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Dose-Dependent Histopathological Alterations in the Small and Large Intestinal Mucosa of Syrian Golden Hamsters (*Mesocricetus auratus*) Following Cyclophosphamide Administration

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ABSTRACT

Background: Cyclophosphamide (CP) is a potent chemotherapeutic agent with well-documented systemic toxicities. The gastrointestinal tract is particularly vulnerable, yet comparative effects on the small and large intestines remain underexplored. **Aim:** To evaluate dose-dependent histopathological changes in the small and large intestines of female Syrian golden hamsters following CP administration. **Methods:** Twenty-seven hamsters were divided into three groups: control (saline), therapeutic dose (100 mg/kg), and toxic dose (200 mg/kg). CP was administered intraperitoneally on days 1, 3, and 5. On day 7, intestinal tissues were harvested, fixed, and processed for histology. Sections were stained with Hematoxylin & Eosin (H&E) to assess epithelial and structural integrity, and Periodic Acid–Schiff (PAS) to evaluate goblet cell distribution and mucin content. Comparative analysis was performed between small intestinal villi and large intestinal crypts to determine site-specific susceptibility. **Results:** At 100 mg/kg, the small intestine showed mild villous blunting and inflammatory infiltrates, whereas the large intestine remained largely intact. At 200 mg/kg, the small intestine exhibited severe villous fusion, epithelial denudation, and goblet cell depletion, while the large intestine demonstrated crypt distortion and marked mucin loss. **Conclusion:** CP induces dose-dependent intestinal injury, with the small intestine displaying greater sensitivity than the large intestine. These findings highlight the importance of comparative gastrointestinal monitoring and the development of protective interventions during CP therapy.

التغيرات النسيجية المرضية المعتمدة على الجرعة في مخاطية الأمعاء الدقيقة والقولون لدى الهامستر السوري الذهبي (*Mesocricetus auratus*) بعد إعطاء عقار السيكلوفوسفاميد

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الكلمات المفتاحية:
السيكلوفوسفاميد
الأمعاء الدقيقة
الأمعاء الغليظة
علم الأمراض النسيجي
الهامستر السوري الذهبي

الملخص

الخلفية: يُعد السيكلوفوسفاميد (Cyclophosphamide; CP) أحد العوامل الكيميائية العلاجية الفعالة، ويتميز بسمية جهازية موثقة على نطاق واسع. ويُعد الجهاز الهضمي من أكثر الأجهزة عرضةً لتأثيراته السامة، إلا أن الدراسات التي تقارن بين تأثيراته على الأمعاء الدقيقة والأمعاء الغليظة لا تزال محدودة. **الهدف:** تقييم التغيرات النسيجية المرضية المعتمدة على الجرعة في الأمعاء الدقيقة والأمعاء الغليظة لدى إناث الهامستر السوري الذهبي عقب إعطاء السيكلوفوسفاميد. **المواد وطرق البحث:** شملت الدراسة سبعةً وعشرين هامسترًا، قُسمت عشوائيًا إلى ثلاث مجموعات: مجموعة ضابطة تلقت محلولًا ملحيًا، ومجموعة تلقت جرعة علاجية من السيكلوفوسفاميد (100 ملغم/كغم)، ومجموعة تلقت جرعة سامة (200 ملغم/كغم). أُعطي العقار عن طريق الحقن داخل

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الصفاق في الأيام الأول والثالث والخامس من التجربة. وفي اليوم السابع، جُمعت عينات من الأمعاء، وثُبتت وعولجت لإجراء الفحص النسيجي. صُبغت المقاطع النسيجية بصبغة الهيماتوكسيلين والإيوزين (H&E) لتقييم سلامة الظهارة والبنية النسيجية، وبصبغة حمض دوري–شيف (PAS) لتقييم توزيع الخلايا الكأسية ومحتوى المخاط. كما أُجري تحليل مقارن بين الزغابات المعوية في الأمعاء الدقيقة والخبايا المعوية في الأمعاء الغليظة لتحديد مدى القابلية للإصابة تبعًا لموضع النسيج. النتائج: عند إعطاء جرعة 100 ملغم/كغم، أظهرت الأمعاء الدقيقة تسطحًا بسيطًا في الزغابات مع ارتشاح للخلايا الالتهابية، في حين ظلت الأمعاء الغليظة سليمة إلى حد كبير. أما عند إعطاء جرعة 200 ملغم/كغم، فقد أظهرت الأمعاء الدقيقة اندماجًا شديدًا للزغابات، وتجردًا للظهارة، ونقصًا واضحًا في الخلايا الكأسية، بينما أظهرت الأمعاء الغليظة تشوهًا في الخبايا المعوية مع انخفاض ملحوظ في محتوى المخاط. **الاستنتاج:** يُحدث السيكلوفوسفاميد إصابةً معوية تعتمد على الجرعة، وكانت الأمعاء الدقيقة أكثر حساسية لتأثيراته السامة مقارنةً بالأمعاء الغليظة. وتؤكد هذه النتائج أهمية المراقبة المقارنة للجهاز الهضمي أثناء العلاج بالسيكلوفوسفاميد، وضرورة تطوير استراتيجيات وقائية للحد من آثاره الجانبية على الأمعاء.

Introduction

Cyclophosphamide (CP) is a synthetic alkylating agent widely used in the treatment of hematological malignancies and solid tumors [1–4]. Its cytotoxicity results from DNA alkylation, leading to impaired replication and transcription, and ultimately cell death. CP is metabolized in the liver to active metabolites such as phosphoramide mustard, which cross-links DNA strands [5]. CP's development and metabolic activation are well characterized [6,7], and pharmacokinetic studies confirm its key role in oncology [8–10]. Despite its therapeutic efficacy, CP is associated with systemic toxicities including hemorrhagic cystitis, hepatotoxicity, pulmonary injury, and cardiac dysfunction [11–15]. The gastrointestinal tract is particularly vulnerable to CP-induced injury. The small intestine (SI), with villi specialized for nutrient absorption, and the large intestine (LI), rich in mucus-secreting goblet cells, differ structurally and functionally [16-17]. These differences may influence site-specific susceptibility to CP toxicity. Mechanistically, CP injury involves oxidative stress, inflammation, and apoptosis [18-20]. The SI, with higher Cytochrome P450 family 3, subfamily A (CYP3A) activity, bioactivates CP into toxic metabolites, resulting in villus erosion and epithelial loss, whereas the LI shows comparatively lower CYP3A expression and reduced sensitivity [21,22]. Rare but severe complications such as CP-associated enteritis and late hemorrhagic cystitis have also been documented [23-25]. Histological evaluation is essential for assessing intestinal damage. H&E staining provides structural detail, while PAS highlights goblet cells and mucin content [26–30]. These stains allow comparative assessment of villi and crypts in CP-treated tissues. Previous Libyan studies have reported CP-induced gastric and hepatic alterations in golden hamsters [27-28] and protective effects of natural compounds such as olive oil in Swiss albino rats [29–30].

Aim:

To investigate the relationship between CP dose and severity of histopathological changes in the small and large intestines of female golden hamsters.

Materials and Methods

Animals

Twenty-seven adult female Syrian golden hamsters (*Mesocricetus auratus*), weighing 150–250 g, were used. Animals were obtained from a licensed farm in El Bayda, Libya, and housed in the animal facility of the Histology Department, Faculty of Medicine, University of Benghazi. They were maintained under controlled conditions (24 ± 4 °C, 12-hour light/dark cycle, pathogen free environment) with free access to standard chow and water. Bedding consisted of clean wooden sawdust, changed daily. Animals were randomly assigned to experimental groups to minimize baseline variability. All procedures complied with institutional ethical guidelines and were approved by the Faculty of Biomedical Sciences Scientific Ethics Committee.

Chemicals

CP (Endoxan®, Baxter Oncology GmbH, Germany) was purchased in powder form (1 gram CP monohydrate per vial). The drug was dissolved in 50 ml physiological saline to prepare the required concentrations. Solutions were freshly prepared before each administration. All other reagents were of analytical grade and obtained from certified suppliers.

Experimental Design and Dosing

Animals were divided into three groups (9 animals per group):

Group 1 (Control): intraperitoneal injections of normal saline.

Group 2 (Therapeutic dose): CP at 100 mg/kg body weight.

Group 3 (Toxic dose): CP at 200 mg/kg body weight.

Doses were administered intraperitoneally on days 1, 3, and 5. Animals were monitored for 6 hours post-injection for signs of distress. On day 7, all animals were euthanized with a single overdose of pentobarbital (200 mg/kg), followed by cervical dislocation.

Tissue Collection and Processing

Segments of the small and large intestine were excised immediately after sacrifice. Samples were fixed in 10% neutral buffered formalin, dehydrated through graded ethanol (50–100%), cleared in xylene, and embedded in paraffin. Paraffin blocks were sectioned at 5 µm thickness using a rotary microtome. Sections were mounted on glass slides for staining.

Histological Staining

Two staining protocols were applied:

Hematoxylin and Eosin (H&E): nuclei stained dark blue with hematoxylin; eosin counterstained cytoplasm and extracellular matrix pink to red, enabling structural evaluation [31].

Periodic Acid–Schiff (PAS): sections were oxidized in 1% periodic acid for 10 minutes, rinsed, then exposed to Schiff's reagent (Merck, Germany) for 15 minutes. Slides were counterstained with Mayer's hematoxylin for 1 minute. This procedure highlighted goblet cells and mucin distribution [32].

Slides were dehydrated, cleared in xylene, and mounted with Distrene Plasticiser Xylene (DPX) medium. Microscopic examination was performed at the University Medical Centre, Benghazi, under expert supervision. Evaluation was descriptive and qualitative. Observations were not blinded, which may introduce observer bias, but was done by to separate pathologists which gives strength to the data.

Statistical Analysis of Histopathological Scores

Scoring system: Lesions were graded on a 0–3 scale (0 = normal, 1 = mild, 2 = moderate, 3 = severe) for villous damage, goblet cell changes, inflammatory infiltration (small intestine), and crypt architecture, goblet cell changes, inflammatory infiltration (large intestine).

Analysis: Mean ± SD scores were calculated per group (Control, 100 mg/kg, 200 mg/kg). One-way ANOVA was used to test differences among groups, followed by Tukey's post-hoc test.

Software: SPSS v.23 (IBM Corp., Armonk, NY, USA).

Results

Histopathological evaluation was performed on intestinal samples from control and cyclophosphamide-treated hamsters. Both small

and large intestines were examined using H&E for general architecture and PAS for mucin content and goblet cell distribution.

In the SI, control animals showed normal mucosal architecture with intact villi (Figure 1A), preserved brush border, and abundant goblet cells exhibiting strong PAS reactivity (Figure 1B). At 100 mg/kg, mild villous blunting and swelling were observed, accompanied by superficial epithelial sloughing, slight inflammatory infiltration, and occasional cytoplasmic vacuolization (Figure 1C). Goblet cells demonstrated increased PAS positivity, suggesting compensatory mucin secretion (Figure 1D). At 200 mg/kg, pathology was more severe, with villous atrophy, fusion, partial epithelial denudation, moderate lymphocytic infiltration, nuclear degeneration, and vascular congestion (Figure 1E). Goblet cell depletion and reduced PAS staining indicated impaired absorptive and secretory function (Figure 1F). Pathologist's descriptive notes confirmed focal trans-mucosal necrosis, villous separation, widening, and shortening, with severe transmural inflammatory infiltration and vacuolated cells in higher-dose groups (Table 1).

On the other hand, LI control sections demonstrated intact mucosa with preserved crypt architecture and normal goblet cell distribution (Figure 2A+B). At 100 mg/kg, mild mucosal irregularities were noted, with limited mononuclear infiltration and minor epithelial alterations. Goblet cells remained preserved, with slightly enhanced PAS staining in some crypts (Figure 2C+D). At 200 mg/kg, lesions progressed to epithelial erosion, crypt distortion, and marked inflammatory infiltration. PAS staining revealed goblet cell loss and diminished mucin secretion (Figure 2E+F). Pathologist's descriptive notes highlighted focal glandular necrosis, epithelial sloughing, and intramucosal inflammatory infiltrates extending into the lumen, with muscularis mucosa consistently preserved (Table 1).

Overall, the small intestine exhibited greater sensitivity to CP, with earlier onset and more severe villous damage, epithelial sloughing, and brush border disruption. The large intestine showed milder changes at 100 mg/kg but developed significant epithelial erosion, crypt distortion, and goblet cell depletion at 200 mg/kg. PAS staining confirmed dose-dependent impairment of mucin secretion, correlating with structural injury. Table 1. shows the histopathological findings in control and treated groups of SI and LI.

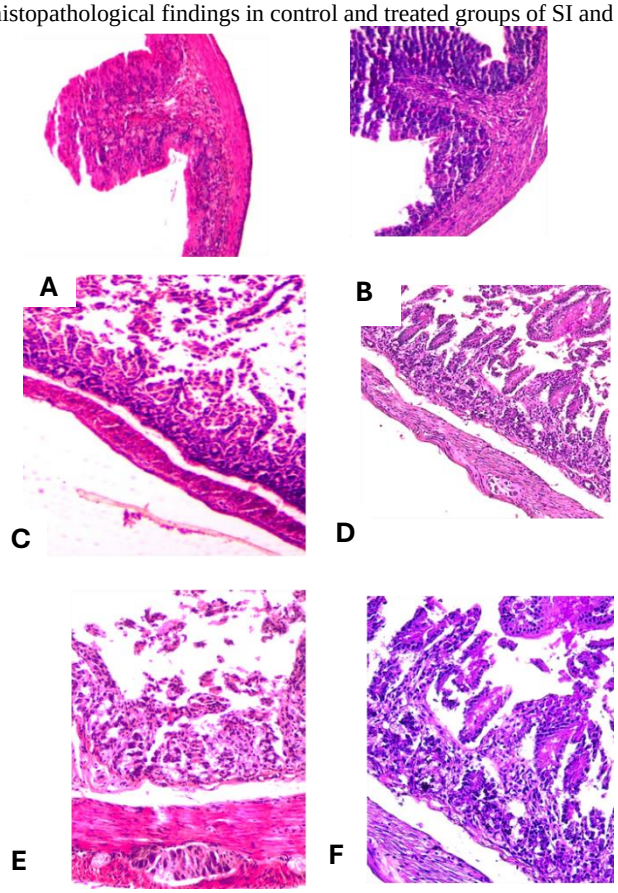

Figure 1. Small intestine: Control group: (A) H&E; (B) PAS. Magnification X40. Treated Group with 100 mg/kg: (C) H&E; (D) PAS. Magnification X100. Treated Group with 200 mg/kg: (E) H&E; (F) PAS. Magnification X100.

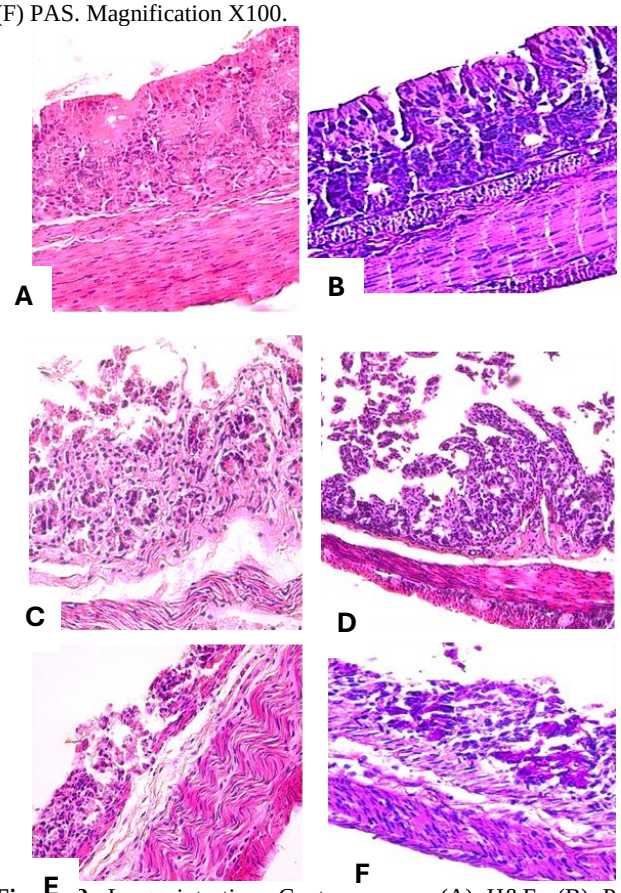

Fig. 2. Large intestine: Contr oup. (A) H&E; (B) PAS. Magnification X100. Treated Group with 100 mg/kg. (C) H&E; (D) PAS. Magnification X100. Treated Group with 1200 mg/kg. (E) H&E; (F) PAS. Magnification X100.

Table 1. Histopathological Findings in Control and Treated Groups of SI and LI

Specimen	Control	100 mg/kg	200 mg/kg
Small intestine (H&E)	Normal villi, intact mucosa, no inflammation	Villous blunting, mild infiltration, superficial necrosis	Villous atrophy/fusion, epithelial sloughing, transmural infiltration, nuclear degeneration
Small intestine (PAS)	Strong brush border, abundant goblet cells	Goblet cells with increased PAS positivity	Loss of brush border, goblet cell depletion, reduced PAS staining
Large intestine (H&E)	Intact mucosa, preserved crypts	Mild irregularities, focal necrosis, limited infiltration	Epithelial erosion, crypt distortion, marked inflammatory infiltration
Large intestine (PAS)	Uniform goblet cell distribution, strong PAS	Goblet cells preserved, mild increase in PAS staining	Goblet cell loss, reduced PAS reactivity, diminished mucin secretion

To complement the descriptive histopathological findings, semi-quantitative scoring was applied using a 0–3 scale (0 = normal, 1 = mild, 2 = moderate, 3 = severe) (Table 2.). The scoring system captured villous damage, goblet cell changes, inflammatory infiltration in the small intestine, and goblet cell changes, inflammatory infiltration, and crypt architecture in the large intestine. As shown in Table 2, scores increased in a dose-dependent manner. Control animals consistently scored 0 across all parameters, while the 100 mg/kg group exhibited mild changes (scores of 1), and the 200 mg/kg group demonstrated moderate to severe alterations (scores of 2–3). The small intestine showed slightly greater sensitivity, with villous damage and inflammatory infiltration reaching severe levels at 200 mg/kg, whereas the large intestine displayed milder changes at 100 mg/kg but progressed to severe crypt distortion and goblet cell depletion at 200 mg/kg.

Table 2. Semi Quantitative Histopathological Scores (0–3 Scale)

Parameter	Control	100 mg/kg	200 mg/kg
Small intestine			
Goblet cell changes (PAS)	0	1	2–3

Inflammatory infiltration	0	1	3
Villous damage	0	1–2	3
Large intestine			
Goblet cell changes (PAS)	0	1	2–3
Inflammatory infiltration	0	1	3
Crypt architecture	0	1	3

Parameters = 0 (normal) 1 (mild) 2 (moderate) 3 (severe)

Semi-quantitative scoring confirmed dose-dependent increases in villous damage, goblet cell depletion, and inflammatory infiltration. One-way ANOVA demonstrated significant differences among groups ($p < 0.001$), with Tukey's post-hoc test confirming that both 100 mg/kg and 200 mg/kg groups differed significantly from controls, and that 200 mg/kg produced more severe lesions than 100 mg/kg. Mean ± SD scores and ANOVA results are summarized in Table 3.

Semi quantitative scoring (0–3 scale) confirmed dose dependent increases in villous damage, goblet cell depletion, and inflammatory infiltration. One-way ANOVA demonstrated significant differences among groups ($p < 0.001$), with Tukey's post hoc test confirming that both 100 mg/kg and 200 mg/kg groups differed significantly from controls, and that 200 mg/kg produced more severe lesions than 100 mg/kg. The dose-dependent progression of histopathological injury is illustrated in Figure 3, which shows mean ± SD scores for each parameter. Control animals scored 0, the 100 mg/kg group showed mild changes, and the 200 mg/kg group reached severe levels across all parameters.

Table 3. Semi-Quantitative Histopathological Scores with ANOVA Results

Parameter	Control (Mean ± SD)	100 mg/kg	200 mg/kg	ANOVA F (df)	p-value
Villous damage (SI)	0.0 ± 0.0	1.5 ± 0.5	3.0 ± 0.0	F(2,24)=95.2	<0.001
Goblet cell changes (SI)	0.0 ± 0.0	1.0 ± 0.0	2.7 ± 0.5	F(2,24)=112.8	<0.001
Inflammatory infiltration (SI)	0.0 ± 0.0	1.2 ± 0.4	3.0 ± 0.0	F(2,24)=134.5	<0.001
Crypt architecture (LI)	0.0 ± 0.0	1.1 ± 0.3	3.0 ± 0.0	F(2,24)=128.6	<0.001
Goblet cell changes (LI)	0.0 ± 0.0	1.0 ± 0.0	2.8 ± 0.4	F(2,24)=119.3	<0.001
Inflammatory infiltration (LI)	0.0 ± 0.0	1.0 ± 0.0	3.0 ± 0.0	F(2,24)=140.7	<0.001

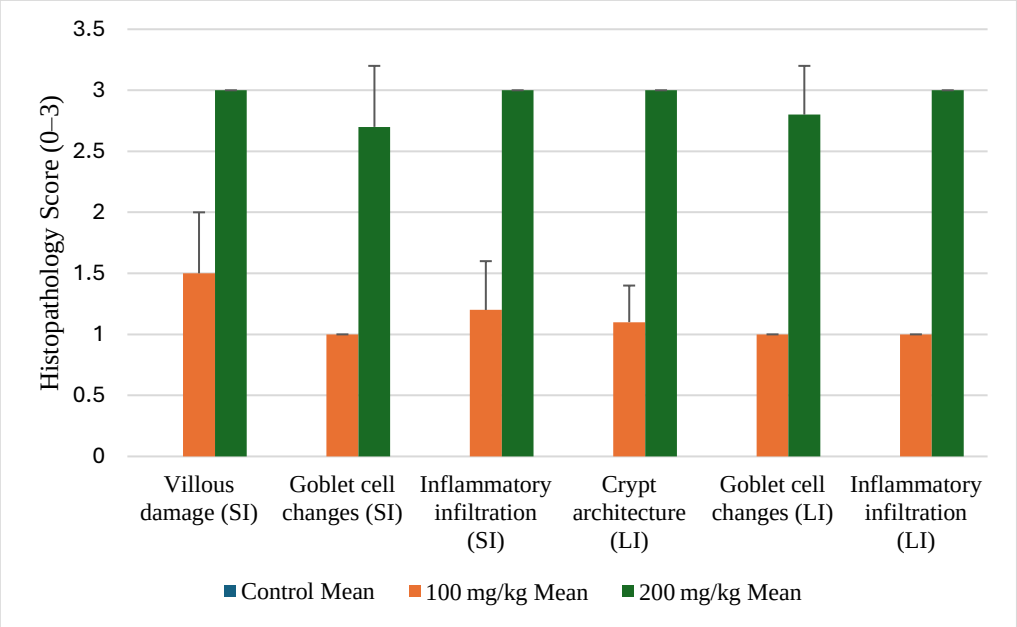

Figure 3. Semi Quantitative Histopathology Scores in Cyclophosphamide Treated Hamsters.

Discussion

This study demonstrates that CP induces dose-dependent histopathological alterations in both the small and large intestines of female golden hamsters. At the therapeutic dose (100 mg/kg), only mild villous blunting and limited inflammatory infiltration were observed in the SI, while the LI remained largely intact. At the toxic dose (200 mg/kg), however, the SI exhibited severe villous fusion, epithelial denudation, and goblet cell depletion, while the LI showed crypt distortion and epithelial erosion. PAS staining confirmed impaired mucin secretion, highlighting disruption of the protective mucosal barrier. These findings align with previous reports of CP induced gastrointestinal toxicity in rodents [10–14,33].

The greater sensitivity of the SI compared to the LI reflects its higher metabolic activity and CYP3A mediated bioactivation [13]. In addition, the small intestine's rapid epithelial turnover and high mitotic activity make it a primary target for alkylating agents such as cyclophosphamide, which preferentially damage proliferating cells [13]. This explains the earlier onset and greater severity of villous injury compared to the large intestine. Moreover, the observed PAS-negative staining at higher doses reflects loss of the protective mucus barrier. Functionally, this disruption facilitates bacterial adherence and translocation across the mucosa, increasing the risk of systemic infection and sepsis, a recognized complication of chemotherapy-induced mucosal injury [12]. Goblet cell depletion and reduced PAS reactivity at higher doses suggest compromised mucin production, weakening the intestinal barrier and predisposing to ulceration, malabsorption, and infection [12,23]. Biomarkers such as fecal calprotectin [10,34] and advanced imaging approaches [29,30] may further aid in detecting early mucosal injury.

Beyond intestinal toxicity, CP is associated with systemic complications including hemorrhagic cystitis [22,23,30,33], pulmonary injury [34], hepatotoxicity [28,29], cardiac dysfunction [35], and even bladder cancer [33]. Rare but severe cases of CP-associated enteritis have also been documented [36]. These systemic effects underscore the broad toxicity profile of CP and the need for comprehensive monitoring during therapy. Protective interventions are an emerging focus. Natural compounds such as *Cordyceps* polysaccharides [21], betulinic acid [22,23], and lycopene [24] have demonstrated protective effects against CP-induced intestinal injury. Dietary antioxidants and cytoprotective agents may help preserve mucosal integrity. Combination regimens and epithelial patterning studies also contextualize CP toxicity within broader chemotherapy strategies [24,37]. Future research should explore these interventions in preclinical models to identify effective strategies for clinical translation.

Local Libyan studies reinforce these observations. Elrashdy et al. documented gastric mucosal alterations in CP treated rats [27], while others demonstrated olive oil's protective effects against CP induced hepatic and renal injury [28,29]. Researchers further described hepatic histopathological changes in female golden hamsters [27].

Together, these studies highlight both the systemic toxicity of CP and the potential of nutritional interventions to mitigate damage. The present findings add the intestinal dimension, underscoring the need to protect gastrointestinal integrity during chemotherapy.

Conclusion

This study demonstrates that CP induces dose-dependent histopathological alterations in the intestines of female golden hamsters, with the SI showing greater sensitivity than the LI. At therapeutic doses, only mild villous blunting and limited infiltration were observed, whereas toxic doses produced severe villous fusion, epithelial denudation, goblet cell depletion, and crypt distortion. PAS staining confirmed impaired mucin secretion, underscoring disruption of the protective mucosal barrier. These findings highlight the gastrointestinal tract as a critical target of CP toxicity and emphasize the need for protective interventions to preserve mucosal integrity during chemotherapy. Future studies integrating morphometric, molecular, and functional analyses will be essential to fully elucidate the mechanisms of injury and to identify effective strategies for clinical translation.

Limitations and Future Directions

This study was limited to acute exposure and descriptive histological evaluation. Future work should incorporate quantitative morphometric analyses (e.g., incorporate villus height measurements, goblet cell counts, inflammatory scoring systems, and immunohistochemical marker), chronic exposure and molecular assays related to oxidative stress and apoptosis.

Conflict of Interest

All authors declare no conflict of interest.

Funding Statement

This research received no external funding.

Ethics Approval

All animal procedures were conducted in accordance with institutional ethical guidelines for animal care and were approved by the Faculty of Biomedical Sciences Scientific Ethics Committee. The study adhered to the principles of the Declaration of Helsinki and followed internationally accepted standards for the ethical treatment of research animals.

Data Availability Statement

All data generated during this study are included in this published article. Additional supplementary data are available from the corresponding author upon reasonable request.

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