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Detection of Periodic Acid – Schiff (PAS) in Gastric Mucosa of an Ulcer Model Treated with Manila

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ABSTRACT

Background: *With the practical benefit of being less expensive than immunohistochemistry, periodic Acid – Schiff (PAS) histochemistry may reveal pathological alterations in the stomach mucosa and detect tissue carbohydrates, glycogen, and neutral mucopolysaccharides.*
Objective: *To find out how Manilkara zapota (sapodilla) extract affects ethanol-induced gastric ulceration and to compare the PAS staining of healthy and ulcerated gastric mucosa.*
Methods: *Wistar rat gastric tissue paraffin blocks from ethanol-ulcer groups with and without sapodilla extract were used in an experimental histochemical study. Sections were stained with H&E and PAS , and PAS features were evaluated using semi-quantitative scoring (0-3) using Shapiro-Wilk and Kruskal-Wallis ($p<0.05$).*
Results: *Although the Pass-positive mucosal length showed a tendency to increase the higher-dose groups, the difference was not statistically significant (Kruskal – Wallis test, $p=0.487$; $p>0.05$) and was accompanied by considerable inter-individual variability. In contrast, lesion scores differed significantly among groups (Kruskal-Walls test $p=0.026$; $p<0.05$), with healthy animals consistently exhibiting a score of 0.00±0.00, whereas the ulcer-induced groups demonstrated higher lesion scores.*
Conclusion: *PAS-based metrics indicate a potential mucosal-defense recovery trend with higher-dose sapodilla extract. However, the effect was not statistically confirmed, indicating the need for additional dosing/time point optimization and complementary results.*

Keywords: *Periodic acid–Schiff (PAS); Manilkara zapota; ethanol-induced; gastric ulcer; glycoproteins; Wistar rat*

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INTRODUCTION

Histochemical staining techniques, particularly the Periodic Acid–Schiff (PAS) method, have long been recognized as a vital tool for identifying tissue carbohydrates such as glycogen, neutral mucopolysaccharides, and basement membrane structures. This approach plays a central role in morphological and diagnostic tissue studies, bridging biomedical research and histological education.[1] Early applications demonstrated its diagnostic value in placental tissues from patients with gestational diabetes mellitus, revealing significant carbohydrate accumulation undetectable by routine staining.[2] Similarly, PAS staining has elucidated carbohydrate localization in diverse tissues, including the proventriculus during pre- and post-hatching phases,[3] intestinal mucosa,[4] and testicular tissue where distinct glycoprotein patterns were identified.[5,6]

Beyond research, PAS staining has proven pedagogically valuable in histology training by providing clearer visualization and structural contrast.[7] Experimental investigations in rodents further confirmed its reliability for assessing tissue integrity across organ systems.[8] In gastric ulcer models, PAS staining effectively visualizes mucosal glycoprotein alterations and mucous depletion, allowing evaluation of mucosal recovery following ethanol-induced injury.[9] Moreover, herbal extracts have been investigated as natural therapeutic agents using PAS-based mucin analysis: *Glycyrrhiza glabra* increased mucopolysaccharide deposition and epithelial glycogen density;[10] *Curcuma longa* preserved mucosal integrity and promoted more uniform glycoprotein distribution;[11] *Aloe vera* enhanced mucous thickness and intensified polysaccharide deposition;[12] and *Zingiber officinale* protected mucosal glycoprotein architecture.[13] Collectively, these findings highlight PAS staining as both a sensitive histochemical marker and a valuable educational and investigative technique for studying tissue carbohydrate dynamics and natural gastroprotective mechanisms. While PAS staining is well-established as a highly sensitive histochemical marker for evaluating mucosal integrity, its application in this study specifically serves as the primary evaluation tool to investigate the gastroprotective potential of Manilkara zapota (sapodilla) extract.

Manilkara zapota was selected due to its rich bioactive compounds, which theoretically contribute to antioxidant and anti-inflammatory mechanisms necessary for mitigating ethanol-induced gastric mucosal injury. Therefore, the primary objective of this study is to determine the efficacy of Manilkara zapota extract in protecting and healing the gastric mucosa, utilizing the methodological advantages of PAS staining to objectively visualize and quantify mucus depletion and glycoprotein recovery.

METHODS

This is an experimental study employing a histochemical approach using PAS staining on gastric tissue samples. The study includes evaluation of staining intensity and distribution, as well as comparative analysis of histopathological findings that are statistically relevant.

RESULTS

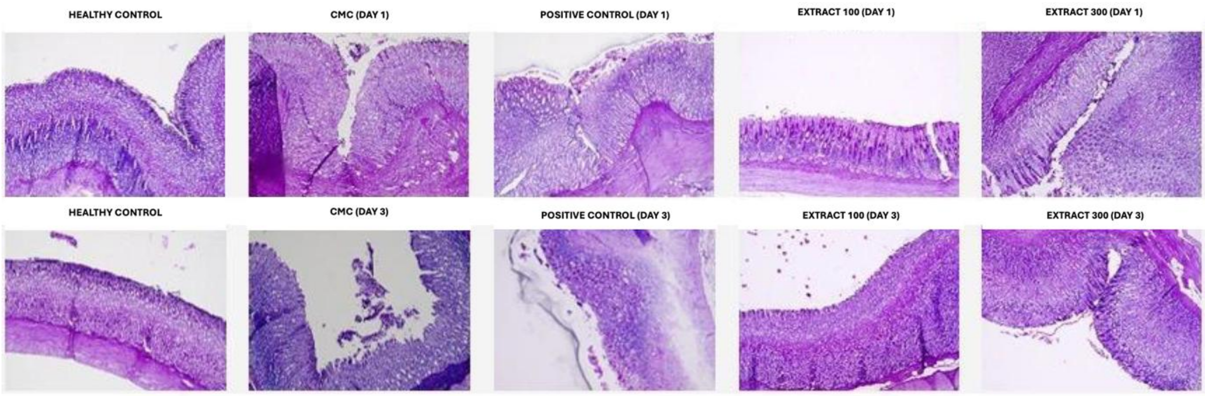

Figure 1. Histopathological features of rat gastric mucosa across treatment groups (H&E and PAS staining)

Abbreviations: Healthy Control, normal intact gastric mucosa; CMC, Carboxymethyl Cellulose serving as the negative vehicle control (evaluated at Dose/Day 1 and 3); Positive Control, standard treatment group (evaluated at Day 1 and Day 3); Extract 100 and Extract 300, groups treated with Manilkara zapota extract at doses of 100 mg/kg and 300 mg/kg, respectively (evaluated at Day 1 and Day 3)."

The data demonstrate the comparative mean lesion scores (±SD) among different treatment groups. The healthy (control) group maintained a score of 0.00 ± 0.00, indicating intact gastric mucosa. Ethanol-induced groups such as CMC(1), CMC(3), and KP(1) exhibited higher mean lesion scores of 3.20 ± 1.10, 3.00 ± 1.00, and 3.40 ± 1.34, respectively, reflecting substantial mucosal injury. Treatment groups receiving *Manilkara zapota* extract (K100 and K300) demonstrated moderate lesion scores ranging from 2.25 ± 1.20 to 2.80 ± 1.30, suggesting partial mucosal protection. However, the K300(3) group exhibited a higher mean score (3.20 ± 1.30), indicating possible variability in dose or treatment duration effects. Overall, the pattern underscores a significant difference across groups (Kruskal–Wallis, $p = 0.026$), driven primarily by the contrast between healthy and induced groups, while treatment optimization may require further adjustment in concentration and exposure time.

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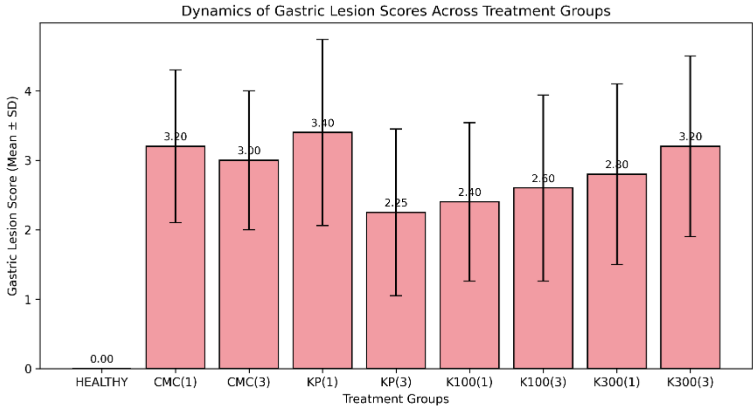

Figure 2. Dynamics of Gastric Lesion Scores Across Treatment Groups

The main findings for the current year indicate that gastric lesion scores differed significantly across groups (Kruskal–Wallis, p = 0.026). Descriptively, the healthy group consistently remained at 0.00 ± 0.00, whereas the induction groups exhibited higher scores, including CMC(1) 3.20 ± 1.10, CMC(3) 3.00 ± 1.00, and KP(1) 3.40 ± 1.34. In the treatment groups, lesion scores tended to fall within a moderate range—for example, K100(1) 2.40 ± 1.14 and K100(3) 2.60 ± 1.34—while K300(3) increased again to a relatively high level (3.20 ± 1.30). Overall, this pattern suggests that the significant global difference is likely driven primarily by the contrast between the healthy group and the injured/induced groups, whereas treatment-related modulation has not yet appeared optimal, potentially reflecting suboptimal dosing and/or duration of administration at specific stages of mucosal healing.

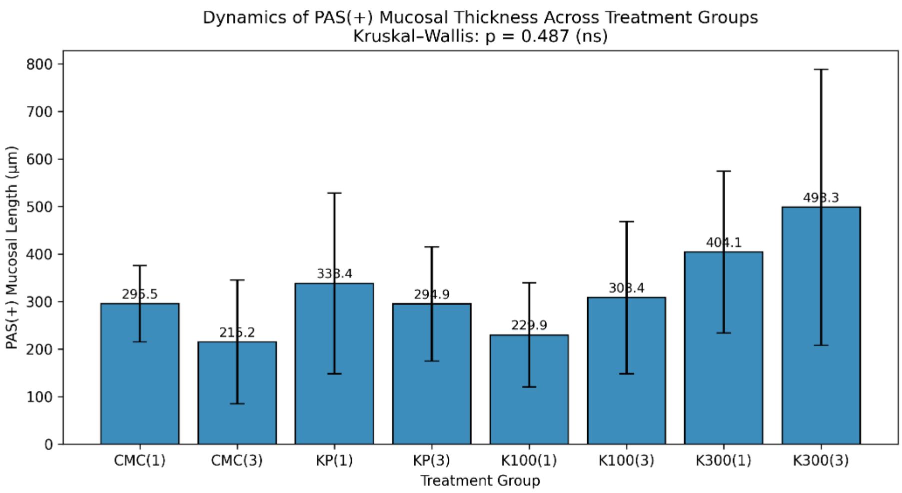

Figure 3. ***Dynamics of PAS(+) mucosal thickness across treatment groups***

Meanwhile, the length of PAS-negative mucosa (PAS[−]) also did not differ significantly across groups (Kruskal–Wallis p = 0.220). However, an interesting pattern emerged: KP(3) 20.40 ± 40.80 µm, K300(3) 49.26 ± 70.95 µm, and K300(1) 65.93 ± 66.58 µm tended to be lower than some other groups, such as CMC(3) 207.15 ± 109.85 µm and KP(1) 197.77 ± 154.11 µm. If PAS(−) is interpreted as reflecting areas of mucus component loss, then a reduction in PAS(−) could conceptually align with improvement of the mucosal barrier. Nevertheless, because the statistical test was not significant, the most defensible interpretation for the current year is that these findings indicate a possible trend but do not yet provide sufficient evidence to claim a meaningful effect. including minimum values of 0 in several groups—suggests that variability is substantial, indicating high inter-individual heterogeneity in mucosal injury severity and the subsequent repair process.

DISCUSSION

The ethanol-induced gastric ulcer model is a clinically relevant model of acute mucosal injury for evaluating potential gastroprotective therapies, because ethanol can disrupt the mucus–bicarbonate barrier, weaken antioxidant defenses, impair microcirculation, and trigger inflammation, resulting in lesions ranging from erosions to ulcers and mucosal bleeding.[18,19] Meanwhile, PAS staining is used to visualize the expression of surface mucosal glycoprotein/mucopolysaccharide components (*mucus*) as an indicator of mucosal defense, because changes in the PAS-stained area can reflect mucus depletion or recovery under gastric injury conditions.[17] *Manilkara zapota was* selected because multiple reports suggest pharmacological potential in gastrointestinal disorders and the presence of bioactive compounds that could theoretically contribute to antioxidant and anti-inflammatory effects. In this context, the primary focus of this research is to prove the gastroprotective efficacy of *Manilkara zapota* extract. To robustly demonstrate these therapeutic effects, the PAS staining method was utilized; its distinct advantage in visualizing minute changes in mucosal glycoproteins serves as the ideal framework to validate the extract's capacity for mucosal defense restoration. However, its efficacy must still be demonstrated quantitatively through histopathological outcomes and rigorous statistical analysis.[14]

The histochemical outcome assessment (PAS-positive and PAS-negative mucosal lengths) did not differ significantly across ulcer groups; nevertheless, observed trends and wide variability (including minimum values of 0 in some groups) suggest substantial inter-individual heterogeneity in mucosal injury severity and repair dynamics. Biologically, increased PAS(+) may reflect restoration of mucosal defense factors, as shown in ethanol-injury models where interventions that reduce oxidative stress and inflammation are accompanied by improvements in mucosal defense indicators such as mucin/PAS staining.[17,18] Ethanol-induced gastric mucosal injury is a complex phenomenon; therefore, an intervention may reduce macroscopic lesion severity without necessarily producing a readily detectable change in a single histochemical parameter at a specific time point. Several studies have linked gastroprotective effects in ethanol models to activation of the Nrf2/HO-1 pathway[18,19] and interventions modulating ROS and inflammatory signaling may improve mucosal integrity. For example, lactoferrin has been reported to mitigate ethanol-induced gastric ulcers through modulation of the ROS/ICAM-1/Nrf2 axis.[15]

CONCLUSION

PAS histochemical staining clearly differentiated normal gastric mucosa from ulcerated or treated mucosa. The healthy control group demonstrated preserved PAS reactivity, whereas the ulcer groups exhibited greater variability in PAS staining patterns, consistent with depletion of the mucosal mucus–glycoprotein layer and ongoing reparative changes. However, when analyses were restricted to ulcer groups, neither PAS-positive mucosal length nor PAS-negative mucosal length differed significantly across treatment arms (Kruskal–Wallis $p = 0.487$ and $p = 0.220$, respectively), indicating that the observed tendency toward mucus restoration was not statistically supported.

Conflicts of Interest

The authors declare no conflicts of interest.

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