JBAAR

Journal of Bioscience and Applied Research
https://jbaar.journals.ekb.eg

SPBH

Biobran/MGN-3: A Promising Natural Strategy for Mitigating Kidney Damage and Immune Modulation During Etoposide Therapy

Ali M. Eldib[1], Haytham M. Abdellatif[2,3], Osman Suliman[2], Faris M. Elmahdi[2], Ibrahim H. Babikir[2], Shimaa B. Hemdan[2,4], Reem M. Emam[2,5], Mohamed S. El-Gerbed[1], Mamdooh H Ghoneum[6,7], Attalla F. El-kott[1,8], Sara M. Altom[2]

[1] Department of Zoology, Faculty of Science, Damanhur University, Damanhur, Egypt.

[2] Al Rayan National College of Medicine, Al-Madinah, P.O. Box 41411, Hijrah Street, Madinah, Kingdom of Saudi Arabia

[3] Department of Pharmacology, Sohag Faculty of Medicine, Sohag University, Egypt

[4] Biochemistry Department, Faculty of medicine, Sohag university, Egypt

[5] Physiology Department, Mansoura University, Egypt

[6] Department of Surgery, Charles Drew University of Medicine and Science, Los Angeles, CA 90059, USA.

[7] Department of Surgery, University of California, Los Angeles, Los Angeles, CA 90095, USA

[8] Department of Biology, College of Science, King Khalid University, Saudi Arabia

DOI:10.21608/jbaar.2025.434075

Abstract

Although chemotherapy is one of the pillars in cancer treatment, its side effects have extremely harmful impacts on many of the body's organs. This study investigates the potential protective effect of the natural product, Biobran/MGN-3, against kidney damage and immune modulation caused by the chemotherapeutic agent Etoposide in rats. Etoposide impairs kidney function, demonstrated by high levels of urea, and imbalances in electrolytes Na and K, and low calcium levels. Etoposide also increased parameters reflecting oxidative stress, malondialdehyde (MDA) and nitric oxide (NO), while reduced the enzyme-mediated antioxidant defense activity, Glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT). Immunostaining of kidney tissues revealed that Etoposide caused inflammation by increasing pro-inflammatory cytokines IL-1β, TNF-α, and TGF-β expression. In addition, it increased apoptosis by raising levels of Bax, Caspase-3, and Cytochrome C, which was evident by qPCR. Comet assay revealed DNA damage in kidney cells following Etoposide treatment. It was evident by flow cytometry that Etoposide increased CD8, CD4, CD3, and CD19, which indicates lymphocyte infiltration into the kidney.

On the other hand, Biobran/MGN-3 helped protect kidney function, lowering oxidative stress, restoring antioxidant enzyme activity, and reducing pro-inflammatory substances. Biobran/MGN-3 also counteracted the Etoposide-induced increase in Bax, Caspase-3, and Cytochrome C, lowering the cell death rate in the kidneys. Additionally, the results showed that Biobran/MGN-3 adjusted immune cell levels by increasing CD8+, CD4+, CD3+, and CD19+ cells, boosting both T-cell and B-cell activity.

These findings suggest that Biobran/MGN-3 could be a helpful treatment addition to reduce kidney damage and mitigate immune impairment from Etoposide.

Keywords: Biobran, Etoposide, impaired immunity, nephrotoxicity, qPCR, Comet assay, flow cytometry

Received: February 1, 2024. Accepted: May 5, 2025. Published: June 19, 2025

1. Introduction

Chemotherapy, commonly used in cancer treatment, despite its potential to effectively treat cancer by killing cancer cells, is known for its harmful side effects on various organs, including the kidneys (1). Moreover, the toxic effects caused by the metabolism of chemotherapeutic drugs may contribute to the development of secondary pathologic conditions, such as acute kidney injury (AKI) and immune-related nephritis (2).

Etoposide is a chemotherapeutic agent employed in the therapeutic management of multiple malignancies, including lung cancer as well as lymphomas. It is also used for Hodgkin's lymphoma, AIDS, and cancers of the reproductive organs (3). Etoposide exerts its anticancer effects by targeting topoisomerase II, inducing DNA double-strand breaks, and triggering programmed cell death (apoptosis) in highly proliferative cancer cells, but it can cause significant off-target effects (4). In several studies, it has also been proven that topoisomerase II inhibitors like etoposide, Teniposide, and Doxorubicin lead to potential renal damage and nephrotoxicity during their metabolism in the kidney (5). In addition, Etoposide treatment has been associated with elevated levels of blood urea nitrogen (BUN) and proteinuria, indicators of kidney stress (6), nephrotoxicity (7), elevated serum creatinine levels, and acute kidney injury in some patients (8). Furthermore, etoposide has been linked to immune system modulation, which can exacerbate kidney dysfunction (9).

Review studies mentioned guidelines that suggest reducing the dose of etoposide for patients with kidney issues. This shows that it's important to monitor kidney function and adjust the dosage accordingly (10). The nephrotoxic and immunomodulatory effects of etoposide cause great difficulty in the management of patients suffering from cancer, which presents a significant challenge in creating chemotherapy protocols for patients with pre-existing kidney conditions (11).

To lessen the impact of these adverse effects, combining pharmaceutical treatments with natural or synthetic agents has become a recommended practice (12). Recent studies have revealed the potential protective role of Biobran/MGN-3, a modified arabinoxylan derived from rice bran, in counteracting the nephrotoxicity and immune dysregulation caused by etoposide (13).

Research studies indicate that Biobran enhances immune function by augmenting the activity of key immune cells, such as T lymphocytes, B lymphocytes, natural killer (NK) cells, and macrophages. This immunostimulatory effect suggests its potential as an adjuvant therapy to support immune competence in patients undergoing chemotherapy. Previous research demonstrated that Biobran improved the immune response and protected against organ damage in different cancers and in other diseases (14). It was also found that Biobran can modulate cytokine production and enhance the activity of T cells and macrophages, further asserting its role in immune support during chemotherapy (15, 16).

Given Biobran's immunomodulatory effects and potential to reduce organ toxicity caused by chemotherapy, our study aims to investigate its protective effects on the kidneys against the nephrotoxic effects of Etoposide treatment. By exploring Biobran’s capacity to lessen etoposide-induced kidney damage and immune suppression, this research will help provide a new therapeutic strategy for improving kidney function and immunologic resilience in patients undergoing etoposide chemotherapy. This combination of Biobran and etoposide holds potential for improving treatment outcomes, minimizing nephrotoxicity, and enhancing immune function, ultimately benefiting patients suffering from cancer and kidney diseases.

2. Materials and Methods

2.1 Drugs and Chemicals

Etopul 100 mg/5 mL (Etoposide) solution vial for infusion was obtained from EIMC Pharmaceuticals Company (Cairo, Egypt) (Product Code: 11204). All other reagents employed in this investigation were obtained from commercial sources and met analytical-grade standards, ensuring optimal purity for experimental use.

2.2 Biobran/MGN-3

Biobran is a bioactive compound derived from the hydrolysis of Shiitake mushroom rice bran using enzymes. Its major chemical structure is arabinoxylan, having an xylose backbone and an arabinose side chain. The biobran utilized in this investigation was freshly produced in a 0.9% saline solution and administered for six weeks. It was provided by Daiwa Pharmaceutical Co., Ltd. in Tokyo, Japan.

2.3 Animals

A group of forty healthy adult male albino rats was used. They were procured from the National Research Center's Animal House in El-Dokki, Cairo, Egypt. They were between 6-7 weeks of age and weighing between 135±5 grams. The rats were raised in a pathogen-free environment and were housed in sterile plastic cages. They were given an unlimited supply of tap water and a conventional pellet diet. For two weeks before the trial, the temperature was kept at 24 ± 2 °C with a relative humidity of 60-70% and a 12-hour light-dark cycle.

Ethical approval:

The study protocols were ethically approved by the Research Ethics Committee of the Faculty of Science, Damanhour University, Egypt, under the number: DMU-SCICSRE-240204.

2.4 Experimental design

The rats were randomly divided into four groups, each group made of 10 rats: (G1) Control Group, receiving no etoposide or Biobran; (G2) Biobran Group, receiving intraperitoneal injections of Biobran at 40 mg/kg body weight every two days for six weeks; (G3) Etoposide Group, receiving intraperitoneal injections of etoposide at 1 mg/kg body weight daily for six weeks; and (G4) Dual treatment, receiving both Biobran and etoposide at the same dosages as G2 and G3.

2.5 Sample collection

Following the completion of the experimental period, rats underwent an overnight fasting period and were subsequently anesthetized using inhalant anesthesia with isoflurane.

2.6 Blood collection

Essential materials included anesthetics, sterile solutions, disinfectants, collection tubes, needles, and warming devices. The rats were immobilized under anesthesia, ensuring a lack of response to stimuli, and the tail was warmed to dilate blood vessels. Blood was collected from the tail vein using sterile needles, avoiding bone injury, and stored in sterile tubes for analysis, with the volume tailored to experimental requirements.

2.7 Kidney tissues

The kidneys were dissected, washed using phosphate-buffered saline (PBS) to wash away red blood cells, and divided into portions for analysis. Sections for apoptotic marker analysis (Bax, caspase 3, Cytochrome C, PD-1, and TNF-α) were stored at -80 °C. Other sections were similarly stored for evaluating cytokines, antioxidants, and oxidative stress indicators.

2.8 Tissue Homogenate Preparation

Frozen renal tissues were homogenized in cold lysis buffer (100 mM potassium phosphate, containing 2 mM EDTA), centrifuged for 15 min, and stored at -20°C for further use after homogenization.

2.9 Quantitative assessment of renal biomarkers in rat serum

Plasma levels of urea and creatinine (protein metabolism markers) and electrolyte balance indicators (sodium, potassium, and calcium) were assessed using standardized protocols to ensure measurement validity of renal health across treatment

groups (17). Each assay was performed in triplicate, and any outliers were retested to guarantee data reliability. The parameters were measured with a Photometer 5010 v5 (RIELE GmbH & Co KG, Berlin, Germany), following standardized techniques and the manufacturer's instructions. **Urea:** Urea/BUN Liquizyme (Modified Urease-Berthlot Method), Spectrum Diagnostics, MOSS GmbH, Schiffgraben 41, 30175 Hannover, Germany. **Creatinine:** Jaffe, Spectrum Diagnostics, MOSS GmbH, Schiffgraben 41, 30175 Hannover, Germany. **Sodium** (Enzymatic Method): Linear Chemicals, S.L.U. Joaquim Costa 18, 2ª planta, 08390 Montgat (Barcelona), Spain. **Potassium:** (Enzymatic Method), Linear Chemicals, S.L.U. Joaquim Costa 18, 2ª planta, 08390 Montgat (Barcelona), Spain. **Calcium:** Calcium (Colorimetric Method, O-Cresolphthalein Complexone), Randox Laboratories Ltd., 55 Diamond Road, Crumlin, County Antrim, BT29 4QY, United Kingdom.

2.10 Determination of oxidative stress and antioxidant biomarkers in kidney tissues

The concentrations of malondialdehyde (MDA) and nitric oxide (NO) were determined using the previously published thiobarbituric acid-reactive substances (TBARS) assay (18). The activities of glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT) were assessed using previously described techniques (19).

2.11 Expression of immunohistochemistry of IL-1β, TNF-α and TGF-β in liver

Paraffinized kidney tissue sections, histologically acquired using a manual microtome, were processed using the streptavidin-biotin peroxidase method for immunohistochemistry. Sections were collected on slides treated with 3-aminopropyltriethoxysilane adhesive solution, improving tissue adhesion by overnight oven incubation at 60 °C. Deparaffinization was carried out with xylene, followed by ethanol hydration in descending concentrations. Endogenous peroxidase was blocked with three 15-minute incubations in 3% hydrogen peroxide. Retrieval of antigens was done using a 0.1 M citrate buffer and Tween 20 at 90 °C for 20 min. Sections were subsequently permeated with a 0.1% saponin solution, blocked in skimmed milk, and incubated with primary antibodies overnight at 4°C. Sections were then washed and treated with secondary antibody and streptavidin-peroxidase complex for 30 minutes each at 37 °C. Diaminobenzidine chromogen solution was applied, followed by counterstaining with Harris hematoxylin. Sections were dehydrated in ethanol and prepared for cytokine analysis through immunohistochemistry (20).

2.12 DNA damage by comet assay

In the comet assay described by Collins et al. (2023), the kidney tissues were minced in an ice-cold buffer (2ml PBS containing EDTA-Na2 and DMSO) to get a cell suspension, cells were mixed with molten low melting point agarose, pH 7.4 at 37 °C and applied to microscope slides then embedded in a low-melting agarose gel. The slides had been immersed in a cold lysis solution (Tris-HCl [pH 10], NaCl, EDTA-Na2, freshly added 1% Triton X-100, and DMSO for 60 min at 4 °C in the dark to remove cell membranes, leaving the DNA and nuclear structures.

The slides were set in an electrostatic precipitation chamber with prechilled electrophoretic buffer (NaOH and EDTA-Na2 (pH > 13)) to unwind the DNA for 20 minutes at 25 volts. The slides were cleaned three times for five minutes with neutralizing buffer (0.4 M Tris-HCl, pH 7.5). A fluorescent dye, ethidium bromide, was applied for staining, then the slides were visualized under a fluorescence microscope, where damaged DNA appears as a "comet tail," as the length and intensity of the tail indicate the degree of DNA damage. The Komet Assay V software (Perspective Instruments) is then used to analyze the images, quantifying comet elements (tail length (µm), tail DNA (%), tail moment, tailed cells (%), and untailed cells) in kidney cells. to measure DNA strand breaks and damage (21)

2.13 Quantitative Real-Time PCR (qPCR)

Primer Design:

Gene	*forward (5'->3)*	*reverse (5'->3)*	*Accession number*
β-actin (Actb)	ATGTGGCTGAGGACTTTGATT	ATCTATGCCGTGGATACTTGG	XM_039089807.1
Bax	CACGTCTGCGGGGAGTC	CCTGGATGAAACCCTGTAGC	NM_017059.2
Caspase3	CTTGGAACGCGAAGAAAAGT	AGCCCATTTCAGGGTAATCC	NM_012922.2
Cytochrome C	GAAAGGGCAGACCTAATAGC	TTACTTAAATCGGGGCTGTCC	NM_012839.2
PD-L1	CTCGCCTACAGGTAAGTCT	TGTGATGGTAAATGCCGCTA	NM_001191954.2
TNF-α	CTACTGAACTTCGGGGTGAT	TGATCTGAGTGTGAGGGTCT	NM_012675.3

Kidney cells were cultured in DMEM with 10% FBS and 1% penicillin-streptomycin, maintained at 37°C in 5% CO_2. At 80% confluence, cells were treated for 24 hours in four groups: (1) untreated control, (2) Biobran (40 mg/kgm), (3) Etoposide (1mg/kgm), and (4) combination of Biobran and Etoposide. RNA was extracted using TRIzol, quantified with NanoDrop, and reverse transcribed into cDNA. Quantitative real-time PCR (qPCR) was performed with SYBR Green Master Mix to measure mRNA levels of apoptotic markers (Bax, Caspase-3, Cytochrome c), immune checkpoint protein PDL-1, and pro-inflammatory cytokine TNF-α. Gene expression was analyzed using the 2^-ΔΔCt method with β-actin as the control, and results from three distinct experiments were presented as mean ± SD. (22).

2.13 Flow cytometric analysis:

Flow cytometry was used for detecting apoptosis markers for T cell tags CD8, CD4, CD3, CD3, 4, and CD19. Kidney cells were initially fixed and permeabilized to preserve cellular structure and enable the detection of markers. Following blocking to minimize nonspecific binding, cells were stained with fluorescently labeled primary antibodies against CD8, CD4, CD3, CD3, 4, and CD19 molecules. Unbound antibodies were washed away before secondary antibody staining. CD8, CD4, CD3, CD3,4, and CD19. were dialyzed against coupling and after adding SO pmol/L FITC isomer I (Sigma) to the buffer (S0 mmol/L sodium borate/NaOH, pH 9.0, 150 mmol/L NaCl, and 1 mmol/L EDTA), the mixture underwent incubation for two hours at 37 °C. Glycine at a concentration of 100 mmol/L was then added to halt the coupling reaction. After additional washing steps, the labeled cells were examined utilizing flow cytometry, and the data were used to determine the percentage of cells positive for CD8, CD4, CD3, CD3, 4, and CD19. The mixture was first dialyzed against 50 mm VL Tris/HCI, pH 8.0.80 mmol/L NaCl, and 1 mmol/L EDTA and then applied to a Mono Q column (Pharmacia; Uppsala, Sweden). The identified proteins were extracted using a NaCl gradient. Protein concentrations and absorbance were determined at 492 nm. To detect phosphatidylserine exposure on cells, FITC-labeled was administered at a final concentration of 2.5 pg/mL to cells incubated in HEPES buffer (IO mmol/L). (23).

2.14 Statistical analysis:

Statistical analysis was done using the SPSS program (version 26). The data are calculated and presented as means associated with the standard

deviation (SD). The normality was tested using the Kolmogorov–Smirnov test, and the data were normally distributed. The difference among the groups has been determined using one-way ANOVA (F test) and post hoc Duncan's test. Statistical significance was defined as a P-value below 0.05.

3. Results:

3.1 *Biochemical analysis for kidney function*

The results are summarized in **Table 1**, with significant differences ($p < 0.05$) between the groups indicated by superscript numbers.

Urea:

The control group (G1) had a mean urea level of 16.3 ± 1.5 mg/dL. Treatment with Etoposide (G3) caused a significant rise in urea levels (24.3 ± 3.0 mg/dL) in comparison with the control ($p < 0.05$). Biobran-treated rats (G2) showed urea levels of 17.6 ± 1.5 mg/dL, which showed no significant difference from the control group. The Dual treated group (G4) had urea levels of 18.0 ± 3.0 mg/dL, which were significantly less than the Etoposide group ($p < 0.05$) but non significantly higher than the control.

Creatinine:

Intergroup comparisons revealed no significant differences in serum creatinine concentrations. The control group (G1) had a creatinine level of 0.34 ± 0.02 mg/dL, while the Biobran (G2), Etoposide (G3), and Dual treated (G4) groups had creatinine levels of 0.41 ± 0.005 mg/dL, 0.40 ± 0.05 mg/dL, and 0.37 ± 0.04 mg/dL, respectively.

Sodium (Na):

Sodium levels in the control group (G1) were 146.3 ± 3.5 nmol/L. Etoposide treatment (G3) significantly reduced sodium levels to 126.3 ± 5.1 nmol/L ($p < 0.05$). Sodium levels in the Biobran group (G2) were 141.6 ± 3.2 nmol/L, and in the Dual treated group (G4), sodium levels were 141.3 ± 3.0 nmol/L, both of which were significantly different from the Etoposide group ($p < 0.05$).

Potassium (K):

The control group (G1) had a potassium level of 4.9 ± 0.2 nmol/L. Etoposide treatment (G3) significantly elevated potassium levels to 6.1 ± 0.05 nmol/L ($p < 0.05$). Potassium levels in the Biobran group (G2) were 4.8 ± 0.2 nmol/L, and in the Dual treated group (G4), levels were 4.8 ± 0.4 nmol/L, both significantly less than the Etoposide group ($p < 0.05$).

Calcium (Ca):

Calcium levels in the control group (G1) were 9.5 ± 0.5 nmol/L. Etoposide treatment (G3) significantly decreased calcium levels to 7.6 ± 0.4 nmol/L ($p < 0.05$), as indicated by superscript [1]. Biobran-treated rats (G2) had calcium levels of 9.4 ± 0.3 nmol/L, and the Dual-treated group (G4) had levels of 9.3 ± 0.3 nmol/L, both of which exhibited significantly greater levels than the Etoposide group ($p < 0.05$).

3.2 *Quantitative analysis of renal oxidative stress biomarkers in different groups*

The oxidative stress markers Malondialdehyde (MDA) and Nitric Oxide (NO) levels were assessed in rat kidney tissue following treatment with Biobran, Etoposide, or a combination of both (Dual). The results are summarized in **Table 2,** with significant differences between groups indicated by superscript numbers.

Malondialdehyde (MDA):

MDA levels in the control group (G1) were 2.7 ± 0.4 nmol/g tissue. Etoposide treatment (G3) caused a significant elevation in MDA levels, reaching 4.6 ± 0.5 nmol/g tissue relative to the untreated control group ($p < 0.05$). The Biobran group (G2) had MDA levels of 2.3 ± 0.1 nmol/g tissue, which showed a significant decrease as contrasted with the Etoposide group ($p < 0.05$), though not statistically different from controls. The Dual treated group (G4) had MDA levels of 2.5 ± 0.2 nmol/g tissue, demonstrating significantly reduced levels relative to the Etoposide-treated cohort ($p < 0.05$), while remaining comparable to control values.

Nitric Oxide (NO):

The NO levels in the control group (G1) were 25.0 ± 3.0 nmol/g tissue. Etoposide treatment (G3)

significantly increased NO levels to 43.0 ± 4.5 nmol/g tissue ($p < 0.05$). The Biobran-treated group (G2) exhibited NO levels of 24.6 ± 1.5 nmol/g tissue, which showing marked significant reduction than the Etoposide group ($p < 0.05$), and comparable to the control group. The Dual treated group (G4) had NO levels of 29.6 ± 2.0 nmol/g tissue, with values significantly diminished versus the Etoposide group ($p < 0.05$) but remained elevated non significantly compared to the control group.

3.2 *Quantification of antioxidants SOD, CAT and GSH in rat kidney*

The levels of antioxidant markers—superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH)—in the rat kidney were assessed across four experimental groups: the control group (G1), the Biobran-treated group (G2), the Etoposide-treated group (G3), and the group receiving both Biobran and Etoposide (Dual treatment, G4). The results of the ELISA assays are detailed in **Table 3**.

Superoxide Dismutase (SOD)

The control group (G1) showed an average SOD activity of 41.3±3.0 nmol/g protein, while the Biobran-treated group (G2) had slightly higher SOD levels (45.6±4.0 nmol/g protein). The Etoposide-treated group (G3) exhibited a significant reduction in SOD activity (20.6±2.5 nmol/g protein), exhibiting statistically lower values than both the control and Biobran-treated groups ($p<0.05$). The group receiving dual treatment (G4) displayed a moderate recovery in SOD activity (31.3±3.0 nmol/g protein), which was significantly higher than the Etoposide group.

Catalase (CAT)

The control group (G1) had a mean CAT activity of 37.6±5.0 nmol/g protein, with the Biobran group (G2) showing a similar level of activity (35.0±3.0 nmol/g protein). The Etoposide group (G3) demonstrated a marked reduction in CAT levels (13.0±2.6 nmol/g protein), significantly lower than both the control and Biobran groups ($p<0.05$). The dual treatment group (G4) showed a partial improvement in CAT activity (25.6±2.0 nmol/g protein), displaying a significant elevation relative to Etoposide treatment ($p<0.05$).

Glutathione Peroxidase (GSH)

GSH levels in the control group (G1) were 2.9±0.5 nmol/g protein, similar to the Biobran-treated group (G2) (2.8±0.4 nmol/g protein). The Etoposide-treated group (G3) exhibited significantly reduced GSH activity (1.3±0.2 nmol/g protein) versus both control and Biobran groups ($p<0.05$). The dual treatment group (G4) had GSH levels of 2.7±0.2 nmol/g protein, indicating a marked recovery as contrasted with the Etoposide group ($p<0.05$).

3.3 *Immunohistochemical analysis of proinflammatory cytokines*

Figure 1 shows immunohistochemical analysis of IL-1β, TNF-α, and TGF-β expression in rat kidney tissues.

Control and Biobran-treated Groups

In the control group, the minimal expression of IL-1β, TNF-α, and TGF-β was observed, with only a few cells showing positive staining (Figures A1, A2, and A3 representing the control and B1, B2, and B3 representing the Biobran-treated group). This indicates a baseline level of inflammatory markers in healthy kidney tissues.

Etoposide Group

The etoposide-treated rats showed a statistically significant upregulation of IL-1β, TNF-α, and TGF-β as contrasted with the baseline control group ($p < 0.05$). Specifically, Figures C1, C2, and C3 illustrate that the majority of kidney cells demonstrated heightened immunoreactivity for these inflammatory markers, indicating that etoposide induces nephrotoxic effects characterized by increased inflammation.

Dual Treatment Group

In contrast, rats that received both etoposide and Biobran treatment showed a significant reduction in IL-1β, TNF-α, and TGF-β expression relative to the

etoposide-only group ($p < 0.05$). Figures D1, D2, and D3 demonstrate that Biobran treatment mitigated the inflammatory response induced by etoposide, as evidenced by the reduced number of positively stained cells for these markers.

3.4 *DNA damage in renal cells by comet assay*

The comet assay illustrated in **Table 4** and **Figure 2** was conducted to assess DNA damage in kidney cells across the four experimental groups.

Tail Length (µm)

The average tail length, a measure of DNA strand breaks, was significantly increased in the Etoposide-treated group (15.4 ± 2.0 µm) compared to the control (5.2 ± 1.1 µm, $p < 0.05$). Both the Biobran (7.8 ± 1.5 µm) and Dual treatment (12.3 ± 1.8 µm) groups also showed increases in tail length relative to the control, but these increases were less pronounced (nonsignificant) than in the Etoposide group.

Tail DNA (%)

The tail DNA% was statistically higher in the Etoposide group (30.5 ± 4.1%, $p < 0.05$) in comparison with the control (10.3 ± 2.0%, $p < 0.001$). The Biobran group (15.6 ± 3.2%) and the Dual treated group (25.1 ± 3.7%) also exhibited increased tail DNA, though the Dual treated group showed a partial reduction compared to Etoposide alone.

Tail Moment

The tail moment, which combines tail length and the DNA% in the tail, was significantly elevated in the Etoposide group (3.67 ± 0.45, $p < 0.05$) relative to the control group (0.53 ± 0.12, $p < 0.001$). Both the Biobran (1.02 ± 0.22) and Dual treated (2.56 ± 0.30) groups showed increased tail moments, with the Dual treated group exhibiting significant improvement compared to Etoposide alone.

Tailed Cells (%)

A significantly higher percentage of cells exhibited DNA damage (tailed cells) in the Etoposide group (45% ± 5%) in contrast with the control group (12% ± 3%, $p < 0.05$). The Dual treated group (38% ± 4%) also exhibited an increased percentage of tailed cells but was reduced compared to Etoposide alone, while the Biobran group (20% ± 4%) showed a mild increase over the control group.

Untailed Cells (%)

The percentage of untailed cells, indicating intact DNA, was significantly decreased in the Etoposide group (55% ± 5%) than the control group (88% ± 3%, $p < 0.05$). The Dual-treated group (62% ± 4%) showed a partial recovery, while the Biobran group (80% ± 4%) maintained a higher percentage of untailed cells, closer to the control group.

3.4 *qPCR analysis for the expression of apoptotic markers, immune checkpoint proteins, and pro-inflammatory cytokines in kidney cells*

The qPCR analysis of kidney cells subjected bo Biobran, Etoposide, or a combination of both demonstrated significant differential expression of apoptotic markers, immune checkpoint proteins, and pro-inflammatory cytokines **Table 5.**

Apoptotic Markers:

Bax: The Control group results revealed Bax expression of 1.04 ± 0.03, while Etoposide significantly upregulated Bax expression (3.94 ± 1.07, $p < 0.05$), indicating enhanced apoptosis. In contrast, Biobran (1.03 ± 0.02) did not significantly alter Bax levels. The Dual Treatment group significantly downregulated compared to Etoposide (2.03 ± 0.31, $p < 0.05$).

Caspase-3: The expression of Caspase-3 was 1.09 ± 0.06 in the control. A significant increase in Caspase-3 expression (2.96 ± 0.26, $p < 0.05$) was observed following Etoposide treatment compared to the control, confirming apoptosis activation. No significant changes were noted in the Biobran (1.14 ± 0.02) while the Dual Treatment group significantly downregulated Caspase-3 compared to the Etoposide group (1.38 ± 0.14, $p < 0.05$).

Cytochrome C: The expression in the control group was 1.04 ± 0.08. Biobran showed similar close

results to the control (1.12 ± 0.01). Etoposide significantly elevated Cytochrome C expression compared to the control (3.53 ± 0.32, $p < 0.05$). The Dual Treatment group significantly downregulated Cytochrome C compared to Etoposide (1.82 ± 0.18, $p < 0.05$).

Immune Checkpoint Protein:

PDL-1: The control and Biobran groups showed nearly close expression results of PD-1 (1.04 ± 0.04) and (1.09 ± 0.07), respectively. Etoposide led to a significant downregulation of PDL-1 expression (0.49 ± 0.05, $p < 0.05$), suggesting a potential enhancement of anti-tumor immune responses. However, the Dual treatment group did not show a notable impact on PDL-1 expression (0.89 ± 0.02).

Pro-Inflammatory Cytokine:

TNF-α: The expression in the control group was 1.04 ± 0.06. Etoposide treatment significantly increased TNF-α expression (3.70 ± 0.52, $p < 0.05$) relative to the control, reflecting an inflammatory response. Biobran showed a close TNF-α expression to the control (0.99 ± 0.05). Interestingly, the Dual Treatment group showed a protective anti-inflammatory effect indicated by 1.82 ± 0.41 expression (significantly downregulated compared to Etoposide).

3.5 ***Flow cytometry analysis of CD8, CD4, CD3, and CD19 molecules in rat kidney***

Figure 3 shows flow cytometry analysis of CD8, CD4, CD3, and CD19 molecules in rat kidneys under different treatment conditions. For all molecules, the control group represents the baseline expression in the kidney of untreated rats. This group is used as a reference point for comparison with the other treatment groups.

The percentage of CD8, CD4, CD3, and CD19 molecules in control group were 22.1%, 23.7%, 18.1%, and 19%, respectively. The Biobran group shows a significant rise in CD8, CD4, CD3, and CD10 molecule expression relative to the control group, as indicated by the percentages of the CD8 +ve population (42.5%), CD4 +ve population (57.8%), CD3 +ve population (53.1%), and CD19 +ve population 63%. The etoposide treated group also showed a significant increase in the CD8 +ve population (48.6%), CD4 +ve population (40.8%), CD3 +ve population (54.1%) and CD19 +ve population (48%) (statistical analysis revealed that the mean difference indicated significance in the four molecules versus the control, $p < 0.05$). As a counterpoint, the dual-treated group (D) shows no significant change in +ve populations of CD8 (28.1%), CD4 (29.2%), CD3 (32.14%), and CD19 (25.6%) compared to the control.

Overall, these results demonstrate that Biobran treatment significantly enhances the infiltration and activation of immune cells in the kidney, particularly T cells. Etoposide treatment also enhanced immune cell populations. The combination of Biobran and Etoposide does not appear to have any synergistic or additive effects on the immune cell population compared to the control.

Table 1. Evaluation of biochemical markers in rat serum, including major waste products of protein metabolism, Urea, and Creatinine, in addition to Electrolyte Balance indicated by Sodium (Na), Potassium (K), and Calcium (Ca).

Serum Biomarkers	Measure	(G1) Control	(G2) Biobran	(G3) Etoposide	(G4) Dual Treatment
Urea (mg/dl)	Mean ±SD	16.3±1.5 [3]	17.6±1.5 [3]	24.3±3.0 [1, 2, 4]	18.0±3.0 [3]
	Range	18-15	19-16	27-21	21-15
Creatinine (mg/dl)	Mean ±SD	0.34±0.02	0.41±0.005	0.40±0.05	0.37±0.04
	Range	0.36-0.32	0.42-0.41	0.47-0.37	0.41-0.33
Na (nmol/L)	Mean ±SD	146.3±3.5 [3]	141.6±3.2 [3]	126.3±5.1 [1, 2, 4]	141.3±3.0 [3]
	Range	147-144	144-138	132-122	144-138
K (nmol/L)	Mean ±SD	4.9±0.2 [3]	4.8±0.2 [3]	6.1±0.05 [1, 2, 4]	4.8±0.4 [3]
	Range	5.2-4.8	5.1-4.6	6.2-5.9	5.3-4.6
Ca (nmol/L)	Mean ±SD	9.5±0.5 [3]	9.4±0.3 [3]	7.6±0.4 [1, 2, 4]	9.3±0.3 [3]
	Range	10.2-9.1	9.8-9.2	8.1-7.3	9.5-9.1

Significance is indicated at $p<0.05$ for the mean difference of ([1]) group 1 (Control), ([2]) group 2 (Biobran), ([3]) group 3 (Etoposide), and ([4]) group 4 (Dual Treatment).

Table 2. Assessment of oxidative stress markers Malondialdehyde (MDA) and Nitric oxide (NO) in rat kidney.

oxidative stress markers	Measure	(G1) Control	(G2) Biobran	(G3) Etoposide	(G4) Dual Treatment
MDA nmol/g protein	Mean ±SD	2.7±0.4 [3]	2.3±0.1 [3]	4.6±0.5 [1, 2, 4]	2.5±0.2 [3]
	Range	3.2-2.4	2.5-2.2	5.2-4.1	2.8-2.3
NO nmol/g protein	Mean ±SD b*	25.0±3.0 [3]	24.6±1.5 [3]	43.0±4.5 [1, 2, 4]	29.6±2.0 [3]
	Range	28-22	26-23	47-38	32-28

Significance is indicated at $p<0.05$ for the mean difference of ([1]) group 1 (Control), ([2]) group 2 (Biobran), ([3]) group 3 (Etoposide), and ([4]) group 4 (Dual Treatment).

Table 3. Assessment of antioxidant markers superoxide dismutase (SOD), (B) catalase (CAT) and glutathione peroxidase (GSH) in rat kidney.

antioxidant markers	Measure	(G1) Control	(G2) Biobran	(G3) Etoposide	(G4) Dual Treatment
SOD nmol/g protein	Mean ±SD	41.3±3.0 [3]	45.6±4.0 [3]	20.6±2.5 [1, 2, 4]	31.3±3.0 [3]
	Range	44-38	50-42	23-18	34-28
CAT nmol/g protein	Mean ±SD	37.6±5.0 [3]	35.0±3.0 [3]	13.0±2.6 [1, 2, 4]	25.6±2.0 [3]
	Range	43-33	38-32	16-11	28-24
GSH Nmol/g protein	Mean ±SD	2.9±0.5 [3]	2.8±0.4 [3]	1.3±0.2 [1, 2, 4]	2.7±0.2 [3]
	Range	3.5-2.4	3.3-2.4	1.6-1.1	2.9-2.5

Significance is indicated at $p<0.05$ for the mean difference of ([1]) group 1 (Control), ([2]) group 2 (Biobran), ([3]) group 3 (Etoposide), and ([4]) group 4 (Dual Treatment).

Table 4: A comet assay showing DNA damage parameters (tail length (µm), tail DNA (%), tail moment, tailed cell (%), and untailed cells) in kidney cells following treatment with Biobran, Etoposide, or a combination of both.

Comet Parameters	Measure	(G1) Control	(G2) Biobran	(G3) Etoposide	(G4) Dual Treatment
Tail length (µm)	Mean ±SD	1.44±0.02 [3, 4]	1.40±0.02 [3, 4]	6.45±0.08 [1, 2, 4]	2.38±0.04 [1, 2, 3]
	Range	1.46-1.43	1.42-1.38	6.52-6.37	2.42-2.34
Tail DNA (%)	Mean ±SD	1.55±0.05 [3, 4]	1.52±0.04 [3, 4]	4.29±0.04 [1, 2, 4]	1.91±0.18 [1, 2, 3]
	Range	1.6-1.4	1.6-1.4	4.3-4.1	2.3-1.4
Tail moment (unit)	Mean ±SD	2.24±0.10 [3, 4]	2.13±0.04 [3, 4]	27.6±0.20 [1, 2, 4]	4.5±0.35 [1, 2, 3]
	Range	2.4-1.9	2.2-2.0	28.1-27.2	5.4-3.6
Tailed cell (%)	Mean ±SD	3.3±0.58 [3, 4]	2.67±0.58 [3, 4]	14.3±2.5 [1, 2, 4]	10.0±1.0 [1, 2, 3]
	Range	4.7-1.8	4.1-1.2	20.5-8.0	12.4-7.5
Untailed (%)	Mean ±SD	96.6±0.58 [3, 4]	97.3±0.58 [3, 4]	83.6±2.0 [1, 2, 4]	90.0±1.0 [1, 2, 3]
	Range	98.1-95.2	98.7-95.8	88.8-78.4	92.4-87.5

Significance is indicated at $p<0.05$ for the mean difference of ([1]) group 1 (Control), ([2]) group 2 (Biobran), ([3]) group 3 (Etoposide), and ([4]) group 4 (Dual Treatment).

Table 5: qPCR results showing relative mRNA expression (2^-ΔΔCt) of apoptotic markers (Bax, Caspase-3, and Cytochrome c), immune checkpoint protein (PDL-1), and pro-inflammatory cytokine (TNF-α), in kidney cells following treatment with Biobran, Etoposide, or a combination of both.

Markers	Measure	(G1) Control	(G2) Biobran	(G3) Etoposide	(G4) Dual (Mix)
Bax	Mean ±SD	1.0±0.03 [3]	1.03±0.02 [3]	3.94±1.07 [1, 2, 4]	2.03±0.31 [3]
	Range	1.02-0.97	1.04-1.00	5.18-3.26	2.37-1.78
Caspase 3	Mean ±SD	1.0±0.06 [3]	1.1±0.02 [3]	2.96±0.26 [1, 2, 4]	1.38±0.14 [3]
	Range	1.05-0.94	1.11-1.08	3.20-2.69	1.53-1.25
Cytochrome C	Mean ±SD	1.0±0.08 [3, 4]	1.12±0.01 [3]	3.58±0.32 [1, 2, 4]	1.62±0.18 [1, 3]
	Range	1.07-0.92	1.13-1.11	3.95-3.37	1.82-1.46
PDL-1	Mean ±SD	1.0±0.04 [3]	1.09±0.07 [3, 4]	0.49±0.05 [1, 2, 4]	0.88±0.02 [2, 3]
	Range	1.03-0.96	1.17-1.03	0.54-o.44	0.90-0.86
TNF-α	Mean ±SD	1.0±o.o6 [3]	0.99±0.05 [3]	3.12±0.52 [1, 2, 4]	1.64±0.21 [3]
	Range	1.04-0.94	1.05-0.95	3.70-2.69	1.82-1.41

Significance is indicated at $p<0.05$ for the mean difference of ([1]) group 1 (Control), ([2]) group 2 (Biobran), ([3]) group 3 (Etoposide), and ([4]) group 4 (Dual Treatment).

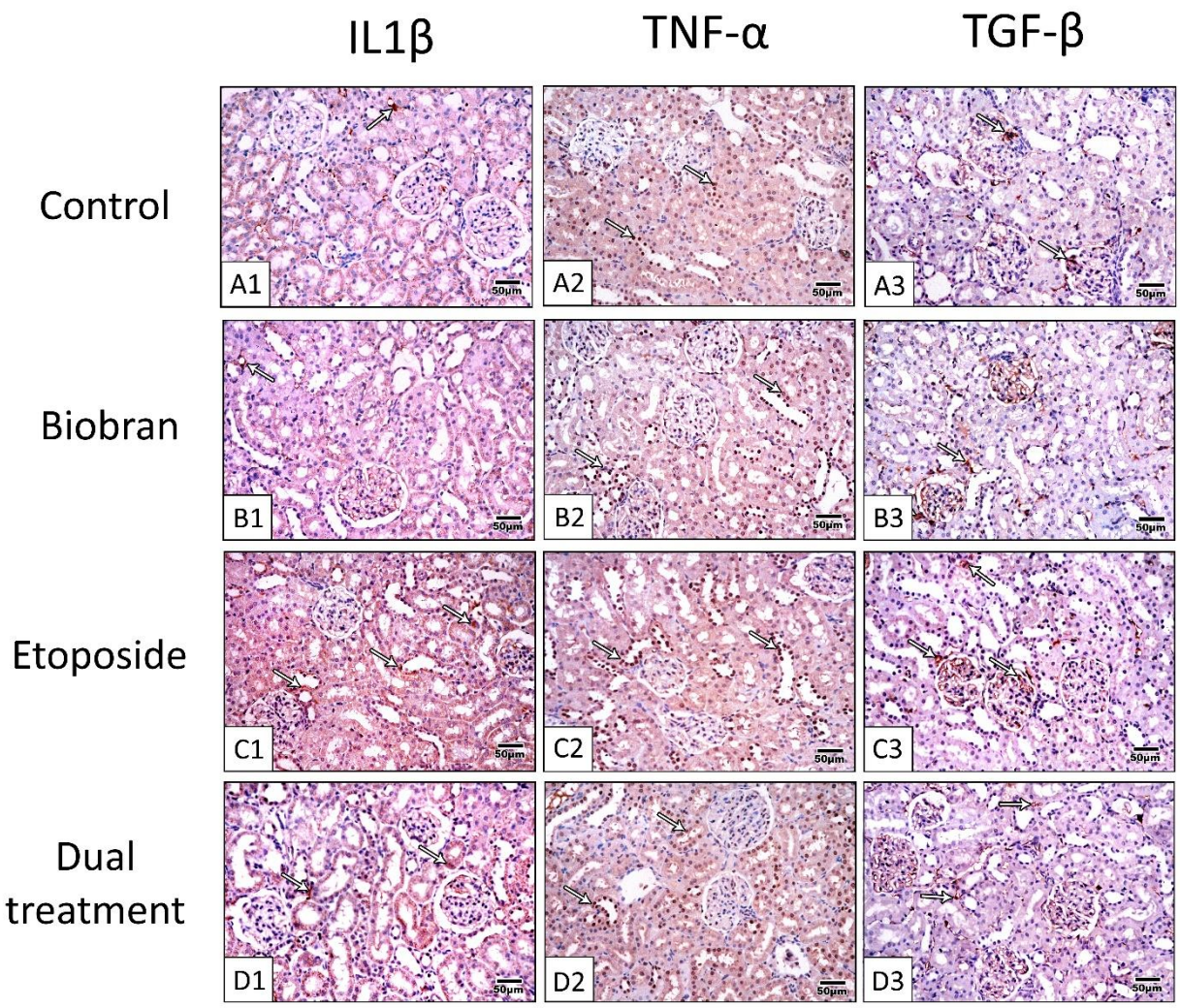

Figure 1: Light micrographs showing the effects of Biobran treatment on rat kidney tissues following etoposide-induced nephrotoxicity, showing IL-1β, TNF-α, and TGF-β expression in kidney cells. Rats in the control group showed a few cells that were positively stained for IL-1β (A1), TNF-α (A2), and TGF-β (A3) expression. Biobran-treated rats also demonstrated that IL-1β (B1), TNF-α (B2), and TGF-β (B3) are expressed in a few cells. The etoposide-treated rats showed an increase of IL-1β (C1), TNF-α (C2), and TGF-β (C3) compared with the control group. Rats treated with both etoposide and Biobran showed kidney cells with a decrease in IL-1β (D1), TNF-α (D2), and TGFβ (D3) relative to the etoposide group.

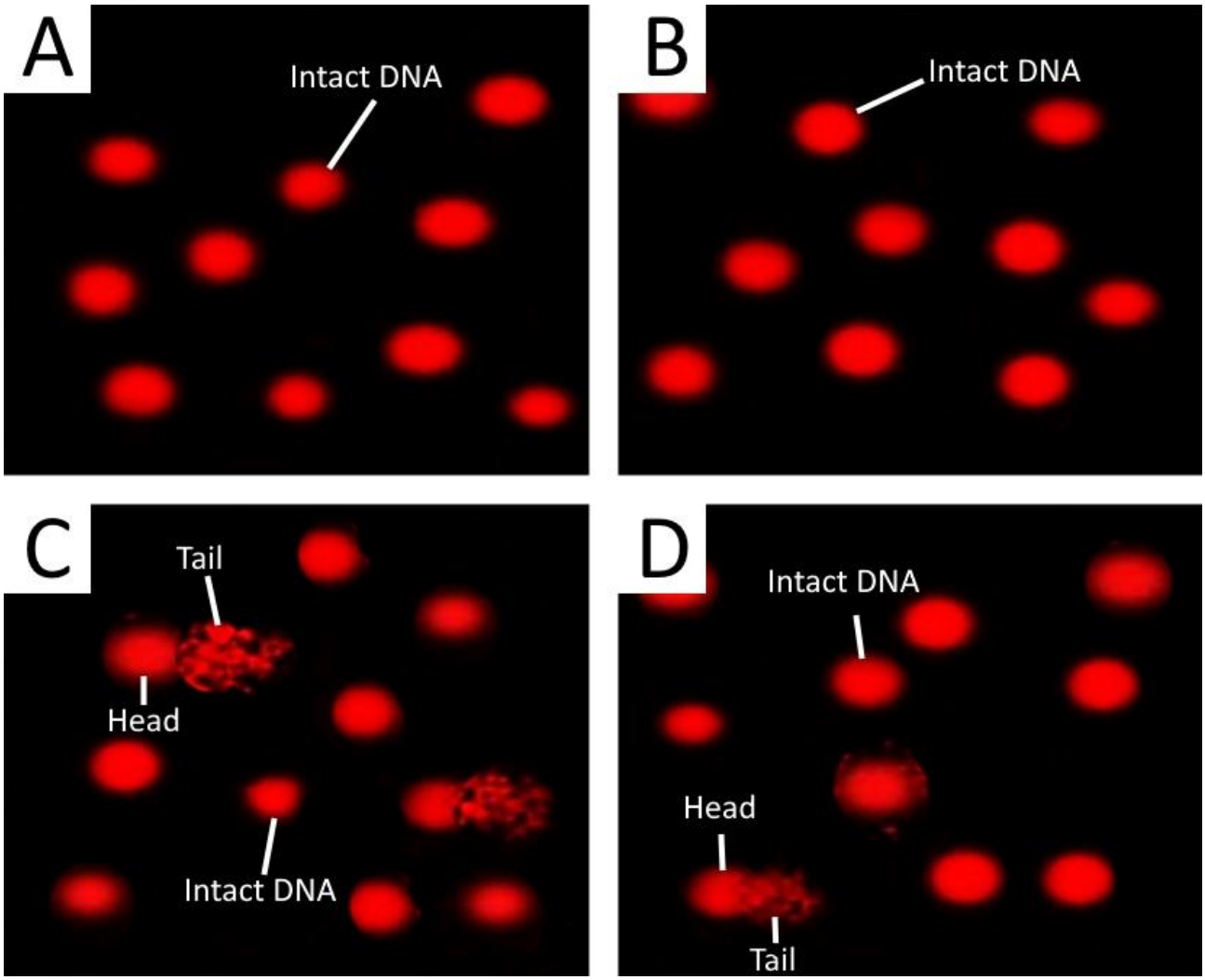

Figure 2 shows the results of a comet assay for DNA damage performed on renal cells treated with different experimental conditions. A: Control group, B: Biobran-treated group, C: Etoposide-treated group, and D: Dual-treated group: Cells treated with a combination of Biobran and Etoposide. The red fluorescent signal represents intact DNA, while the "tail" formation indicates DNA damage. A longer tail suggests more extensive DNA damage.

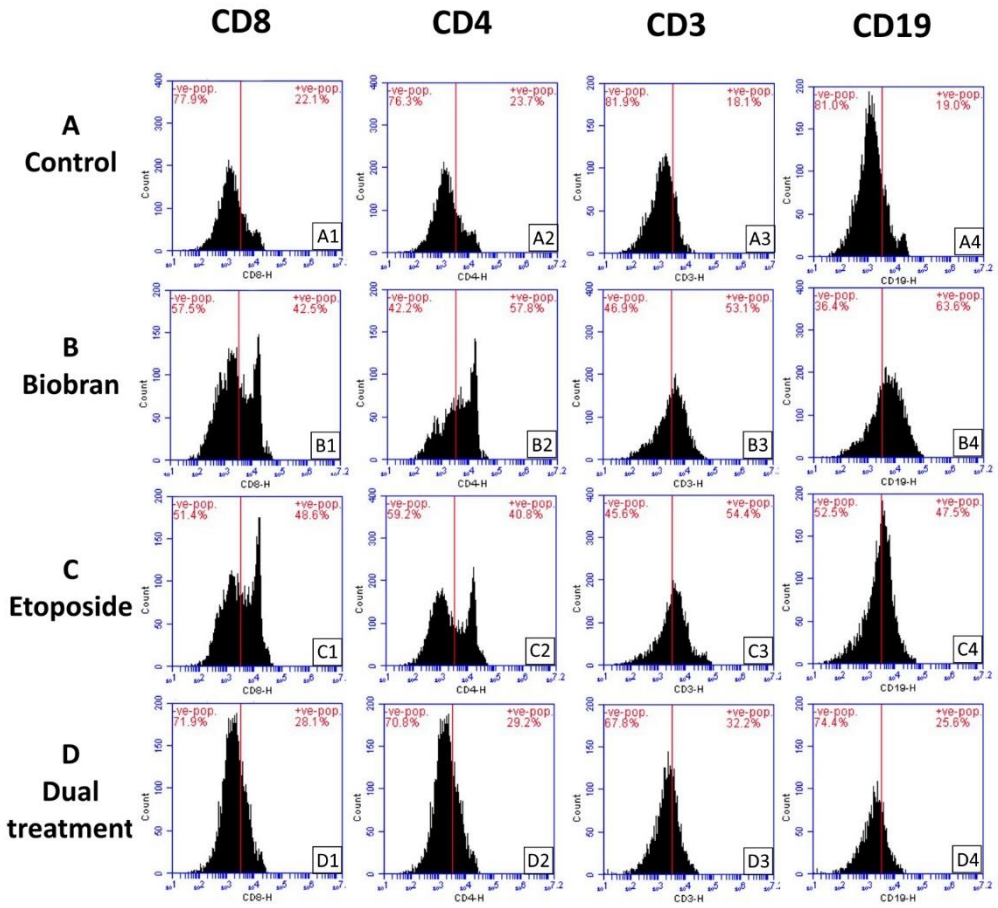

Figure 3. Flow cytometry analysis of CD8, CD4, CD3, CD4,3 and CD19 molecules in rat kidney under different treatment conditions. (A1, A2, A3, A4, and A5) Representative flow cytometry plots of baseline expression of CD8 (A1), CD4 (A2), CD3 (A3), CD3,4 (A4) and CD19 (A5) molecules in the kidney of untreated control rats. (B1, B2, B3, B4, and B5) Representative flow cytometry plots illustrating a significant increase in the expression of CD8 (B1), CD4 (B2), CD3 (B3), CD3,4 (B4), and CD19 (B5) molecules in the kidney of rats treated with Biobran compared to control. (C1, C2, C3, C4, and C5) Representative flow cytometry plot demonstrating an increase in the expression of CD8 (C1), CD4 (C2), CD3 (C3), CD3,4 (C4), and CD19 (C5) molecules in the kidney of rats treated with etoposide compared to control. (D1, D2, D3, D4, and D5) Representative flow cytometry plots depicting no significant change in the expression of CD8 (D1), CD4 (D2), CD3 (D3), CD3,4 (D4), and CD19 (D5) molecules in the kidney of rats with a dual treatment of both compounds compared to control. Data are presented as mean fluorescence intensity (MFI) ± standard error of the mean (SEM) from n=4 rats.

Discussion

The utilization of chemotherapeutic protocols for cancer treatment is considered one of the pillars in cancer treatment due to their wide spectrum of action. Many chemotherapeutic agents have shown effective results in curing the disease and preventing its spread. Unfortunately, the side effects of such treatments can be devastating and can have extremely harmful effects on many of the body's organs (24). Among those side effects are compromising the immune system and nephrotoxic effects that harm the tissues of the kidney and damage their ability to perform their functions of filtration and purification. These effects can reduce the efficacy of the treatment in the long run. That is why the alleviation and reduction of such side effects have received special attention from researchers in many studies and research (25).

Etoposide, a common chemotherapeutic agent, highlights this disparity between effective treatment and dangerous side effects, as it causes immunosuppression and significant renal toxicity (26).

On the flip side, Biobran, a natural mixture of hemicelluloses produced from rice bran, has shown potential in the mitigation of the nephrotoxic and immunosuppressive effects of etoposide. That is mainly due to its potent immunomodulatory properties (27).

Previous studies have examined the chemotherapy supporting role of Biobran, particularly its effects on the immune system, including innate as well as adaptive cells, and chemical components such as cytokines, and its protective effects on the body's organs, especially the kidney and liver. These studies have demonstrated the undeniable protective ability of Biobran and its ability to increase immune capabilities (28).

Initially, we examined renal function by measuring serum creatinine, urea, and other important renal parameters such as sodium, potassium, and calcium. An increase in the levels of rat serum, urea, sodium, potassium, and calcium was found in the current investigation when examining the effects of etoposide. This indicates significant damage to renal cells, which is supported by previous studies (29, 30). Remarkably, in the dual treatment group (etoposide and Biobran) the urea levels were significantly less than in the etoposide group. That highlights the protective antioxidant abilities of Biobran, which alleviate the side effects of etoposide. Conversely, Creatinine levels did not vary greatly between the treatment groups. Suggesting that the nephrotoxic effects of etoposide were not severe enough to affect the creatinine filtration or that the duration of exposure was not long enough for any changes to manifest in creatinine levels **(36)**.

It is well known that maintaining the right balance of electrolytes is crucial for kidney and body functions. Our results revealed that Etoposide treatment caused important changes in electrolyte levels. Sodium and calcium levels dropped, while potassium levels increased. The lower sodium indicates impaired reabsorption. The high potassium levels point to possible damage to the tubules, which can lead to hyperkalemia, a serious condition if not treated. The drop in calcium may result from poor calcium reabsorption in the renal tubules or loss of calcium from bones, which can happen with chemotherapy-related kidney damage. Sodium and potassium levels in the dual treatment group significantly improved as compared to the Etoposide group, confirming Biobran's function in reversing electrolyte disruptions caused by Etoposide. Moreover, Biobran's capacity to keep calcium levels stable points to a preventive impact against hypocalcemia brought on by etoposide, which may be related to Biobran's promotion of cellular health and defense against oxidative damage (31).

The ability of Biobran to modulate oxidative stress markers was obvious by evaluating malondialdehyde (MDA) and nitric oxide (NO) levels in kidney tissues. Raising MDA levels with etoposide is in line with earlier research linking chemotherapy drugs to the formation of reactive oxygen species (ROS) and free radicals, which in turn induce oxidative stress. The idea that this treatment induces nitrosative and oxidative stress is definitively confirmed by the elevated NO levels in the etoposide group, and more evidence that Etoposide induces nitrosative and oxidative stress comes from the increase in NO levels in the etoposide group (6). Meanwhile, the MDA and NO levels in the dual-treated group were comparable to the control, indicating that Biobran effectively mitigated lipid peroxidation caused by Etoposide (32).

To fully comprehend the antioxidant defense system, we investigated the activity of the antioxidant markers glutathione peroxidase (GSH), catalase (CAT), and superoxide dismutase (SOD), all of which decreased following Etoposide administration (33). All three marker activities showed a significant recovery in the dual treatment group when compared to the Etoposide group, indicating that Biobran aids in the restoration of antioxidant enzyme function (34). The explanation of Biobran to modulate oxidative stress markers and enhance antioxidant defenses may be attributed to its composition, which includes arabinoxylan and other bioactive polysaccharides known for their immunomodulatory and antioxidant properties (35, 36).

Critical insights into the inflammatory response in rat kidney tissues after Etoposide treatment and the protective benefits of Biobran are obtained from the immunohistochemistry study of proinflammatory cytokines, namely IL-1β, TNF-α, and TGF-β. The Proinflammatory cytokines (IL-1β, TNF-α, and TGF-β) were minimally expressed in healthy kidney tissues in both the control and Biobran-treated groups, suggesting that Biobran does not normally cause an inflammatory response. On the other hand, the administration of Etoposide markedly elevated these cytokines, resulting in oxidative stress-related inflammation and perhaps fibrosis, which is in line with previous studies (37). Nevertheless, co-administration of Biobran resulted in a reduction of IL-1β, TNF-α, and TGF-β levels, indicating its anti-inflammatory and protective properties against nephrotoxicity generated by Etoposide. It is possible that Biobran can lessen kidney damage by blocking the NF-κB pathway and reducing fibrosis (1, 38).

The comet assay was performed to measure the degree of DNA damage caused by Etoposide and to evaluate Biobran's capacity for protection. The analysis showed that renal cells treated with Etoposide had substantial DNA damage, as evidenced by increased tail length, tail DNA percentage, and tail moment, all of which pointed to numerous DNA strand breaks., Etoposide, known for its genotoxic properties, caused considerable genomic instability and probably aided in the necrosis or death of the cells (39, 40). Biobran's protective impact was highlighted by the low damage to DNA. The combination treatment of Etoposide and Biobran reduced but did not completely prevent the DNA damage caused by Etoposide. The antioxidant properties of Biobran are thought the protect by lowering oxidative stress and maybe improving DNA repair processes (41, 42).

Through qPCR analysis, the molecular processes by which Biobran and Etoposide affect apoptotic markers, immune checkpoint proteins, and pro-inflammatory cytokines in kidney cells were investigated. Etoposide was found to play a role in triggering apoptosis via mitochondrial pathways, as validated by the marked overexpression of apoptotic markers Bax, Caspase-3, and Cytochrome C, which leads to exacerbated kidney damage. This seems to be in line with the fact that

etoposide functions as a topoisomerase II inhibitor, destroying DNA (43, 44). Biobran only treatment, on the other hand, did not exhibit apoptotic induction but dramatically decreased the expression of apoptotic markers in the dual treatment group compared to the Etoposide effect, indicating that it provided some protection against apoptosis produced by etoposide via antioxidant mechanisms (45). Additionally, Etoposide inhibited PDL-1, which may have improved immune responses against tumors but also increased the possibility of immune-mediated tissue injury. Conversely, Biobran decreased the risk of kidney damage by controlling this reaction through the maintenance of balanced PDL-1 expression (46). Furthermore, kidney tissues had an inflammatory reaction due to the considerable upregulation of TNF-α by Etoposide, whereas Biobran preserved kidney function by lowering TNF-α levels and exerting an anti-inflammatory impact (12,47). The potential of biobran as a therapeutic adjunct is highlighted by its capacity to control immunological responses and downregulate pro-inflammatory cytokines, which serve to protect against Etoposide-induced nephrotoxicity.

The results of flow cytometry analysis of CD8, CD4, CD3, and CD19 molecules in rat kidneys highlighted important data in regard to the effects of Biobran and etoposide on the immune cell count. The control group indicated a baseline for immune cell counts. The Biobran group showed an increase in all immune markers (CD4, CD8, CD3, and CD19). This highlights Biobran's ability to enhance immune cell infiltration and activation, particularly T-cells. These observations align with previous studies' reports on the immunomodulatory properties of biobran. The rise of CD8 and CD4 indicates an enhancement of cytotoxic T-cells and helper T-cells, both of which are invaluable for the immune defense (48).

Etoposide treatment also showed an increase in CD8, CD4, CD3, and CD19. This suggests that etoposide has immunostimulatory properties. However, this could be due to etoposide inducing apoptosis in many of the body's tissues, thereby increasing the recruitment and activation of immune cells (9).

Interestingly, the combination of etoposide and Biobran showed no notable increases in the levels of immune cells. This lack of synergistic additive action suggests a potential antagonistic reaction between the two substances.

Conclusion

The combined analysis addressing nephrotoxicity and immune modulation in rats receiving Etoposide included increased biomarkers of oxidative stress, DNA damage, apoptosis, and inflammation, demonstrating that etoposide worsens kidney injury. Etoposide promotes renal cell death, raising the risk of nephrotoxicity by the upregulation of apoptotic markers, including Bax, Caspase-3, and pro-inflammatory cytokine (TNF-α). Biobran, an adjunct treatment, showed protective effects by attenuating DNA damage and inflammation caused by Etoposide. Biobran enhanced immunological regulation and reduced inflammatory response without intensifying immune activation by reducing TNF-α levels. Biobran was also associated with increased infiltration of CD8+, CD4+, CD3+, and CD19+ immune cells, which is consistent with its role in boosting immunological surveillance.

These findings have important clinical implications by using Biobran as an adjuvant treatment to prevent kidney damage, enhancing the renal safety profile during chemotherapy for cancer patients, especially for those who are more susceptible to nephrotoxicity, without lowering the therapeutic benefit.

Authorship Contributions:

Conceptualization: Ali M. Eldib, **Methodology:** Ali M. Eldib, Mohamed S. El-Gerbed. **Formal**

Analysis: Mohamed S. El-Gerbed, Ali M. Eldib **Investigation:** Osman Suliman, Sara. M. Altom, Faris M. Elmahdi, Ibrahim H. Babikir Shimaa B. Hemdan, Reem M. Emam **Data Curation:** Ali M. Eldib, Mohamed S. El-Gerbed, Mamdooh H Ghoneum, Noha El-Anwar. **Writing - Original Draft:** Ali M. Eldib. **Writing - Review & Editing:** Mamdooh H Ghoneum, Noha El-Anwar. **Supervision:** Ali M. Eldib, Mohamed S. El-Gerbed, Mamdooh H Ghoneum.

Funding

The current research was carried out without any external financial support from public, private, or non-profit funding sources.

Declaration of Competing Interest

The authors state that they have no known competing financial interests or personal relationships that could have influenced the work presented in this study.

Data Availability

Data is available on reasonable request from the corresponding author.

Declaration of Manuscript Submission

By submitting a manuscript, the author confirms that the work has not been previously published, is not currently being considered for publication elsewhere, and has received approval from all co-authors, if applicable, and from the relevant authorities—whether implicitly or explicitly—at the institution where the research was conducted.

References

1. Santos MLC, de Brito BB, da Silva FAF, Botelho A, de Melo FF. Nephrotoxicity in cancer treatment: An overview. World J Clin Oncol. 2020;11(4):190-204.
2. Stewart T, Dowling M, Janson B, Siderov J, Xie J, Grigg A, et al. Acute kidney injury in patients receiving high-dose etoposide phosphate as conditioning prior to hematopoietic stem cell transplantation in hematologic malignancies may be associated with elevated body mass index. Leuk Lymphoma. 2023;64(1):253-5.
3. Zhang W, Gou P, Dupret JM, Chomienne C, Rodrigues-Lima F. Etoposide, an anticancer drug involved in therapy-related secondary leukemia: Enzymes at play. Transl Oncol. 2021;14(10):101169.
4. Stefansdottir A, Johnston ZC, Powles-Glover N, Anderson RA, Adams IR, Spears N. Etoposide damages female germ cells in the developing ovary. BMC Cancer. 2016;16(1):482.
5. Matias-Barrios VM, Radaeva M, Song Y, Alperstein Z, Lee AR, Schmitt V, et al. Discovery of New Catalytic Topoisomerase II Inhibitors for Anticancer Therapeutics. Front Oncol. 2020;10:633142.
6. Wang S, Chan SY, Deng Y, Khoo BL, Chua SL. Oxidative stress induced by Etoposide anti-cancer chemotherapy drives the emergence of tumor-associated bacteria resistance to fluoroquinolones. J Adv Res. 2024;55:33-44.
7. Anai S, Iwama E, Yoneshima Y, Otsubo K, Tanaka K, Nakanishi Y, et al. Association of nephrotoxicity during platinum-etoposide doublet therapy with UGT1A1 polymorphisms in small cell lung cancer patients. Lung Cancer. 2018;126:156-61.
8. Beyer J, Rick O, Weinknecht S, Kingreen D, Lenz K, Siegert W. Nephrotoxicity after high-dose carboplatin, etoposide and ifosfamide in germ-cell tumors: incidence and implications for hematologic recovery and clinical outcome. Bone Marrow Transplant. 1997;20(10):813-9.
9. Johnson TS, Terrell CE, Millen SH, Katz JD, Hildeman DA, Jordan MB. Etoposide selectively ablates activated T cells to control the immunoregulatory disorder hemophagocytic lymphohistiocytosis. J Immunol. 2014;192(1):84-91.
10. Anand U, Dey A, Chandel AKS, Sanyal R, Mishra A, Pandey DK, et al. Cancer

chemotherapy and beyond: Current status, drug candidates, associated risks and progress in targeted therapeutics. Genes Dis. 2023;10(4):1367-401.
11. Nicolette J, Luijten M, Sasaki JC, Custer L, Embry M, Froetschl R, et al. Utility of a next-generation framework for assessment of genomic damage: A case study using the pharmaceutical drug candidate etoposide. Environ Mol Mutagen. 2021;62(9):512-25.
12. Elsaid AF, Agrawal S, Agrawal A, Ghoneum M. Dietary Supplementation with Biobran/MGN-3 Increases Innate Resistance and Reduces the Incidence of Influenza-like Illnesses in Elderly Subjects: A Randomized, Double-Blind, Placebo-Controlled Pilot Clinical Trial. Nutrients. 2021;13(11).
13. Agrawal S, Agrawal A, Ghoneum M. Biobran/MGN-3, an Arabinoxylan Rice Bran, Exerts Anti-COVID-19 Effects and Boosts Immunity in Human Subjects. Nutrients. 2024;16(6).
14. Badr El-Din NK, Noaman E, Ghoneum M. In vivo tumor inhibitory effects of nutritional rice bran supplement MGN-3/Biobran on Ehrlich carcinoma-bearing mice. Nutr Cancer. 2008;60(2):235-44.
15. Henderson AJ, Ollila CA, Kumar A, Borresen EC, Raina K, Agarwal R, et al. Chemopreventive properties of dietary rice bran: current status and future prospects. Adv Nutr. 2012;3(5):643-53.
16. Ooi SL, McMullen D, Golombick T, Nut D, Pak SC. Evidence-Based Review of BioBran/MGN-3 Arabinoxylan Compound as a Complementary Therapy for Conventional Cancer Treatment. Integr Cancer Ther. 2018;17(2):165-78.
17. Gowda S, Desai PB, Kulkarni SS, Hull VV, Math AA, Vernekar SN. Markers of renal function tests. N Am J Med Sci. 2010;2(4):170-3.
18. Marrocco I, Altieri F, Peluso I. Measurement and Clinical Significance of Biomarkers of Oxidative Stress in Humans. Oxid Med Cell Longev. 2017;2017:6501046.
19. Weydert CJ, Cullen JJ. Measurement of superoxide dismutase, catalase and glutathione peroxidase in cultured cells and tissue. Nat Protoc. 2010;5(1):51-66.
20. de Souza Dantas Oliveira SH, de Souza Aarão TL, da Silva Barbosa L, Souza Lisbôa PG, Tavares Dutra CD, Margalho Sousa L, et al. Immunohistochemical analysis of the expression of TNF-alpha, TGF-beta, and caspase-3 in subcutaneous tissue of patients with HIV lipodystrophy syndrome. Microb Pathog. 2014;67-68:41-7.
21. Collins A, Møller P, Gajski G, Vodenková S, Abdulwahed A, Anderson D, et al. Measuring DNA modifications with the comet assay: a compendium of protocols. Nat Protoc. 2023;18(3):929-89.
22. Chen Z, Halford NG, Liu C. Real-Time Quantitative PCR: Primer Design, Reference Gene Selection, Calculations and Statistics. Metabolites. 2023;13(7).
23. Wlodkowic D, Telford W, Skommer J, Darzynkiewicz Z. Apoptosis and beyond: cytometry in studies of programmed cell death. Methods Cell Biol. 2011;103:55-98.
24. Pathak S, Zajac KK, Annaji M, Govindarajulu M, Nadar RM, Bowen D, et al. Clinical outcomes of chemotherapy in cancer patients with different ethnicities. Cancer Rep (Hoboken). 2023;6 Suppl 1(Suppl 1):e1830.
25. Sharma A, Jasrotia S, Kumar A. Effects of Chemotherapy on the Immune System: Implications for Cancer Treatment and Patient Outcomes. Naunyn Schmiedebergs Arch Pharmacol. 2024;397(5):2551-66.
26. Cordero C, Loboda C, Clerc-Urmès I, Clément L, Pochon C, Chastagner P. Unexpected acute renal injury after high-dose etoposide phosphate and total body irradiation in children undergoing hematopoietic stem cell transplantation. Pediatr

Blood Cancer. 2017;64(12).
27. Ooi SL, Pak SC, Micalos PS, Schupfer E, Lockley C, Park MH, et al. The Health-Promoting Properties and Clinical Applications of Rice Bran Arabinoxylan Modified with Shiitake Mushroom Enzyme: A Narrative Review. Molecules. 2021;26(9).
28. Elsaid AF, Shaheen M, Ghoneum M. Biobran/MGN-3, an arabinoxylan rice bran, enhances NK cell activity in geriatric subjects: A randomized, double-blind, placebo-controlled clinical trial. Exp Ther Med. 2018;15(3):2313-20.
29. Obadipe JA, Samuel TA, Jimoh MA, Folorunso SA. Chemotherapy-Induced Electrolyte Disorder and Nephrotoxicity in Cancer Patients from Selected Nigerian Tertiary Health Care Hospitals. Niger Med J. 2022;63(3):196-203.
30. Abbasi B, Hayat A, Lyons M, Gupta A, Gupta S. Serum protein and electrolyte imbalances are associated with chemotherapy induced neutropenia. Heliyon. 2022;8(7):e09949.
31. Ooi SL, Micalos PS, Kim J, Pak SC. Rice bran arabinoxylan compound as a natural product for cancer treatment - an evidence-based assessment of the effects and mechanisms. Pharm Biol. 2024;62(1):367-93.
32. Abdou HM, Hamaad FA, Abd Elmageed GM, Ghoneum MH. Efficiency of Biobran/MGN-3, an Arabinoxylan Rice Bran, in Attenuating Diabetes-Induced Cognitive Impairment of the Hippocampus via Oxidative Stress and IR/Akt/NF-κB in Rats. Evid Based Complement Alternat Med. 2023;2023:8248576.
33. Zhou J, Nie RC, Yin YX, Cai XX, Xie D, Cai MY. Protective Effect of Natural Antioxidants on Reducing Cisplatin-Induced Nephrotoxicity. Dis Markers. 2022;2022:1612348.
34. Noaman E, Badr El-Din NK, Bibars MA, Abou Mossallam AA, Ghoneum M. Antioxidant potential by arabinoxylan rice bran, MGN-3/biobran, represents a mechanism for its oncostatic effect against murine solid Ehrlich carcinoma. Cancer Lett. 2008;268(2):348-59.
35. Zhao Z, Cheng W, Qu W, Wang K. Arabinoxylan rice bran (MGN-3/Biobran) alleviates radiation-induced intestinal barrier dysfunction of mice in a mitochondrion-dependent manner. Biomed Pharmacother. 2020;124:109855.
36. Ghoneum M, Badr El-Din NK, Abdel Fattah SM, Tolentino L. Arabinoxylan rice bran (MGN-3/Biobran) provides protection against whole-body γ-irradiation in mice via restoration of hematopoietic tissues. J Radiat Res. 2013;54(3):419-29.
37. Wang CI, Chu PM, Chen YL, Lin YH, Chen CY. Chemotherapeutic Drug-Regulated Cytokines Might Influence Therapeutic Efficacy in HCC. Int J Mol Sci. 2021;22(24).
38. Domingo IK, Latif A, Bhavsar AP. Pro-Inflammatory Signalling PRRopels Cisplatin-Induced Toxicity. Int J Mol Sci. 2022;23(13).
39. Shin HJ, Kwon HK, Lee JH, Anwar MA, Choi S. Etoposide induced cytotoxicity mediated by ROS and ERK in human kidney proximal tubule cells. Sci Rep. 2016;6:34064.
40. Menendez D, Anand JR, Murphy CC, Bell WJ, Fu J, Slepushkina N, et al. Etoposide-induced DNA damage is increased in p53 mutants: identification of ATR and other genes that influence effects of p53 mutations on Top2-induced cytotoxicity. Oncotarget. 2022;13:332-46.
41. Ghoneum M, Badr El-Din NK, Ali DA, El-Dein MA. Modified arabinoxylan from rice bran, MGN-3/biobran, sensitizes metastatic breast cancer cells to paclitaxel in vitro. Anticancer Res. 2014;34(1):81-7.
42. Badr El-Din NK, Abdel Fattah SM, Pan D, Tolentino L, Ghoneum M. Chemopreventive Activity of MGN-3/Biobran Against Chemical Induction of Glandular Stomach Carcinogenesis in Rats and Its Apoptotic Effect in Gastric Cancer

Cells. Integr Cancer Ther. 2016;15(4):Np26-np34.

43. Servais H, Ortiz A, Devuyst O, Denamur S, Tulkens PM, Mingeot-Leclercq MP. Renal cell apoptosis induced by nephrotoxic drugs: cellular and molecular mechanisms and potential approaches to modulation. Apoptosis. 2008;13(1):11-32.
44. Day TW, Wu CH, Safa AR. Etoposide induces protein kinase Cdelta- and caspase-3-dependent apoptosis in neuroblastoma cancer cells. Mol Pharmacol. 2009;76(3):632-40.
45. Ghoneum MH, El Sayed NS. Protective Effect of Biobran/MGN-3 against Sporadic Alzheimer's Disease Mouse Model: Possible Role of Oxidative Stress and Apoptotic Pathways. Oxid Med Cell Longev. 2021;2021:8845064.
46. Xue L, Chen B, Lin J, Peng J. Anti-PD-L1 immune checkpoint inhibitors in combination with etoposide and platinum for extensive-stage small cell lung cancer: a case report. Ann Palliat Med. 2021;10(1):828-35.
47. Yameny, A., Alabd, S., Mansor, M. Serum TNF-α levels as a biomarker in some liver diseases of Egyptian patients. *Journal of Medical and Life Science*, 2023; 5(1): 1-8. doi: 10.21608/jmals.2023.329303
48. Ghoneum M, Agrawal S. Mgn-3/biobran enhances generation of cytotoxic CD8+ T cells via upregulation of dec-205 expression on dendritic cells. Int J Immunopathol Pharmacol. 2014;27(4):523-30.