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Lactiplantibacillus plantarum-derived postbiotic preparation attenuates dexamethasone-associated testicular injury in adult rats

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Article

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Abstract

Glucocorticoid exposure can disrupt hepatic metabolism and injure the testis through direct and indirect pathways, while steatotic liver disease is associated with impaired male reproductive function. We evaluated whether a *Lactiplantibacillus plantarum*-derived postbiotic preparation attenuates testicular injury in dexamethasone-treated rats. Twenty young adult Wistar male rats (8–10 weeks old) were randomized to four groups (n = 5/group): control, postbiotic, dexamethasone, and combined treatment. Dexamethasone was administered intraperitoneally at 8 mg/kg daily during week 1 and every other day during week 2; the preparation was administered by gastric gavage at 200 mg/kg/day for 14 days. Testes were evaluated by haematoxylin and eosin histopathology, Johnsen and Cosentino scoring, and 8-hydroxy-2'-deoxyguanosine (8-OHdG) immunohistochemistry with digital image analysis. Dexamethasone caused marked seminiferous tubular atrophy and germ-cell depletion. Johnsen scores were 8.53 ± 0.18, 8.73 ± 0.21, 1.50 ± 0.27, and 7.37 ± 0.29, respectively; corresponding Cosentino scores were 1.08 ± 0.02, 1.03 ± 0.01, 3.61 ± 0.04, and 1.49 ± 0.05. Combined treatment was associated with lower 8-OHdG immunoreactivity and preserved seminiferous architecture. Hepatic vacuolation was qualitatively less pronounced with combined treatment. Further studies using chemically characterised preparations are needed to clarify the underlying mechanisms.

1 Introduction

Steatotic liver disease is increasingly recognized as a systemic metabolic disorder rather than an isolated hepatic condition. Hepatic lipid accumulation develops when fatty-acid uptake, *de novo* lipogenesis, oxidation, and export become imbalanced, and the resulting metabolic stress can progress toward inflammation and cellular injury [1–2]. Extrahepatic associations include abnormalities of glucose metabolism, cardiovascular disease, and male reproductive function. Clinical and experimental studies have reported associations between fatty liver and lower reproductive hormone concentrations, altered semen parameters, and impairment of the germinal epithelium [3–4].

Dexamethasone is a synthetic glucocorticoid widely used for its anti-inflammatory and immunosuppressive properties. At experimental doses, however, it can disturb carbohydrate and lipid metabolism and induce hepatic steatosis. In rats, intraperitoneal dexamethasone at 4–16 mg/kg produced fatty changes in the liver, supporting the use of this exposure for experimental hepatic injury [5]. Glucocorticoid exposure can also adversely affect the testis. Experimental studies have described germ-cell loss, oxidative stress, lipid peroxidation, apoptosis, and histopathological deterioration after dexamethasone treatment [6–8].

The intestinal microbiota and its products provide an additional biological interface linking metabolism and systemic organ function. The gut-liver axis integrates microbial, epithelial, immune, and metabolic signals, and disruption of these interactions has been implicated in steatotic liver disease [9]. A related gut-testis axis has emerged as an active field of investigation: microbial metabolites and host-derived mediators may connect intestinal barrier integrity and immune signalling with endocrine regulation and

spermatogenesis [10–14]. Experimental studies have implicated dysbiosis-associated inflammation, altered retinoid metabolism, and gut-derived extracellular vesicles in male reproductive changes, although these pathways are not interchangeable and require model-specific testing [12–14]. Postbiotics have consequently attracted attention as non-viable microbial-derived preparations that may retain biologically active cellular components and microbial products. The current consensus emphasizes that a postbiotic preparation should be sufficiently characterised and shown to confer a health benefit; nomenclature should not imply a defined molecular composition that has not been demonstrated [15]. *Lactiplantibacillus plantarum* is a lactic acid bacterium with documented probiotic and food-related applications [16]. Non-viable postbiotic preparations have also been investigated for potential metabolic and anti-inflammatory effects [17], whereas probiotic strategies have separately been explored in the management of fatty liver disease [18].

The present experiment was designed to examine whether a culture-supernatant-based preparation derived from *Lactiplantibacillus plantarum* ATCC 14917 protects the testis during dexamethasone exposure. Because the final preparation was not chemically characterized, the term "*L. plantarum*-derived postbiotic preparation" is used throughout this article rather than assigning the observed effect to a specific metabolite. We hypothesized that concurrent administration of the preparation would preserve seminiferous architecture and spermatogenesis and would reduce immunohistochemical evidence of oxidative DNA damage.

2 Materials and Methods

2.1 Animals and experimental design

Twenty young adult Wistar male rats aged between 8–10 weeks old were acclimatized for one week at approximately 22°C under a 12-h light/dark cycle. Water and commercial standard balanced feed were available ad libitum. After acclimatization, animals were randomly assigned to four groups of five animals each: untreated control, postbiotic alone, dexamethasone alone, and dexamethasone plus postbiotic. Individual body weights were recorded at group allocation. The experimental schedule is summarized in Table 1. The study was approved by the Ethics Committee of the Faculty of Veterinary Medicine, Urmia University (approval reference No. 27937). All methods were performed in accordance with the relevant guidelines and regulations. The euthanasia procedure was conducted with reference to the AVMA Guidelines for the Euthanasia of Animals: 2020 Edition [19].

2.2 Preparation of the *L. plantarum*-derived postbiotic preparation

Lactiplantibacillus plantarum ATCC 14917 was obtained from the microbial collection of the Iranian Research Organization for Science and Technology. Following activation, the bacterium was cultured in de Man, Rogosa and Sharpe (MRS) medium at approximately 10^8 CFU/mL, 37°C, 5% CO2, for 9 h. The culture supernatant was collected and designated as the postbiotic preparation. It was cooled,

transferred to sterile containers, frozen at − 18°C, and lyophilized. The resulting powder was stored in sealed sterile containers in a desiccator containing silica gel under refrigeration and was resuspended in distilled water immediately before oral administration. The general preparation approach is consistent with published postbiotic-processing workflows [20].

2.3 Treatment protocol

Dexamethasone was administered intraperitoneally at 8 mg/kg/day during the first week and every other day during the second week. The L. plantarum-derived preparation was administered by gastric gavage at 200 mg/kg/day for 14 consecutive days, resuspended in distilled water before dosing. The control group received no experimental treatment, the postbiotic group received the postbiotic preparation alone, the dexamethasone group received dexamethasone alone, and the combined group received both treatments. After two weeks, animals were euthanized by intraperitoneal administration of ketamine hydrochloride (300 mg/kg; 10% solution; Alfasan, Woerden, The Netherlands) in combination with xylazine hydrochloride (30 mg/kg; 2% solution; Alfasan, Woerden, The Netherlands). The procedure was conducted with reference to the AVMA guidance on dissociative-agent combinations for rodents [19]. During the procedure, loss of the righting reflex, generalized muscle flaccidity, cessation of spontaneous movement, and absence of corneal and pedal withdrawal reflexes were observed. Death was confirmed individually by the absence of spontaneous respiration and heartbeat on auscultation. Following confirmation of death, liver and testicular tissues were collected, rinsed in physiological saline, and fixed in 10% buffered formalin.

2.4 Histological processing and hematoxylin and eosin staining

Testicular tissues were fixed in 10% buffered formalin for approximately one week. The testicular capsule was then removed, a transverse section was made, and the tissue was returned to formalin for a further week. Samples were dehydrated through graded ethanol, cleared in xylene, embedded in paraffin, and sectioned at 5 μm. Sections were stained with routine Hematoxylin and Eosin (H&E). Microscopic assessment focused on seminiferous tubular architecture, germ-cell organization, germ-cell degeneration and pyknosis, Sertoli-cell integrity, interstitial edema, and vascular congestion. Representative images were acquired with a Canon PowerShot SX230 HS camera.

2.5 Assessment of spermatogenesis and histopathological injury

One hundred seminiferous tubules from each experimental group were assessed for spermatogenic integrity using the 10-point Johnsen scoring system, in which 1 indicates severe loss of germinal elements and tubular atrophy and 10 indicates complete spermatogenesis with abundant spermatozoa [21]. Histopathological severity was additionally graded with the four-point Cosentino system, in which 1 represents essentially normal architecture and 4 represents severe structural injury with hemorrhagic and necrotic changes [22].

2.6 8-OHdG immunohistochemistry and image analysis

Immunohistochemistry was used to assess 8-hydroxy-2'-deoxyguanosine (8-OHdG), a marker of oxidatively modified DNA [23]. Sections were deparaffinized and rehydrated through descending ethanol concentrations (96%, 90%, 80%, and 70%). Antigen retrieval was performed in sodium citrate buffer (pH 7.2) by microwave heating for 3 min at 720 W followed by 10 min at 180 W. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide for 5 min, after which the sections were treated with a protein-blocking solution. The primary anti-8-OHdG antibody (Elabscience, China) was applied at a 1:200 dilution for 18 h at 4°C. MACH-Universal reagent containing horseradish peroxidase (HRP)-conjugated secondary detection was applied for 60 min, followed by 3,3'-diaminobenzidine (DAB) chromogen for 5 min and brief hematoxylin counterstaining. Sections were then dehydrated, cleared, and coverslipped.

Digital image analysis was performed using ImageJ. Percentage-positive cells and the intensity of the brown 8-OHdG immunoreactive signal were assessed within defined image fields. Representative staining and the corresponding group-level comparisons are presented in Fig. 3.

2.7 Statistical analysis

The results were analyzed with SPSS version 18 using one-way analysis of variance followed by Tukey multiple comparisons, with $p < 0.05$ considered statistically significant.

Table 1
Experimental groups and treatment schedule

Group	n	Dexamethasone	Postbiotic
Control	5	None	None
Postbiotic	5	None	200 mg/kg/day by gastric gavage, days 1–14
Dexamethasone	5	8 mg/kg/day i.p., days 1–7; 8 mg/kg every other day, days 8–14	None
Dexamethasone + postbiotic	5	8 mg/kg/day i.p., days 1–7; 8 mg/kg every other day, days 8–14	200 mg/kg/day by gastric gavage, days 1–14
i.p. intraperitoneal			

3 Results

3.1 Dexamethasone-associated testicular histopathology

Untreated control testes showed regularly arranged seminiferous tubules with preserved germ-cell layers and active spermatogenesis. The postbiotic-only group was morphologically similar to the control group, with no obvious treatment-associated structural injury (Fig. 1A, B).

Dexamethasone produced severe disruption of the seminiferous epithelium, as reflected by marked germ-cell depletion, pyknotic nuclei, tubular atrophy, widened interstitial spaces, and vascular congestion. Many tubules contained only a residual basal layer of spermatogonial cells, while primary spermatocytes and more advanced germ-cell stages were markedly reduced or absent. Sertoli-cell depletion or vacuolation was also evident. In the dexamethasone group, mean seminiferous tubular diameter was approximately 153 μm compared with approximately 193 μm in controls, corresponding to a reduction of about 21%.

The combined dexamethasone plus postbiotic group showed substantial preservation of seminiferous architecture (Fig. 1D; additional fields are shown in Fig. 2C, D). Most tubules retained multiple germ-cell layers extending to spermatids and spermatozoa, and the overall appearance was considerably closer to the untreated condition than to dexamethasone alone. The reported mean tubular diameter was approximately 178 μm, corresponding to a substantially smaller reduction from control than that observed after dexamethasone alone.

3.2 Johnsen and Cosentino scores

The quantitative histopathological indices were concordant with the microscopic findings (Table 2). The Johnsen score was 8.53 ± 0.18 in controls, 8.73 ± 0.21 in the postbiotic-only group, 1.50 ± 0.27 after dexamethasone, and 7.37 ± 0.29 after combined treatment. Statistically, dexamethasone + postbiotic group showed differences with others at $p < 0.05$ (Table 2). The Cosentino score was 1.08 ± 0.02 in controls, 1.03 ± 0.01 in the postbiotic-only group, 3.61 ± 0.04 after dexamethasone, and 1.49 ± 0.05 after combined treatment. Thus, dexamethasone produced the most severe histopathological injury, while concurrent postbiotic treatment markedly improved both spermatogenic integrity and the injury score at $p < 0.05$ (Table 2).

Table 2
Johnsen and Cosentino scores

Group	Johnsen score (mean ± SD)	Cosentino score (mean ± SD)
Control	8.53 ± 0.18	1.08 ± 0.02
Postbiotic	8.73 ± 0.21	1.03 ± 0.01
Dexamethasone	1.50 ± 0.27	3.61 ± 0.04
Dexamethasone + postbiotic	7.37 ± 0.29	1.49 ± 0.05

Different superscript letters indicate significant differences within the corresponding column at $p < 0.05$. Values are mean ± SD.

3.3 8-OHdG immunohistochemistry

Testicular 8-OHdG immunoreactivity was significantly higher in dexamethasone-treated animals than in the control and postbiotic-only groups ($p < 0.05$). Concurrent administration of the *L. plantarum*-derived

preparation significantly reduced 8-OHdG immunoreactivity relative to dexamethasone alone ($p < 0.05$) (Fig. 3). The figure combined representative DAB-stained sections with quantitative presentation of the percentage of positive cells and pixel-intensity measurements.

The group-level direction of the 8-OHdG response paralleled the histopathological scores: the group with the greatest seminiferous injury showed the strongest immunoreactivity, whereas combined treatment was associated with both structural preservation and lower 8-OHdG signal.

3.4 Descriptive hepatic findings

Liver sections were also examined to characterize the hepatic phenotype accompanying dexamethasone exposure (Fig. 4). Control and postbiotic-only sections showed generally preserved hepatic architecture with relatively few small optically clear cytoplasmic vacuoles. Dexamethasone-treated livers contained more numerous and larger clear vacuoles, consistent with lipid droplets being dissolved during routine tissue processing. In the combined-treatment group, vacuolation was visibly less extensive than in the dexamethasone-only group. Because hepatic lesions were not assigned a numerical steatosis score and no quantitative lipid measurement was performed, these liver observations are descriptive and are not presented as a statistically tested efficacy endpoint.

4 Discussion

4.1 Dexamethasone-associated testicular injury

The principal finding was a severe testicular injury phenotype following dexamethasone exposure. Seminiferous tubular diameter declined, the germ-cell series was depleted, pyknotic nuclei and Sertoli-cell alterations were apparent, and interstitial edema and vascular congestion accompanied the loss of normal architecture. The Johnsen and Cosentino scores changed in the same direction as the qualitative histology, strengthening the internal consistency of the observed phenotype. These findings accord with experimental reports of glucocorticoid-associated germ-cell injury [6–8]. Glucocorticoids may affect the testis through more than one route: systemic endocrine suppression can alter hypothalamic-pituitary-gonadal signalling, while local changes in redox balance, cellular survival, and vascular homeostasis may contribute to epithelial disruption. In the present experiment, however, gonadotropins, testosterone, direct apoptosis markers, and testicular perfusion were not measured; these pathways therefore remain plausible explanations rather than demonstrated mechanisms.

4.2 Relationship between dexamethasone, hepatic steatosis, and testicular injury

The additional liver sections showed more numerous and larger cytoplasmic vacuoles in dexamethasone-treated animals and less conspicuous vacuolation in the combined-treatment group. This pattern is consistent with experimental evidence that pharmacological dexamethasone exposure can produce hepatic fatty change [5]. It also provides histological context for the established clinical and

experimental association between steatotic liver disease and male reproductive abnormalities [3–4]. Nevertheless, the hepatic assessment in this study was descriptive: no blinded steatosis grade, hepatic triglyceride concentration, serum lipid profile, liver enzyme panel, insulin measurement, or inflammatory marker was obtained. The data therefore do not establish that liver steatosis mediated the testicular lesions or that improvement in the liver caused the testicular response. Direct testicular glucocorticoid effects and indirect consequences of systemic metabolic disturbance may have occurred concurrently. This distinction is important because a short pharmacological dexamethasone model is not equivalent to chronic metabolic dysfunction-associated steatotic liver disease in people. Probiotic-based approaches have also been explored in the management of fatty liver disease [18], but this literature does not establish efficacy for the non-viable culture-supernatant preparation studied here.

4.3 Protective association of the *L. plantarum*-derived preparation

Concurrent administration of the L. plantarum-derived preparation was associated with substantial preservation of testicular structure. The Johnsen score increased from 1.50 after dexamethasone alone to 7.37 with combined treatment, while the Cosentino score decreased from 3.61 to 1.49. The qualitative sections at both lower and higher magnification similarly show more preserved seminiferous epithelium and retention of multiple germ-cell stages in the combined group. The postbiotic-only group was morphologically similar to the control group, with no obvious treatment-associated structural injury over the two-week period. These findings support a protective association in this model, but do not demonstrate restoration of fertility. Sperm concentration, motility, morphology, epididymal sperm reserves, mating success, and offspring outcomes remain other parameters for future investigations.

4.4 8-OHdG and oxidative DNA damage

The decrease in 8-OHdG immunoreactivity after combined treatment provides a second line of evidence that tracks with the histological findings. 8-OHdG is a marker of oxidatively modified DNA [23], and the stronger signal in dexamethasone-treated testes is compatible with increased oxidative DNA damage during severe tissue injury. Its reduction alongside improved seminiferous architecture is consistent with, but does not prove, a role for redox regulation in the observed protection. Immunoreactivity alone cannot establish the rate of reactive oxygen species generation, the balance between oxidant production and antioxidant defence, or the occurrence of apoptosis. No direct measurements of lipid peroxidation, reduced glutathione, superoxide dismutase, catalase, glutathione peroxidase, caspase activation, or TUNEL-positive cells were included. Accordingly, the findings should be described as reduced 8-OHdG immunoreactivity rather than as definitive evidence of a specific antioxidant or anti-apoptotic mechanism.

4.5 Biological plausibility of a postbiotic effect

A biological effect of an orally administered *L. plantarum*-derived preparation is plausible, but several mechanistic routes remain open. Depending on the growth and processing steps, a culture-supernatant-derived preparation may contain soluble microbial metabolites and other secreted products capable of

interacting with intestinal epithelial, immune, and metabolic pathways. These constituents were not chemically profiled in the present experiment, so no individual compound can be assigned responsibility for the observed histological response. The distinction matters for postbiotic research because preparations can differ substantially in composition, stability, and biological activity; reproducible evaluation requires a defined production process, compositional characterisation, and appropriate confirmation of the material administered [15, 17, 24].

The gut-liver axis offers one possible route for systemic effects: intestinal barrier function, microbial metabolites, bile-acid signalling, and host inflammatory responses can influence hepatic metabolism [9]. The gut-testis axis is an increasingly active area of reproductive biology, with experimental work indicating that gut microbial communities and metabolites may affect testicular structure, spermatogenesis, and the hormonal environment through several non-exclusive pathways [10–11]. Proposed mediators include changes in intestinal permeability and circulating microbial products, low-grade inflammation, short-chain fatty acids and other microbial metabolites, altered retinoid or bile-acid signalling, and endocrine crosstalk. These pathways could, in principle, affect Sertoli-cell function, blood-testis barrier integrity, germ-cell survival, or oxidative stress, but none was measured here.

The mechanistic literature supports the plausibility of this framework without proving that it operated in this experiment. In a mouse model of diethylhexylphthalate exposure, viable *L. plantarum* TW1-1 was associated with changes in gut microbiota, intestinal permeability, circulating lipopolysaccharide, inflammation, and testicular injury [13]. A separate metabolic-syndrome model linked disrupted spermatogenesis with altered microbial vitamin A metabolism [12]. More recently, gut-derived circulating exosomes carrying altered microRNA cargo were shown to impair meiosis in mice, illustrating that gut-to-testis communication may involve host-derived circulating mediators as well as classical microbial metabolites [14]. These studies used different exposures and, in some cases, live bacteria rather than an uncharacterised culture-supernatant preparation. They therefore provide biological context, not direct evidence that microbiota modulation, exosome signalling, endocrine normalisation, or barrier repair explains the present findings.

Discriminating among these possibilities will require future studies that characterise the administered preparation and measure intestinal and systemic endpoints alongside reproductive outcomes. Useful additions include microbiome profiling; intestinal tight-junction and permeability measurements; circulating lipopolysaccharide and inflammatory cytokines; short-chain fatty acids and bile-acid profiles; serum testosterone, LH, and FSH; direct testicular redox and apoptosis markers; and sperm quality or fertility endpoints.

5 Conclusions

Under the conditions tested, dexamethasone exposure was associated with severe seminiferous architectural injury, impaired spermatogenesis scores, and increased testicular 8-OHdG immunoreactivity. Concurrent administration of the *L. plantarum*-derived preparation at 200 mg/kg/day

was associated with improved histopathological scores, preservation of seminiferous architecture, and lower 8-OHdG signal. Liver sections showed a qualitatively less conspicuous steatotic pattern in the combined-treatment group, but these observations were not quantitatively scored. The results support further investigation of a standardised preparation, but do not identify its active constituents, establish a gut-testis mechanism, demonstrate improved fertility, or distinguish direct testicular effects from systemic metabolic effects of dexamethasone.

Declarations

Ethics approval and animal welfare

The study was approved by the Ethics Committee of the Faculty of Veterinary Medicine, Urmia University (approval reference No. 27937). All methods were performed in accordance with the relevant guidelines and regulations. The euthanasia procedure was conducted with reference to the AVMA Guidelines for the Euthanasia of Animals: 2020 Edition.

Competing interests

The authors declare no competing interests.

Consent for publication

Not applicable.

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Author Contribution

B. K. M.: Data curation, Formal analysis. Z. H.: . Assistance in methodology.M. H.: Conceptualization, Methodology, Supervision, Writing - review and editing, Correspondence. R. H.: Supervision, Interpretation of histopathology.A. Sh. J.: Supervision, Testicular result interpretation.M. M.: Postbiotic preparation, Supervision, Review and editing.

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Data Availability

The group-level data underlying the reported histopathological outcomes are presented in the manuscript. The underlying study records and any available raw data should be made available by the corresponding author on reasonable request, subject to institutional permissions.

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Figures

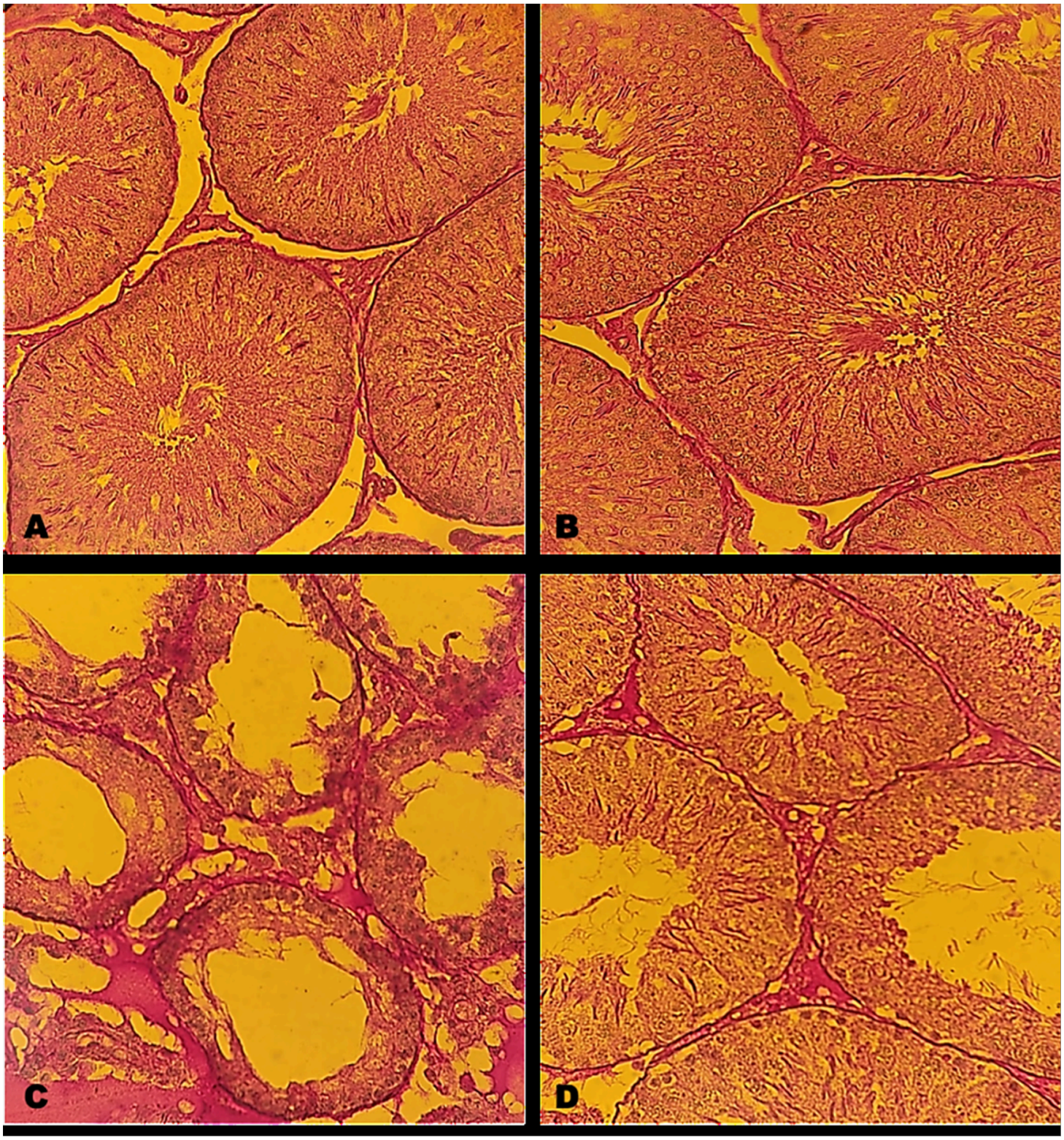


Figure 1

Representative hematoxylin and eosin-stained testicular sections from the four experimental groups: (A) control; (B) postbiotic; (C) dexamethasone; and (D) dexamethasone + postbiotic. Dexamethasone was associated with marked tubular atrophy and loss of the spermatogenic series, whereas combined treatment preserved seminiferous architecture

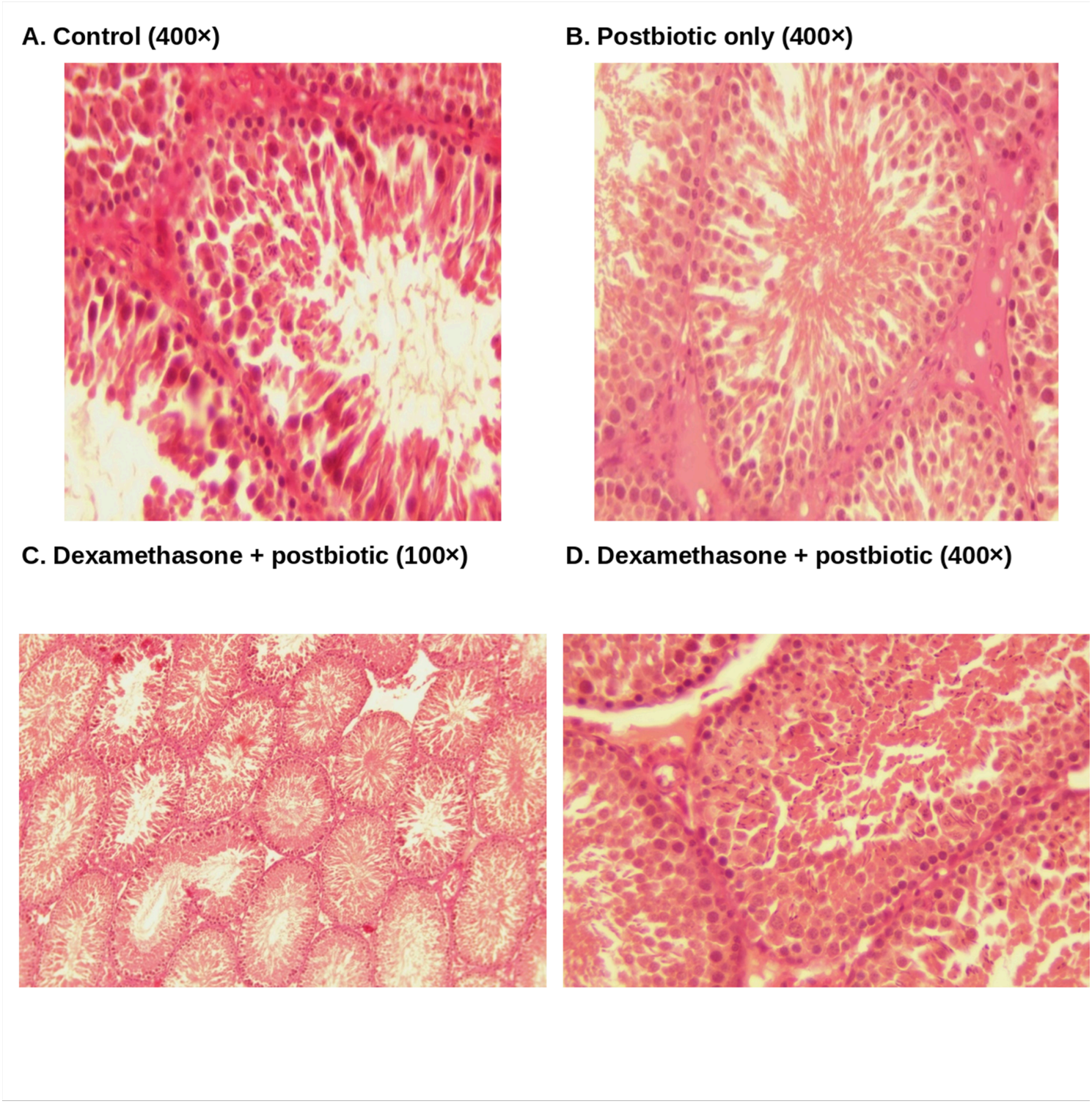


Figure 2

Additional representative hematoxylin and eosin (H&E)-stained testicular micrographs. (A) Control, 400×; (B) postbiotic-only, 400×; (C) dexamethasone + postbiotic, 100×; and (D) dexamethasone + postbiotic, 400×. These panels provide additional views of preserved seminiferous architecture in the control and postbiotic-only groups and of the germinal epithelium in the combined-treatment group. Magnifications follow the recorded descriptions; calibrated scale bars were not available in the supplied micrographs.

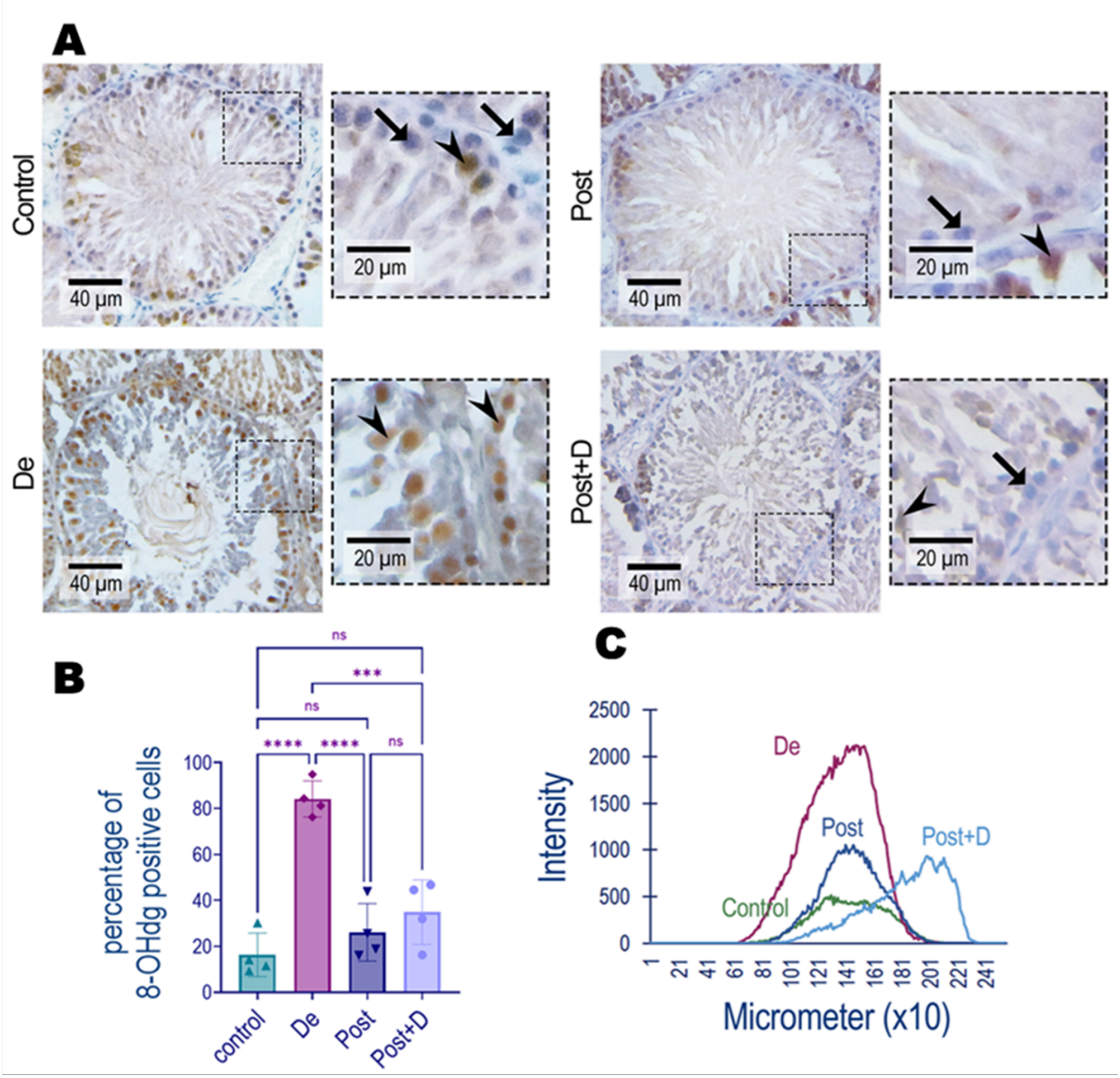


Figure 3

8-hydroxy-2'-deoxyguanosine (8-OHdG) immunohistochemistry and digital image analysis in rat testes after two weeks of treatment. Representative DAB-stained sections are shown with percentage-positive-

cell and pixel-intensity measurements. Dexamethasone increased immunoreactivity, whereas combined treatment reduced the signal relative to dexamethasone alone.

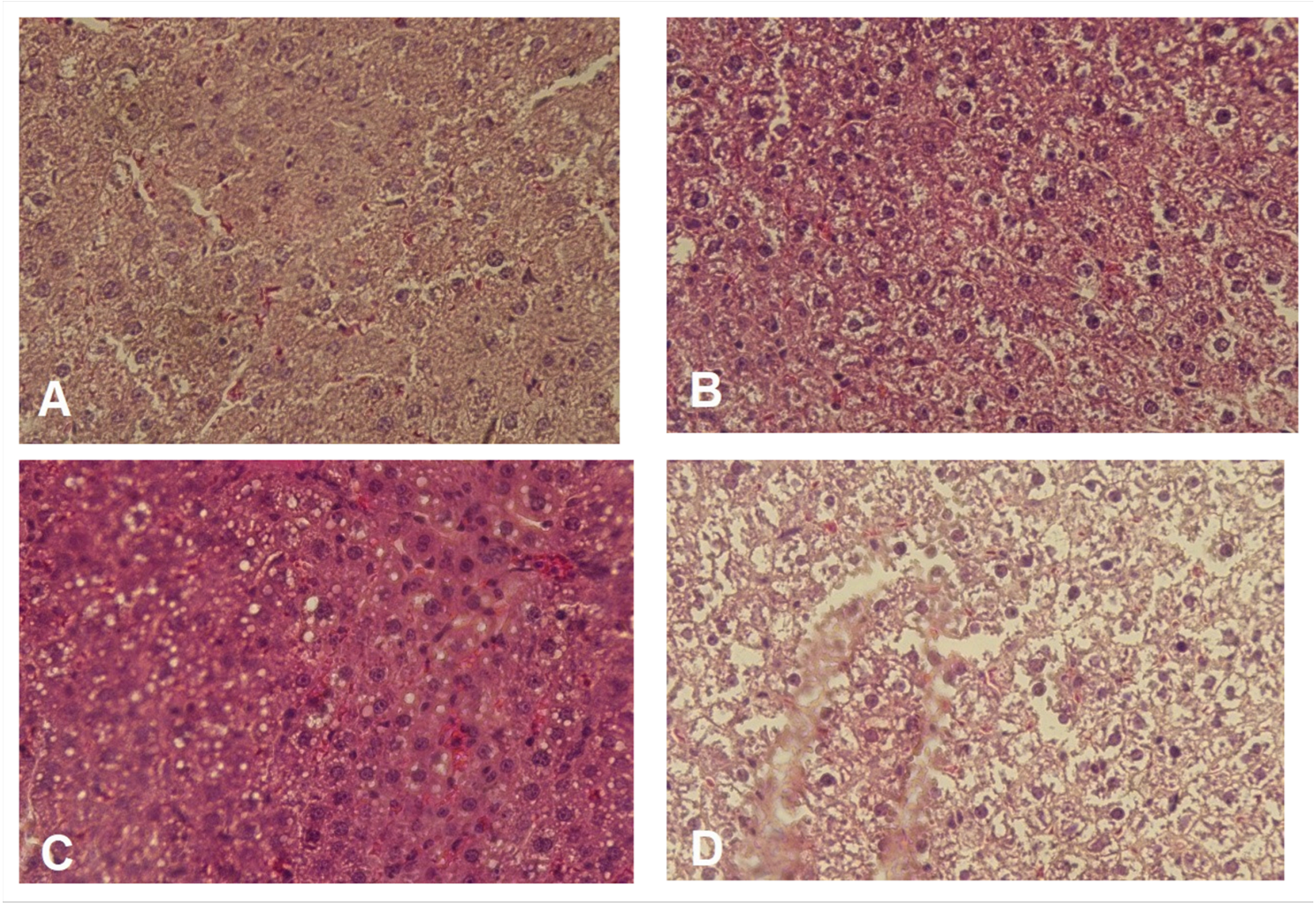


Figure 4

Representative hematoxylin and eosin-stained liver sections at 400×: (A) control; (B) dexamethasone + postbiotic; (C) postbiotic-only; and (D) dexamethasone. Optically clear cytoplasmic spaces represent sites of lipid vacuoles dissolved during routine processing. Larger and more numerous vacuoles are evident in the dexamethasone group, whereas the combined-treatment group shows qualitatively less extensive vacuolation. Liver lesions were not scored or quantified.