MELATONIN ATTENUATES ALCOHOL-INDUCED OXIDATIVE STRESS AND DUODENAL INJURY IN WISTAR RATS

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Abstract

Long-term alcohol intake can damage the lining of the small intestine by increasing harmful oxidative processes. Melatonin is a naturally produced substance in the body known for its strong protective and antioxidant properties. This study examined whether melatonin could protect the duodenum against alcohol-induced injury in adult rats. Sixteen female Wistar rats were divided into four groups and treated for twenty-eight days with 1 ml/kg of 50% alcohol, 5 mg/kg of melatonin, both substances together, or neither. Changes in body weight, antioxidant activity, lipid damage, and duodenal tissue structure were assessed. Alcohol exposure reduced weight gain, weakened antioxidant defenses, increased lipid damage, and caused marked injury to the duodenal lining. Treatment with melatonin significantly improved weight gain, restored antioxidant balance, reduced tissue damage, and preserved normal duodenal structure. Melatonin alone produced no harmful effects. These findings suggest that melatonin protects the duodenum against alcohol-induced damage, possibly through attenuation of oxidative stress.

Keywords: Alcohol-induced injury, Antioxidant enzymes, Duodenum, Melatonin, Oxidative stress.

Introduction

Alcohol-induced gastrointestinal injury remains a significant global health concern, contributing to chronic mucosal inflammation, epithelial erosion, oxidative stress, and ulcer formation (Chen and Haber, 2021). The duodenum, being the first segment of the small intestine exposed to gastric chyme and ingested irritants, is particularly vulnerable to ethanol-induced oxidative damage (Beck, 2017). High concentrations of alcohol have been shown to disrupt mucosal integrity through excessive generation of reactive oxygen species (ROS), lipid peroxidation, and impairment of the endogenous antioxidant defense system (Das and Vasudevan, 2007). These biochemical disturbances result in structural mucosal damage characterized primarily by erosive and inflammatory changes, which may progress to more severe injury with continued exposure. Consistent with this, Akinrinde and Ajibade (2024) reported duodenal alterations more in line with severe erosive and inflammatory mucosal injury, with potential progression toward ulcerative lesions.

Received 6 January 2026; Revised 5 June 2025 Accepted 7 July 2026; Published Online 22 July 2026; Page 1
Published xx September 2026

Superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx), constitute the primary enzymatic barrier against oxidative injury in the gastrointestinal tract (Halliwell, 2012). Ethanol ingestion depletes these antioxidant systems while simultaneously increasing malondialdehyde (MDA), a key marker of lipid peroxidation (Albano, 2006). The resulting redox imbalance plays a critical role in the pathogenesis of alcohol-induced duodenal mucosal injury (Chung et al., 2025). Consequently, research interest has increased in identifying natural or endogenous compounds capable of restoring mucosal protection through antioxidant, anti-inflammatory, or cytoprotective mechanisms.

Melatonin, a neurohormone synthesized by the pineal gland and extensively produced in the gastrointestinal tract, has emerged as a potent mucosal protective agent. Beyond its circadian functions, melatonin exhibits strong free-radical scavenging activity, enhances antioxidant enzyme expression, and stabilizes epithelial tight junctions (Akinci et al., 2013). Experimental evidence demonstrates that melatonin attenuates alcohol-induced gastric and intestinal injury by reducing oxidative stress, suppressing pro-inflammatory cytokines, and promoting epithelial regeneration (Hu et al., 2009; Brzozowska et al., 2014; Karaaslan and Suzen, 2015; Kolli et al., 2013). Its role in improving mucosal microcirculation and preventing ethanol-mediated apoptosis further supports its therapeutic potential in gastrointestinal disorders (de Talamoni et al., 2017).

Despite extensive documentation of melatonin's gastroprotective effects, limited studies have specifically evaluated its protective effect on the duodenum following chronic alcohol exposure, particularly using integrated biochemical and histomorphological endpoints. Understanding this relationship is essential because duodenal injury contributes significantly to malabsorption, dyspepsia, and increased susceptibility to further intestinal pathologies (Montoro-Huguet et al., 2021).

Therefore, this study investigates the effects of melatonin on alcohol-induced duodenal injury, assessing changes in body weight, oxidative stress biomarkers (SOD, CAT, GPx, MDA), and histological architecture in Wistar rats after 28 days of exposure. This will provide additional insight into the protective potential of melatonin in alcohol-related intestinal pathology.

Materials and Methods

Experimental Animals

Sixteen (16) adult female Wistar rats weighing 160–180 g were obtained from the Animal House of the Department of Anatomy. The animals were housed under standard laboratory conditions (12 h light/12 h dark cycle, temperature 22–25 °C, humidity 50–60%) and allowed free access to commercial pellet feed and water *ad libitum*. Animals were acclimatized for 7 days before experimentation. All procedures were conducted according to the Research Ethics Committee of the College of Medical Sciences, University of Benin's guidelines for the care and use of laboratory animals and in compliance with international ethical standards for animal research.

Experimental Design

The rats were randomly divided into four groups (n = 4 per group):

- **Group 1 (Control):** Received distilled water only.
- **Group 2 (Alcohol Only):** Received 1 ml/kg of 50% ethanol daily.
- **Group 3 (Melatonin + Alcohol):** Received melatonin (5 mg/kg) + 1 ml/kg of 50% ethanol.
- **Group 4 (Melatonin only):** Received melatonin only (5 mg/kg body weight) orally.

Melatonin (Nature's Bounty®, 5 mg softgel dietary supplement, manufactured by Bactolac Pharmaceutical Inc., USA) was used in this study. The softgel capsule was punctured and its liquid contents were administered directly by oral gavage at a dose of 5 mg/kg body weight. In

the co-treatment group, melatonin and ethanol were administered simultaneously once daily for 28 consecutive days.

All administrations were given orally once daily for 28 consecutive days using an orogastric cannula.

1 mL/kg body weight of 50% (v/v) ethanol was administered orally once daily using an orogastric cannula. This regimen has been widely used in experimental models of chronic ethanol-induced organ toxicity and oxidative stress in Wistar rats, including hepatotoxicity studies (Obayuwana and Okereke, 2024)

Body Weight Measurement

Body weights were recorded on Day 0 (initial weight) and Day 28 (final weight) using a digital weighing balance. Weight change for each animal was calculated as:

Weight Gain (g) = Final Body Weight – Initial Body Weight.

Tissue Collection

On Day 29, animals were deeply anesthetized with ketamine (80 mg/kg) and sodium pentobarbital (200 mg/kg, diluted 1:1 with normal saline to reduce peritoneal irritation) administered intraperitoneally prior to tissue collection. Adequate anesthetic depth was confirmed before euthanasia and tissue harvesting. Animals were euthanized under deep anesthesia, after which the duodenum was carefully excised for histological and biochemical analyses.

- A portion was fixed in 10% neutral buffered formalin for histology.
- The remaining portion was immediately rinsed in ice-cold saline, blotted dry, weighed, and homogenized for biochemical analyses.

Preparation of Tissue Homogenate

Duodenal tissues were homogenized in ice-cold phosphate-buffered saline (PBS; pH 7.4) using a Teflon homogenizer to yield a 10% (w/v) homogenate. The homogenate was centrifuged at 3000 rpm for 15 minutes at 4°C, and the supernatant was collected for assay of oxidative stress markers.

Biochemical Analysis

Superoxide Dismutase (SOD) Activity

Superoxide dismutase (SOD) activity was determined according to the method described by Misra and Fridovich (1972), based on inhibition of epinephrine autoxidation.

Catalase (CAT) Activity

Catalase (CAT) activity was determined using the method originally described by Sinha (1972), based on the decomposition of hydrogen peroxide and subsequent formation of chromic acetate measured colorimetrically.

Glutathione Peroxidase (GPx) Activity

Glutathione peroxidase (GPx) activity was determined according to the method described by Paglia and Valentine (1967), based on the oxidation of reduced glutathione in the presence of cumene hydroperoxide.

Lipid Peroxidation (MDA Levels)

Malondialdehyde (MDA) activity, an index of lipid peroxidation, was determined according to the method described by Buege and Aust (1978).

Histological Processing

Formalin-fixed duodenal tissues were dehydrated in ascending alcohol grades, cleared in xylene, and embedded in paraffin wax. Sections of 5 µm thickness were cut using a rotary microtome and stained with hematoxylin and eosin (H&E).

Slides were examined under a light microscope at 40x and 100x magnifications. Photomicrographs were captured for documentation and comparison of architectural changes including:

- mucosal villi integrity

- mucosal glands
- submucosa architecture
- ulceration or erosion
- muscularis mucosa and propria

Histological assessment was performed descriptively by blinded examination of tissue sections to compare architectural alterations among experimental groups. Due to the exploratory nature of the study, no semiquantitative histopathological scoring or morphometric analysis was performed.

Statistical Analysis

All data were expressed as mean ± standard error of mean (SEM). Differences among groups were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test for multiple comparisons. Within-group comparison of initial and final body weight was carried out using paired t-test. A p-value < 0.05 was considered statistically significant. Statistical analyses were performed using SPSS version 25.0.

Results and Discussion

This study investigated the effects of alcohol, melatonin, and their combined administration on body weight, oxidative stress parameters, and duodenal histomorphology in Wistar rats. The findings demonstrate that chronic exposure to 50% alcohol induces significant oxidative and structural damage in the duodenum, while melatonin effectively protects against these deleterious effects.

At baseline, there were no significant differences in initial body weights across the experimental groups. After 28 days of treatment, paired comparisons showed a significant increase in final body weight within each group relative to its initial value ($p < 0.05$) as indicated in Table 1.

When weight gain was compared across groups (Table 2), rats treated with 50% alcohol only exhibited a significant reduction in weight gain relative to the control group ($p < 0.05$). Conversely, animals treated with alcohol + melatonin demonstrated significantly greater weight gain compared with the alcohol-only group (#p < 0.05), suggesting partial recovery of growth deficit by melatonin co-administration.

Alcohol administration resulted in markedly reduced weight gain compared to the control group (Table 2), consistent with previous studies demonstrating that ethanol impairs nutrient absorption, increases metabolic burden, and induces gastrointestinal mucosal injury and inflammation (Butt et al., 2023). Chronic ethanol exposure has also been associated with impaired intestinal function and metabolic dysregulation, which may contribute to reduced growth and body weight gain (Keshavarzian and Fields, 2017). In the present study, co-administration of melatonin significantly improved weight gain relative to the alcohol-only group, suggesting that melatonin may support metabolic stability and attenuate alcohol-induced physiological stress.

Table 1: Body Weight Parameters - Within Group Comparisons (Initial vs Final)

Group	Initial Weight (g)	Final Weight (g)	t-value (df)	p-value (Paired t-test)
Control	166.5 ± 0.9	187.0 ± 0.4	t(3) = 41.00	**<0.001***
50% Alcohol	166.8 ± 4.2	171.5 ± 4.1	t(3) = 7.55	**0.005***
5mg/kg Melatonin + 50% Alcohol	173.3 ± 3.3	191.5 ± 2.1	t(3) = 11.79	**<0.001***
5mg/kg Melatonin	165.5 ± 2.3	191.3 ± 1.2	t(3) = 21.80	**<0.001***

*Values are expressed as mean ± SEM. (n = 4) *P <0.05 indicates statistical significance between initial and final weight within each group using paired t-test*

Table 2: Weight Change across Experimental Groups

Parameter/ Group	Control	50% Alcohol	5mg/kg Melatonin + 50% Alcohol	5mg/kg Melatonin	F-value (df)	p-value
Weight Change (g)	20.5 ± 0.5	4.8 ± 0.6 *	18.3 ± 1.6 #	25.8 ± 1.2 #	F(3,12) = 72.13	**<0.001**

*Values are expressed as mean ± SEM. (n = 4) *P <0.05 compared with the control group; #P <0.05 compared with the 50% alcohol only group based on Tukey's HSD post-hoc test following one-way ANOVA.*

The antioxidant assays provide further evidence of alcohol-induced oxidative stress. Rats treated with alcohol showed significant reductions in SOD, CAT, and GPx activities alongside elevated MDA levels (Table 3), confirming increased oxidative stress within the duodenum. These alterations are consistent with previous reports demonstrating that ethanol metabolism generates free radicals and disrupts the enzymatic antioxidant defense system (Contreras-Zentella et al., 2022). Lipid peroxidation, indicated by increased MDA levels, reflects membrane damage and plays a central role in ethanol-induced mucosal injury (Akinrinde and Ajibade, 2024).

Melatonin supplementation significantly reversed these biochemical alterations. Co-treatment restored SOD, CAT, and GPx activities and reduced MDA concentrations toward normal values (Table 3). These findings align with established evidence that melatonin is a potent antioxidant capable of directly scavenging free radicals, upregulating endogenous antioxidant enzymes, and stabilizing cellular membranes (García et al., 2014; Reiter et al., 2016; Hacışevki and Baba, 2018). Melatonin also improves mitochondrial function and reduces the activation of inflammatory pathways, contributing to its protective effects in gastrointestinal tissues (Favero et al., 2017).

Table 3: Oxidative Stress Parameters in Duodenal Tissue

Parameter /Group	Control	50% Alcohol	5mg/kg Melatonin + 50% Alcohol	5mg/kg Melatonin	F-value (df)	p-value
SOD	1.68 ± 0.06	0.58 ± 0.08*	1.68 ± 0.11#	1.73 ± 0.11#	F(3,12) = 37.53	**<0.001**
CAT	1.29 ± 0.01	0.28 ± 0.02*	1.31 ± 0.01#	1.33 ± 0.01#	F(3,12) = 1591.92	**<0.001**
GPx	1.49 ± 0.04	0.79 ± 0.05*	1.13 ± 0.04*#	1.14 ± 0.05#	F(3,12) = 41.33	**<0.001**
MDA	0.46 ± 0.05	1.29 ± 0.03*	0.36 ± 0.02#	0.40 ± 0.04#	F(3,12) = 142.50	**<0.001**

*Values are expressed as mean ± SEM. (n = 4) *P <0.05 compared with the control group; #P <0.05 compared with the 50% alcohol only group based on Tukey's HSD post-hoc test following one-way ANOVA.*

Histologically, alcohol-fed rats exhibited extensive mucosal damage characterized by villus erosion, glandular distortion, and funnel-shaped ulceration (Fig. 1B and Fig. 2B). This agrees with known patterns of ethanol-induced intestinal injury, where oxidative stress, inflammation, and epithelial apoptosis contribute to mucosal breakdown (Brzozowska et al., 2014). In contrast, animals treated with melatonin showed well-preserved villi, intact mucosal glands, and normal submucosal and muscular layers (Fig. 1C and Fig. 2C). The protective histological profile further supports melatonin's role in enhancing mucosal integrity, maintaining epithelial tight junctions, and promoting tissue repair (Karaaslan and Suzen, 2015).

The melatonin-only group (Fig. 1D and Fig. 2D) showed no biochemical or histological abnormalities, confirming its safety and lack of adverse effects on the gastrointestinal tract, a finding also supported by earlier toxicological studies (Kolli et al., 2013).

Overall, the combination of antioxidant restoration, improved weight gain, and preserved histoarchitecture strongly indicates that melatonin possesses significant therapeutic potential in mitigating alcohol-induced duodenal injury. This is particularly relevant because chronic alcohol consumption continues to be a major contributor to gastrointestinal morbidity worldwide (Haber & Kortt, 2021).

Despite these findings, some limitations should be acknowledged. The study employed a relatively small sample size, and histological evaluation was descriptive without semiquantitative lesion scoring or morphometric assessment. In addition, mechanistic pathways underlying melatonin-mediated protection were not explored beyond oxidative stress biomarkers. Therefore, the findings should be interpreted as preliminary preclinical evidence requiring further validation in larger studies using quantitative histopathological approaches and mechanistic analyses.

Histology

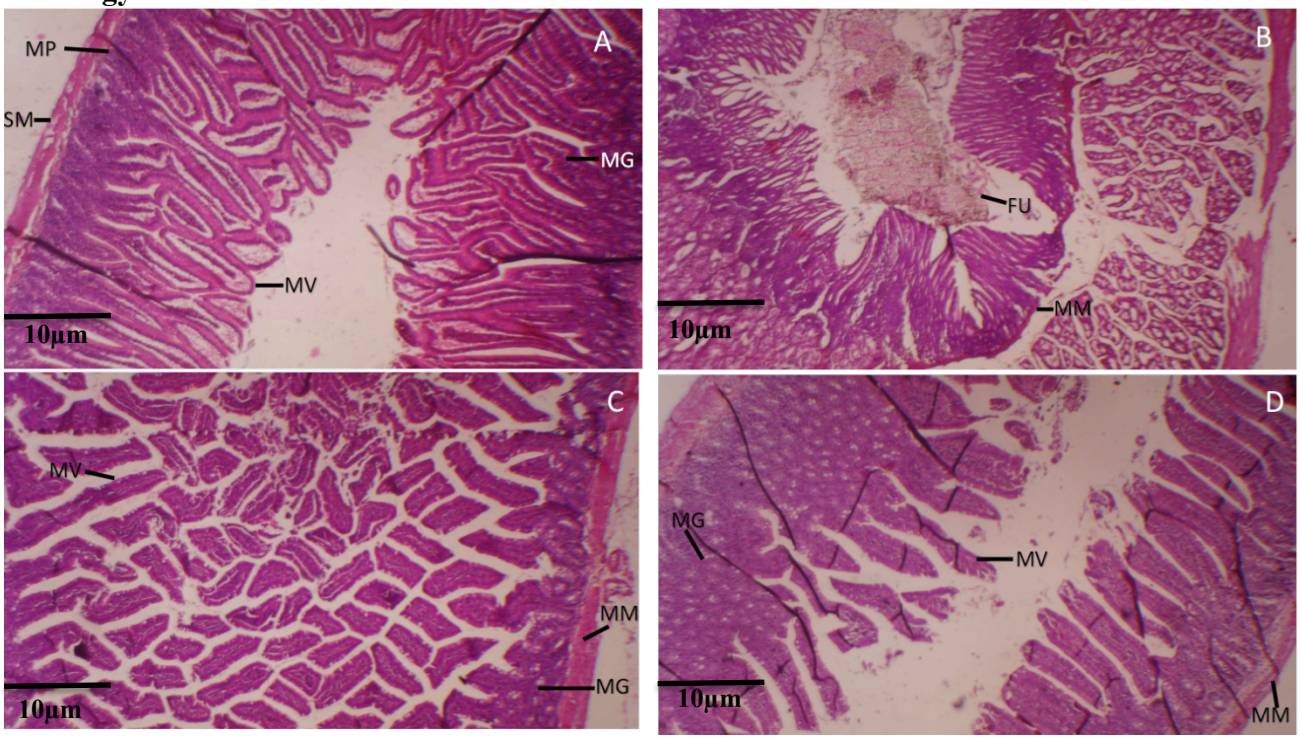

Fig 1: Plates A–D show sections of rat duodenum (H&E, 40X): (A) control with normal architecture comprising mucosal villi (MV), mucosal glands (MG), submucosa (SM), and muscularis propria (MP); (B) alcohol-treated showing a funnel-shaped ulcer (FU) and

muscularis mucosa (MM); (C) alcohol + melatonin-treated with preserved normal architecture of MV, MG, and MM; and (D) melatonin-treated showing normal MV, MG, and MM.

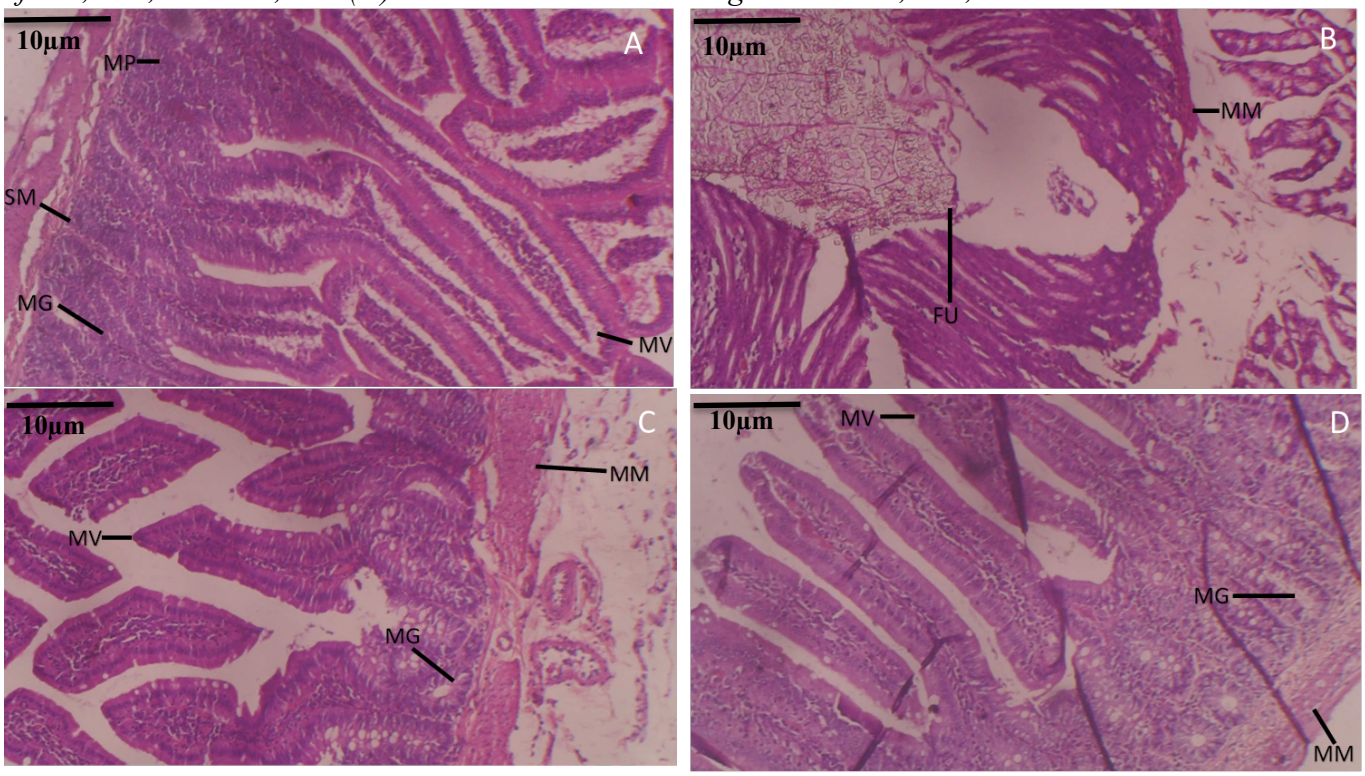

Fig 2: Plates A–D show sections of rat duodenum (H&E, 100X): (A) control with normal architecture comprising mucosal villi (MV), mucosal glands (MG), submucosa (SM), and muscularis propria (MP); (B) alcohol-treated showing a funnel-shaped ulcer (FU) and muscularis mucosa (MM); (C) alcohol + melatonin-treated with preserved normal architecture of MV, MG, and MM; and (D) melatonin-treated showing normal MV, MG, and MM.

Conclusion

Melatonin therefore demonstrated a protective effect against alcohol-induced duodenal injury in this experimental rat model. While these findings suggest potential relevance for alcohol-related intestinal injury, further studies incorporating larger sample sizes, quantitative histological analyses, and mechanistic investigations are required before therapeutic implications can be established.

Availability of data and materials

The data can be obtained from the corresponding author upon reasonable request by contacting them through their email ID.

Acknowledgements

None.

Funding

None.

Authors Contribution

Edobor Obayuwana: Methodology, supervision, project administration, investigation, and resources. Joseph Raymond Enoghase: Data curation, writing—review and editing, and visualization. All authors have read and agreed to the published version of the manuscript.

Ethics declarations

Ethics approval and consent to participate

Ethical approval for this study was obtained from the Research Ethics Committee of the College of Medical Sciences, University of Benin, Nigeria (Approval No. CMS/REC/2025/881; approved on 5 December 2025).

Consent for publication

Not applicable.

Competing interests

None.

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