even in patients with advanced fibrosis and cirrhosis. According to the American Association for the Study of Liver Diseases Guideline published in 2023, although serum AST levels are frequently utilized in clinical settings for the identification of patients with liver disease, it is noteworthy that these levels may be within normal limits in individuals with diabetes, NASH, and advanced hepatic fibrosis. The guidance clarifies that AST levels are not sensitive or specific for identifying NAFLD/NASH with advanced fibrosis, and intermittently elevated ALT or AST may indicate chronic liver

injury (39). Urea levels, which were measured to examine kidney function, were within the reference range for all rats, and no significant differences were among the groups. In agreement with our results, George et al. (40) demonstrated that cisplatin treatment induced kidney injury, although conventional biomarkers such as serum creatinine and blood urea nitrogen remained largely unchanged. While Bartani et al. (4) reported increased urea levels in rats treated with the BEP combination, their protocol utilized considerably higher doses than those in our study. In their study, co-administration of the antioxidant nanocurcumin attenuated this effect, resulting in lower urea levels in comparison to the BEP group.

A noteworthy strength of this study is that, while the intracellular antioxidant and protective effects of GSH supplementation against various chemical toxicities have been previously investigated, this is the first study to evaluate the effects of exogenous GSH supplementation against the organ damage induced by the BEP regimen, which is the standard clinical treatment for testicular and ovarian cancers. The well-controlled experimental design and the concurrent evaluation of multiple organs further strengthen the validity of the findings.

### Study Limitations

Nevertheless, this study has several limitations. Primarily, the use of a male rat model and a dosing regimen that does not fully mimic clinical practice limits the direct translation of these findings to humans. Additionally, because the study primarily focused on histopathological and biochemical assessments, specific molecular markers of fibrosis and OS, including TGF- $\beta$ ,  $\alpha$ -SMA, collagen I, and other OS biomarkers, as well as inflammatory cytokines and downstream molecular pathways involved in fibrogenesis, were not evaluated.

### Conclusion

GSH administration reduced histopathological changes associated with BEP, such as inflammation and interstitial edema, as well as the fibrotic response, thereby helping to preserve tissue integrity in rats exposed to BEP. These findings contribute to our understanding of the effects of GSH on BEP-induced tissue injury and fibrosis in this experimental model. Human studies are required to assess the clinical applicability of GSH.

### Ethics

**Ethics Committee Approval:** All experimental methods were performed in accordance with the guidelines approved by the Maltepe University Experimental Animal Local Ethics Committee (approval no: 2024.02.03, approval date: February 29, 2024).

**Informed Consent:** Not applicable.

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## Footnotes

### Authorship Contributions

Surgical and Medical Practices: H.B., Y.D.Ç., S.G., Concept: H.B., Y.D.Ç., S.G., Design: H.B., Y.D.Ç., S.G., Ş.Y., Data Collection or Processing: H.B., Y.D.Ç., S.G., Ş.Y., Analysis or Interpretation: H.B., Y.D.Ç., Literature Search: H.B., Y.D.Ç., M.C., Writing: H.B., Y.D.Ç., S.G., Ş.Y., M.C.

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