



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

Edobor Obayuwana¹, Joseph Raymond Enoghase^{2*}

¹Department of Anatomy, University of Benin, Benin City, Edo State, Nigeria

²Department of Anatomy, University of Benin, Benin City, Edo State, Nigeria

* Corresponding author e-mail: joseph.enoghase@bmedsci.uniben.edu

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. Twenty rats were divided into four groups and treated for twenty-eight days with alcohol, 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 show that melatonin effectively protects the duodenum from alcohol-induced damage by reducing 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 lead to villus blunting, crypt degeneration, inflammatory infiltration, and in severe cases, ulcerative lesions (Akinrinde and Ajibade 2024). Superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and reduced glutathione (GSH) 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 (Mukhopadhyay et al. 2022). Experimental evidence demonstrates that melatonin attenuates alcohol-induced gastric and intestinal injury by reducing oxidative stress, suppressing pro-inflammatory cytokines, and promoting epithelial regeneration (Kolli et al. 2013, Brzozowska et al. 2014, Karaaslan and Suzen 2015). 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.

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 therapeutic potentials of melatonin in alcohol-related intestinal pathology.

Materials and Methods

Experimental Animals

Twenty (20) adult Wistar rats of both sexes, 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 institutional 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 = 5 per group):

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

- **Group 4 (Melatonin only):** Received melatonin only (5 mg/kg body weight) orally.

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

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 anaesthetized with ketamine (80 mg/kg). The duodenum was carefully excised.

- 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

SOD activity was determined using the method described by Vives-Bauza et al. (2007) based on the inhibition of pyrogallol autoxidation. Results were expressed as units per milligram of protein (U/mg protein).

Catalase (CAT) Activity

CAT activity was measured according to Vives-Bauza et al. (2007) method, which evaluates the rate of decomposition of hydrogen peroxide at 240 nm. Results were expressed in U/mg protein.

Glutathione Peroxidase (GPx) Activity

GPx activity was measured using the method of Vives-Bauza et al. (2007) based on the oxidation of reduced glutathione in the presence of cumene hydroperoxide. Results were reported as U/mg protein.

Lipid Peroxidation (MDA Levels)

Malondialdehyde levels, an index of lipid peroxidation, were quantified using the thiobarbituric acid reactive substances (TBARS) assay (Ghani et al. 2017). MDA concentration was expressed as nmol/mg protein.

Protein Determination

Total protein concentration in homogenates was determined using the Biuret method to normalize enzyme activities.

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 ×40 and ×100 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

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 Discussions

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, consistent with previous studies showing that ethanol impairs nutrient absorption, increases metabolic burden, and suppresses appetite through gastrointestinal inflammation and mucosal damage (Butt et al., 2023). Chronic ethanol exposure is also known to interfere with intestinal motility and hormonal regulation, further contributing to poor weight outcomes (Keshavarzian and Fields, 2017). In the present study, co-administration of melatonin significantly improved weight gain relative to the alcohol-only group, indicating that melatonin supports metabolic stability and may mitigate alcohol-induced nutritional deficits.

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

Group	Initial Weight (g)	Final Weight (g)	p-value (Paired t-test)
Control	166.5 ± 0.9	187.0 ± 0.4	<0.001*
50% Alcohol	161.8 ± 4.2	171.5 ± 3.9	0.003
5mg/kg Melatonin + 50% Alcohol	173.3 ± 3.5	191.5 ± 2.1	<0.001*
5mg/kg Melatonin	165.5 ± 2.3	191.3 ± 1.2	<0.001*

Values are expressed as mean ± SEM. * $P < 0.05$ 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	p-value
Weight Change (g)	20.5 ± 0.5	4.8 ± 0.6 *	18.3 ± 1.6 #	25.8 ± 1.2 #	<0.001

Values are expressed as mean ± SEM. * $P < 0.05$ 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, confirming excessive generation of reactive oxygen species (ROS) 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. 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 Gastric Tissue

Parameter/Group	Control	50% Alcohol	5mg/kg Melatonin + 50% Alcohol	5mg/kg Melatonin	p- value
SOD	1.68±0.06	0.58±0.08*	1.68±0.11#	1.73±0.11#	<0.001
CAT	1.29±0.01	0.28±0.02*	1.31±0.01#	1.33±0.01#	<0.001
GPx	1.49±0.04	0.79±0.05*	1.13±0.04*#	1.14±0.05#	<0.001
MDA	0.46±0.05	1.29±0.03*	0.36±0.02#	0.40±0.04#	<0.001

Values are expressed as mean ± SEM. * $P < 0.05$ 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. 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. 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 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.

Histology

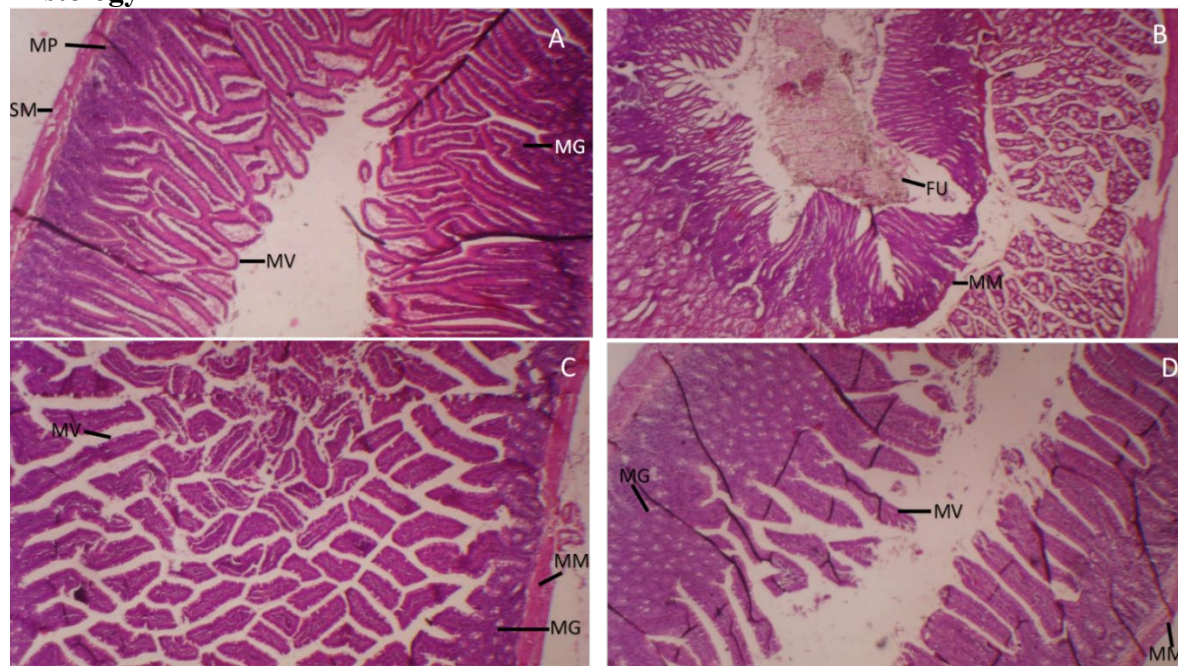


Figure 1. Plates A–D show sections of rat duodenum (H&E, 40X): (A) control with normal architecture comprising mucosal villi (MV), mucosal glands (MG), submucosa (SM), and muscularis propria (MP); (B) alcohol-treated showing a funnel-shaped ulcer (FU) and muscularis mucosa (MM); (C) alcohol + melatonin-treated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

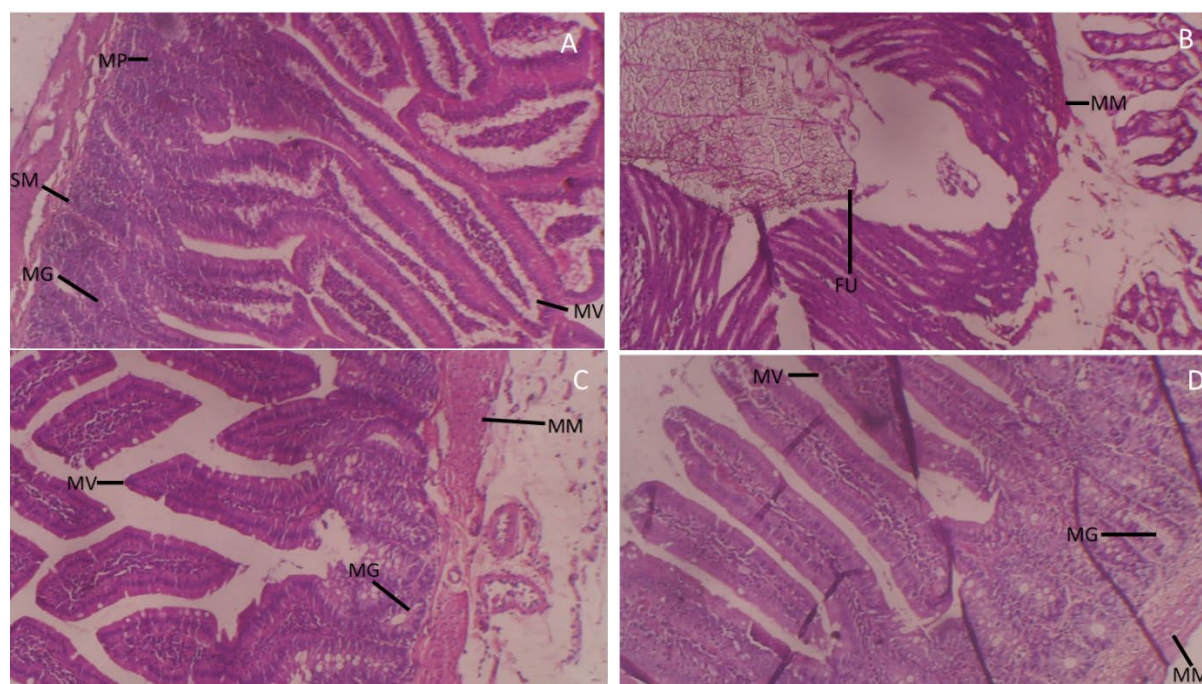


Figure 2. Plates A–D show sections of rat duodenum (H&E, 100X): (A) control with normal architecture comprising mucosal villi (MV), mucosal glands (MG), submucosa (SM), and muscularis propria (MP); (B) alcohol-treated showing a funnel-shaped ulcer (FU) and muscularis mucosa (MM); (C) alcohol + melatonin-treated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

Conclusions

Chronic administration of 50% alcohol induced significant oxidative stress, impaired antioxidant defenses, reduced weight gain, and caused severe duodenal mucosal injury in Wistar rats. Melatonin co-administration effectively countered these effects by restoring antioxidant enzyme activities, reducing lipid peroxidation, preserving duodenal histological architecture, and improving weight outcomes. Melatonin alone produced no deleterious effects, underscoring its safety.

Melatonin therefore demonstrates strong gastroprotective potential against alcohol-induced duodenal toxicity and may represent a beneficial adjunct in preventing or managing alcohol-related intestinal injury.

Availability of data and materials

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

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Authors Contribution

Edobor Obayuwana: Methodology, Supervision and project administration Investigation, Resource. 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

The research ethics committee's guidelines for animal handling and treatment at the University of Benin's College of Medical Sciences was fully implemented.

Consent for publication

Not applicable.

Competing interests

None.

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