Effect of Black Garlic Extract on Aspirin-Induced Gastric Mucosal Injury in Wistar Rats (*Rattus norvegicus*)

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ABSTRACT

Background: Nonsteroidal anti-inflammatory drugs (NSAIDs), including aspirin, are widely used but are associated with gastric mucosal injury through inhibition of prostaglandin synthesis and oxidative stress mechanisms. Black garlic, a processed form of *Allium sativum*, has been reported to possess antioxidant and anti-inflammatory properties that may provide gastroprotective effects.

Objective: To evaluate the effect of black garlic extract on aspirin-induced gastric mucosal injury in Wistar rats (*Rattus norvegicus*).

Methods: This experimental laboratory study used a posttest-only control group design. Male Wistar rats were allocated into a negative control group, a positive control group receiving aspirin, and three treatment groups administered black garlic extract at doses of 50 mg/kg, 100 mg/kg, and 200 mg/kg body weight. Aspirin was administered orally at 104 mg/kg body weight for seven days to induce gastric mucosal injury, followed by seven days of black garlic extract administration in the treatment groups. Gastric tissues were collected for histopathological examination, and epithelial injury was assessed using the Barthel Manja scoring system. Data were analyzed using non-parametric statistical tests.

Results: Histopathological evaluation demonstrated gastric mucosal epithelial injury in the positive control group, predominantly in the form of desquamation and erosion. Administration of black garlic extract was associated with reduced severity of mucosal injury, with progressive improvement observed at higher doses. The group receiving 200 mg/kg body weight showed no detectable gastric mucosal lesions. A significant difference in gastric mucosal injury was observed among groups (p<0.001).

Conclusion: Black garlic extract exhibited a protective effect against aspirin-induced gastric mucosal injury in Wistar rats, with the highest dose demonstrating the most favorable histopathological outcome.

KEYWORDS: black garlic, aspirin, NSAIDs, gastric mucosa, peptic ulcer, histopathology

INTRODUCTION

Nonsteroidal anti-inflammatory drugs (NSAIDs) are among the most commonly prescribed and self-administered medications worldwide due to their analgesic, antipyretic, anti-inflammatory, and antiplatelet properties.[1] Despite their broad clinical utility, NSAID use is strongly associated with gastrointestinal (GI) adverse effects, particularly gastric mucosal injury and peptic ulcer disease.[2] Aspirin, one of the most widely used NSAIDs, remains a major contributor to drug-related gastropathy because of its frequent long-term use in cardiovascular prevention.[3]

NSAID-associated gastric injury represents a significant clinical concern, as mucosal damage may occur even in the absence of prominent symptoms and can progress to complications such as bleeding or perforation.[4] The pathogenesis of aspirin-induced gastric mucosal injury is multifactorial. Systemic inhibition of cyclooxygenase-1 (COX-1) results in decreased prostaglandin synthesis, leading to impairment of essential mucosal defense mechanisms, including mucus and bicarbonate secretion, maintenance of mucosal blood flow, and epithelial restitution.[5] In addition, aspirin exerts topical epithelial toxicity through increased mucosal permeability and disruption of surface epithelial integrity.[6]

Beyond prostaglandin depletion, oxidative stress and inflammatory activation play important roles in amplifying gastric mucosal

injury. Increased production of reactive oxygen species, neutrophil infiltration, and microvascular disturbance contribute to epithelial desquamation and erosion.[5,7] These overlapping mechanisms explain why acid suppression alone may not fully prevent NSAID-related gastric damage, highlighting the need for adjunctive gastroprotective strategies.[8] In recent years, increasing attention has been directed toward natural products with antioxidant and anti-inflammatory properties as potential gastroprotective agents. Black garlic, a processed form of *Allium sativum* produced through controlled aging, exhibits distinct biochemical characteristics compared with fresh garlic. The aging process alters organosulfur compounds and increases stable antioxidant constituents, including S-allyl cysteine, which have been associated with cytoprotective and anti-inflammatory activity.[9,10] Experimental studies have demonstrated that black garlic possesses significant antioxidant capacity and may modulate inflammatory pathways relevant to mucosal injury.[11] Several preclinical investigations have suggested that black garlic preparations may exert protective effects in chemically induced gastric ulcer models.[12] However, evidence regarding its effect on aspirin-induced gastric mucosal injury remains limited. This gap in evidence provides the rationale for the present study, which aimed to evaluate the effect of graded doses of black garlic extract on gastric mucosal epithelial injury in Wistar rats (*Rattus norvegicus*) induced by aspirin.

METHODS

This laboratory experimental study employed a true experimental, posttest-only control group design and was conducted from 19 August to 16 October 2024. Ethical approval was obtained prior to study initiation (No. 108/1.KT/KEPPKP/VII/2024). Male Wistar rats (*Rattus norvegicus*) were used. The initial sample comprised 30 animals meeting the inclusion criteria; two animals died during the experiment (drop-outs), resulting in 28 rats included in the final analysis. The analyzed animals were distributed into five groups: negative control (K−, n=5), positive control (K+, n=5), and three treatment groups (P1, P2, P3; each n=6). During a 7-day acclimatization period, animals received BR-1 standard feed and water (aquades) ad libitum.

After acclimatization, rats in the positive control and all treatment groups received aspirin orally at 104 mg/kg body weight once daily for 7 consecutive days (day 8 to day 14) to induce gastric mucosal injury, while the negative control group did not receive aspirin. On day 15, animals in the positive control group were terminated. The treatment groups then received black garlic extract orally for 7 days (day 15 to day 21) at dose levels of 50 mg/kg body weight (P1), 100 mg/kg body weight (P2), and 200 mg/kg body weight (P3). On day 22, animals in the negative control group and all treatment groups were terminated, and gastric tissue was collected for histopathological preparation.

Black garlic extract was prepared by blending 1 kg of peeled black garlic with 70% ethanol, followed by maceration for 3 days with stirring every 24 hours. The macerate was filtered, and the filtrate was concentrated using a rotary evaporator (40°C; 40 rpm) to obtain a viscous extract.

Histopathological assessment of the gastric mucosa focused on epithelial injury severity. Epithelial changes were evaluated using the Barthel Manja scoring approach on an ordinal scale: 0 (no lesion), 1 (desquamation), 2 (erosion), and 3 (ulceration). Each specimen was examined in five microscopic fields at 400× magnification, and the dominant score (>50% of fields) was used to classify the lesion category per animal.

Statistical analysis followed assessment of distribution normality (Shapiro–Wilk) and homogeneity of variance (Levene's test). Because the data were not normally distributed, group comparisons were performed using the Kruskal–Wallis test, followed by Mann–Whitney post hoc testing to assess between-group differences when applicable. A two-sided significance threshold of $p < 0.05$ was applied.

RESULTS

A total of 30 male Wistar rats were initially allocated to the experimental groups, with six animals assigned to each group. This allocation was determined to accommodate potential attrition during the experimental period.

During the study, two animals were lost due to mortality. Consequently, 28 rats were included in the final analysis. The losses occurred in the negative control group and the positive control group, resulting in final group sizes of five animals in each control group, while all treatment groups retained six animals. All animals that fulfilled the predefined inclusion criteria were included in the final histopathological and statistical analyses.

Histopathological Findings

Microscopic examination of gastric tissue from the negative control group demonstrated intact epithelial architecture without pathological alterations. In contrast, specimens from the positive control group exhibited epithelial disruption characterized by desquamation and focal erosions following aspirin administration. In the treatment group receiving black garlic extract at a dose of 50 mg/kg body weight, epithelial desquamation remained the predominant histopathological feature. Specimens from the 100 mg/kg body weight group demonstrated milder epithelial alterations with partial preservation of mucosal continuity. In animals receiving 200 mg/kg body weight, gastric mucosa showed preserved epithelial architecture without detectable lesions. Representative histopathological findings are presented in Figure 1.
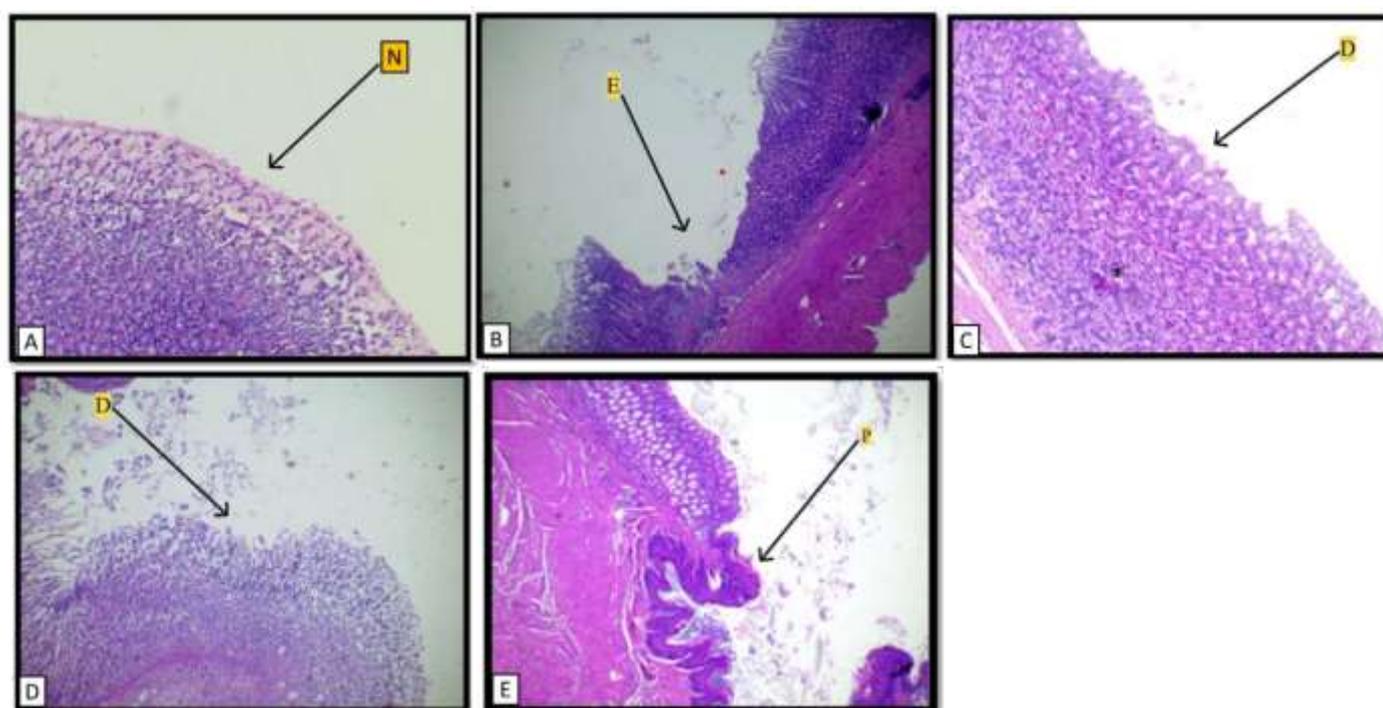

Figure 1. Representative histopathological features of gastric mucosa in experimental groups (hematoxylin–eosin staining, 400× magnification)**.**(A) Gastric mucosa with no histopathological abnormality (N: no finding).(B) Gastric mucosal erosion (E) characterized by superficial epithelial loss.(C) Intact mucosal architecture without pathological alteration (N).(D) Gastric epithelial desquamation (D) showing detachment of superficial epithelial cells.(E) Polypoid mucosal lesion (P) characterized by mucosal protrusion toward the lumen.

Quantitative Findings

Quantitative evaluation of gastric mucosal epithelial injury was performed using ordinal histopathological scoring based on the Barthel Manja classification. Preliminary assessment demonstrated non-normal data distribution and lack of variance homogeneity. Therefore, non-parametric statistical tests were applied for group comparison. Overall comparison using the Kruskal–Wallis test revealed a statistically significant difference in gastric mucosal epithelial injury scores among the five experimental groups ($p < 0.001$). Pairwise comparison using the Mann–Whitney test indicated significant differences between the positive control group and the treatment groups receiving black garlic extract, particularly at doses of 100 mg/kg body weight and 200 mg/kg body weight. The distribution of injury severity showed that the positive control group predominantly exhibited epithelial desquamation and erosion, whereas higher doses of black garlic extract were associated with lower injury scores.

Table 1. Gastric mucosal epithelial injury scores among experimental groups

Group	n	Median (IQR)	p-value*
Negative control (K−)	5	0 (0–0)	
Positive control (K+)	5	2 (1–2)	
P1 – Black garlic 50 mg/kg	6	1 (1–1)	< 0.001
P2 – Black garlic 100 mg/kg	6	0 (0–1)	
P3 – Black garlic 200 mg/kg	6	0 (0–0)	

DISCUSSION

The present study demonstrated that administration of black garlic extract was associated with improved gastric mucosal epithelial integrity in an aspirin-induced injury model, with greater preservation observed at higher doses. These findings are biologically plausible when interpreted within the established pathophysiology of NSAID-related gastric mucosal damage.[5,6]

Aspirin-induced injury primarily results from inhibition of COX-1–mediated prostaglandin synthesis, leading to compromised mucosal defense and increased vulnerability of the gastric epithelium to luminal aggressors.[5] In parallel, aspirin contributes to direct epithelial toxicity and promotes oxidative and inflammatory cascades that further disrupt mucosal integrity.[6,7] The histopathological spectrum observed in this study—ranging from epithelial desquamation to erosion in untreated animals—is consistent with these mechanisms.

The improvement in mucosal architecture observed following black garlic administration may be explained by its antioxidant and anti-inflammatory properties. Black garlic is known to contain higher levels of stable antioxidant compounds compared with raw garlic, particularly water-soluble organosulfur derivatives that can attenuate oxidative stress.[9,10] Oxidative stress has been recognized as a key contributor to aspirin-induced mucosal injury; therefore, reduction of reactive oxygen species may partially restore epithelial defense and limit surface damage.[7,11]

The dose-related pattern observed in this study further supports a potential biological gradient. Lower doses of black garlic extract were associated with residual epithelial desquamation, whereas the highest dose demonstrated preserved mucosal architecture without detectable injury. Similar dose-dependent gastroprotective effects have been reported in experimental ulcer models using black garlic–based interventions and related antioxidant compounds.[12] These findings suggest that sufficient bioactive exposure may be required to counteract the multifactorial injury induced by aspirin.

A polypoid mucosal lesion was identified in one specimen. In experimental gastric injury models, such findings should be interpreted cautiously, as focal polypoid protrusions may represent reactive mucosal changes, edema, or folding artifacts rather than true neoplastic lesions.[13] Conservative terminology is therefore appropriate, and further characterization would require serial sectioning or adjunctive histological techniques.

From a clinical perspective, current guidelines emphasize acid suppression therapy—particularly proton pump inhibitors—as the cornerstone of NSAID- and aspirin-related ulcer prevention.[14] However, acid suppression does not directly address oxidative or inflammatory mechanisms of injury, and long-term use may be associated with additional risks.[8] These limitations have prompted interest in complementary approaches that target non-acid pathways. Although black garlic extract demonstrated promising effects in this experimental model, these findings should be interpreted strictly within a preclinical context and not extrapolated directly to clinical practice.

Several limitations of the present study should be acknowledged. First, sample size was reduced due to mortality-related attrition, resulting in unequal group sizes. Second, assessment of injury relied on ordinal histopathological scoring, which, although appropriate for tissue-level evaluation, remains semi-quantitative. Third, mechanistic biomarkers such as oxidative stress indices or inflammatory mediators were not measured, limiting causal inference. Finally, translation to human aspirin users remains constrained by species-specific differences and experimental conditions.[14,15]

Future studies should incorporate standardized characterization of black garlic extract, quantitative assessment of oxidative and inflammatory pathways, and larger sample sizes to strengthen mechanistic interpretation. Comparative evaluation against established gastroprotective agents may further clarify the translational relevance of black garlic as a potential adjunctive intervention.

CONCLUSION

In this experimental rat model of aspirin-induced gastric mucosal injury, administration of black garlic extract was associated with improved gastric mucosal epithelial integrity, with a clear dose-related pattern. The 200 mg/kg body weight regimen demonstrated the most favorable histopathological profile, showing preserved mucosa without detectable epithelial injury under the study conditions. These findings support black garlic extract as a potential gastroprotective adjunct in preclinical settings; however, confirmation in larger, rigorously controlled studies with standardized extract characterization and mechanistic biomarkers is warranted before any translational inference to human aspirin users.

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