THE PROTECTIVE EFFECT OF ATORVASTATIN AGAINST INDOMETHACIN-INDUCED GASTRIC ULCERATION IN RATS

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ABSTRACT

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| Background: Gastric ulcer, a disruption in the normal integrity of the gastric mucosa poses a significant clinical challenge, necessitating the exploration of novel therapeutic strategies. Atorvastatin, a statin with anti-inflammatory and anti-oxidant properties, may offer a promising approach for managing and preventing gastric ulcers.  Aim: This study investigated the potential of atorvastatin, to exert a gastroprotective effect on indomethacin-induced gastric ulceration in rats.  Methodology: We employed a rat model of indomethacin-induced gastric ulcers to investigate the anti-ulcer potential of atorvastatin. Rats were randomly assigned to nine groups, receiving various doses of atorvastatin (3-19 mg/kg) post-ulcer induction or pre-treated with atorvastatin (10 and 19 mg/kg) prior to ulcer induction.  **Results:** Atorvastatin significantly (p ≤ 0.05) and dose-dependently reduced the ulcer index, with pre-treatment providing superior protection (96% protection at 19 mg/kg) comparable to the standard anti-ulcer drug cimetidine (97%). Notably, pre-treated groups exhibited reduced ulcer indices and enhanced protection compared to treatment groups.  **Conclusion:** Our findings demonstrate the potent anti-ulcer effects of atorvastatin, both in treatment and prevention, suggesting its potential repurposing for gastric ulcer management. These observations warrant further clinical investigation to validate the therapeutic efficacy of atorvastatin in gastric ulcer prevention and treatment. |

*Keywords: Gastric ulcer, atorvastatin, ulcer index, cimetidine, indomethacin [Put four to eight keywords.*

1. INTRODUCTION

Gastric ulcer, a predominant subtype of peptic ulcer disease, remains a significant global health challenge despite advances in gastroenterological research and therapeutics. It is characterized by mucosal sores that extends through the muscularis mucosa into the submucosa or deeper layers. (1) The erosion in the stomach is due to the imbalance between aggressive factors, such as gastric acid and pepsin, and the defensive mechanisms including mucus secretion and mucosal blood flow.(2) Gastric ulcers can lead to severe complications such as: hemorrhage, perforation, gastrointestinal obstruction and malignancy.(3) The pathogenesis of gastric ulcers is complex and multifactorial, involving Helicobacter pylori infection, prolonged use of non-steroidal anti-inflammatory drugs (NSAIDs), excessive alcohol consumption, nutritional deficiencies, and stress.(3)

Pharmacological interventions which are the mainstay of treatment, include: histamine receptor antagonists, prostaglandins analogues, proton pump inhibitors, cytoprotective agents, antacids and anticholinergics, but most of these drugs produce undesirable side effects or drug interactions and may even alter biochemical mechanisms of the body upon chronic usage, thus, emphasizing the need for safer and more effective alternatives.(4,5), Drug repurposing, where existing medications are evaluated for off-label applications has today emerged a promising and productive strategy to mitigate the financial and time-intensive demands of de novo drug development. This approach offers a novel perspective in addressing clinical conditions such as gastric ulcers. The role of statins, particularly atorvastatin, has expanded beyond their conventional use as lipid-lowering agents due to their pleiotropic effects, including anti-inflammatory, antioxidant, and endothelial-protective properties.(6) These properties have sparked interest in their potential therapeutic applications in non-cardiovascular conditions, including gastric mucosal protection. Atorvastatin, a widely prescribed HMG-CoA reductase inhibitor, has been demonstrated to exert beneficial effects on oxidative stress modulation and inflammatory pathways, which are critical in the pathogenesis of gastric ulcers.(7), (8)

Several experimental models are employed in anti-ulcer research, including pylorus-ligated, NSAID-induced, and ethanol-induced gastric ulcer models.(9) The pylorus-ligated model, which involves obstructing the pyloric end of the stomach, causes an accumulation of gastric acid, promoting ulceration and facilitating the measurement of gastric acid secretion. (10) NSAID and ethanol-induced models are pivotal in evaluating the efficacy of anti-secretory and cytoprotective agents, as their mechanisms involve gastric acid secretion and suppression of mucosal prostaglandin synthesis, key contributors to ulcer pathogenesis.(11). This study aims to explore the potential effects of atorvastatin on experimentally induced gastric ulcers using the NSAID, indomethacinl-induced rat model.

2. material and methods

**Experimental animals**

Healthy Wistar rats aged 12 – 16 weeks were used for this study. The rats were obtained from the animal house of the Department of pharmacology and toxicology, faculty of pharmacy, University of Uyo. They were housed in standard cages and maintained on a standard pelleted feed and water *ad libitum.* Permission and approval for animal studies were obtained from the College of Health Science Animal Ethics Committee, University of Uyo. The animals were euthanized using ketamine (25mg/0.5mL) and sacrificed 5 hours following the induction of ulcer.

**Animal groupings and dosing**

The rats were randomly assigned to nine groups, each comprising five animals, and treated as follows: group 1 were healthy rats treated with distilled water (negative control), group 2 was the model group administered indomethacin but untreated (positive control), groups 3, 4, 5, and 6 received atorvastatin at doses of 3 mg/kg, 5 mg/kg, 10 mg/kg, and 19 mg/kg, respectively, one hour after ulcer induction while group 7 was treated with the standard anti-ulcer drug, cimetidine, at a dose of 100 mg/kg one hour after ulcer induction, groups 8 and 9 were pretreated with 10 mg/kg and 19 mg/kg atorvastatin respectively.

**Ulcer induction**

Gastric ulcers were induced with a single oral dose of indomethacin (40mg/kg). Prior to ulcer induction, the rats were deprived of food overnight but had free access to water. Various degrees of ulceration were manifested 5 hours after ulcer induction.

**Evaluation of gastric mucosa for ulcer**

Five hours after the ulcer induction, the rats were sacrificed, and their stomachs were carefully opened along the greater curvature to assess for gastric lesions. The stomachs were rinsed with normal saline to remove residual gastric contents and blood clots. Thereafter, they were examined for ulcerative lesions using a hand lens with 10x magnification. The ulcer index was determined based on a scoring system as described by Reddy et al. (2012).

Normal coloured stomach - - - - - - - - - - - - - - - - - - - - - - - - -- - - - - - - - - - - - - (0.0)

Red colouration - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - (0.5)

Spot ulcer - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - (1.0)

Hemorrhagic streak - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -- - - - (1.5)

Deep ulceration - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - (2.0)

Perforation - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - (3.0)

**Determination of Antiulcer Activity**

The antiulcer activity of the drugs was determined using the relation

Ulcer index (UI) = UN + US + (UP/10)

Were UI = ulcer index

UN =average number of ulcer per animal

US = average of severity score

UP = percentage of animals with ulcer.

The percentage protective ratio was calculated as follow:

% ​of ​protection = ​100 − [ UIPreteated]

[UIControl ] ×100

**Statistical analysis**

The results were expressed as mean ± SEM and statistically analyzed using one-way analysis of variance. Dunnett’s multiple comparison post-hoc test was thereafter employed to compare different treatment groups with respective controls. The level of significance was set at p < 0.05.

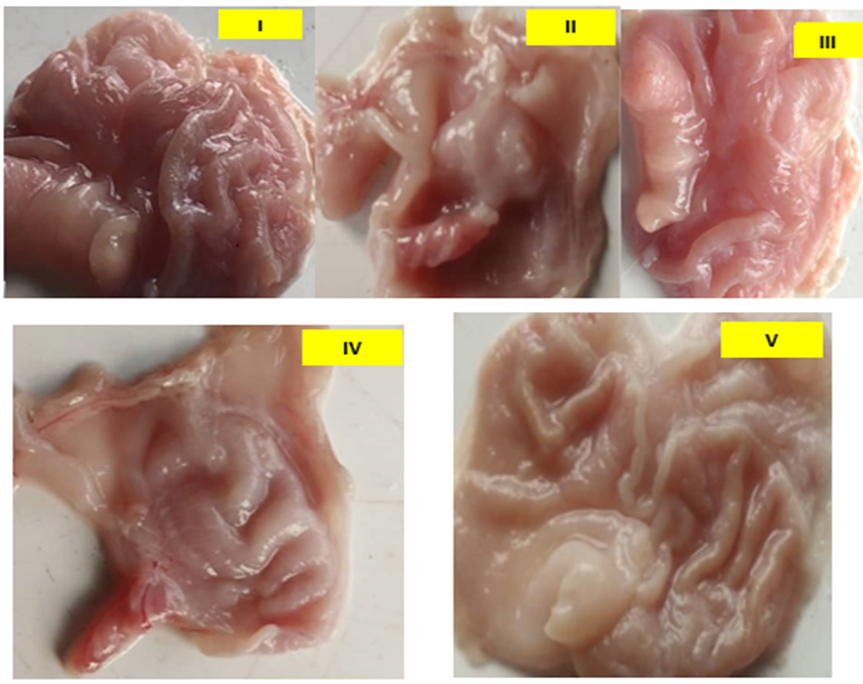
3. results

Gross macroscopic examination of the stomach following ulcer induction by both the ethanol and indomethacin revealed hemorrhagic and eroded tissues. However, morphological examination revealed greater ulcer severity in the indomethacin-induced group, which recorded an ulcer score of 7.4 compared to 6.4 in the ethanol-induced group. Consequently, the indomethacin-induced ulcer model was selected for subsequent experimental procedures. Atorvastatin-pre-treated and treated indomethacin-induced gastric ulcer showed reduced improved gastric mucosa when compared to the untreated group (Figures 1 and 2). Observations revealed that treatment intervention mitigated the severity of red discoloration, hemorrhagic streaks, spot ulcers, deep ulcerations, and perforations. Notably, the therapeutic effects were more pronounced in groups pre-treated with atorvastatin prior to ulcer induction.



**Figure 1.** Evaluation of rat gastric mucosa following ulcer induction

1=Negative control (healthy animals) showing the normal architecture of the gastric mucosa; II=Positive Control (Ulcer model group sowing perforations); III = Atorvastatin, 3mg/kg treated group showing perforations as indicated by the arrow; IV=Atorvastatin treated 5mg/kg, showing deep ulceration. The arrows show spot ulcers.

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**Figure 2.** Evaluation of rat gastric mucosa following ulcer induction

1=10mg/kg atorvastatin-treated group; II=19mg/kg atorvastatin-treated group; III = 10mg/kg atorvastatin pre-treated group; IV=19mg/kg atorvastatin pre-treated group V=100mg/kg cimetidine-treated group.

These ulcer scores were further utilized to calculate the ulcer index and percentage inhibition of ulceration, as earlier described. The influence of therapeutic intervention appeared more prominent in the groups that were pre-treated with Atorvastatin before the ulcer induction.

**Atorvastatin Significantly reduced Ulcer Index in Indomethacin-induced ulcer model**

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**Figure 3.** Effect of different doses of atovastatinon on the ulcer index of indomethacin-induced ulcer model in rat. \*Art =Artovastatin. Cim = Cimitidine. The results are expressed as mean ± SEM (n = 5). One way analysis of variance was employed followed by Dunnett’s multiple comparison post hoc test. The level of significance was set at p ≤0.05 when compared to the positive control group.

The ulcer index was significantly reduced in the atorvastatin-treated groups compared to the positive control group, which recorded an index of 7.4. This reduction was dose-dependent. Interestingly, the group treated with 10 mg/kg atorvastatin exhibited an ulcer index comparable to that of the group pre-treated with the same dose. Among all groups, the 19 mg/kg atorvastatin pre-treated group demonstrated the lowest ulcer index (0.3), closely resembling the group treated with 100 mg/kg cimetidine, which had an ulcer index of 0.2 (Figure 3). All groups treated or pre-treated with atorvastatin showed a significant reduction in ulcer index, indicating a substantial improvement and amelioration of indomethacin-induced ulceration.

**Atorvastatin Improved the percentage of protection of the gastric mucosa in indomethacin-induced gastric ulcer rat model.**



**Figure 4.** Percent ulcer inhibition of Different doses of Atorvastatin on indomethacin-induced ulcer model in rat. \*Pre. Art = pretreated with Artovastatin. Other doses were administered after the ulcer induction.

The percentage of gastric protection significantly improved following intervention with Atorvastatin. As illustrated in Figure 4, the healthy animals in the negative control group demonstrated 100% protection, whereas the untreated ulcer-induced group (ulcer model group) exhibited 0% protection. Treatment with Atorvastatin resulted in a marked and statistically significant improvement in protection (p ≤ 0.05). Notably, the group pre-treated with 19 mg/kg of Atorvastatin achieved 96% protection, comparable to the 97% protection observed in the group treated with the standard anti-ulcer drug, cimetidine. These findings suggest that Atorvastatin is potentially as effective as cimetidine in mitigating gastric ulceration. Furthermore, the percentage of protection increased progressively with higher doses of Atorvastatin, highlighting its promising ulcer-preventive efficacy.

**4. DISCUSSION**

Gastric ulceration, characterized by a localized lesion on the mucosal epithelium, is among the most prevalent gastrointestinal disorders globally, contributing to significant morbidity and mortality, with approximately 15 deaths per 15,000 ulcer-related complications reported annually.(12),(13) Inhibitory action of indomethacin on prostaglandin synthesis coupled with free radicals’ formation has been implicated in the pathogenesis of gastric ulceration. (14) The frequent adverse events, and resistance to existing therapies have encouraged more search into already existing drugs with seemingly positive attributes that can be exploited in the management of gastric ulcer. In this study, we investigated the effect of a statin, atorvastatin in indomethacin-induced gastric ulcer in rats.

Evaluation of the gastric mucosa following atorvastatin intervention greatly and significantly improved the lesion created by indomethacin. The dose dependent improvement of the indomethacin-eroded gastric mucosa was similar to a previous report by Ahmed et al., 2022. (9) Previous works had reported the antioxidant, anti-inflammatory, immunomodulatory, antithrombotic, vascular protective, and neuroprotective pleiotropic effects of atorvastatin which goes beyond their lipid-lowering effects. Atorvastatin, a widely prescribed statin, exhibits anti-inflammatory and antioxidant properties beyond its lipid-lowering effects. These pleiotropic effects have been demonstrated in various studies, highlighting atorvastatin's potential in modulating inflammatory pathways and oxidative stress. For instance, atorvastatin has been shown to exert anti-inflammatory effects by modulating the NLRP3 inflammasome and toll-like receptors (TLRs), which are implicated in cardiovascular diseases. By influencing these pathways, atorvastatin contributes to the reduction of vascular inflammation, thereby offering protective benefits in atherosclerotic conditions.(15) Additionally, atorvastatin's antioxidant properties have been observed in studies focusing on oxidative damage and inflammation. In models of ischemia–reperfusion injury, atorvastatin demonstrated a protective effect by reducing oxidative stress and tissue damage, underscoring its potential in mitigating conditions associated with oxidative injury.(16) Furthermore, atorvastatin has been reported to activate antioxidant pathways, such as the Nrf2/HO-1 axis, enhancing the body's defense mechanisms against oxidative stress. This activation contributes to its vascular protective effects, making atorvastatin a valuable therapeutic agent in managing diseases characterized by heightened oxidative stress and inflammation.(17) These findings suggest that atorvastatin's anti-inflammatory and antioxidant effects play a significant role in its therapeutic efficacy, extending its benefits beyond cholesterol reduction. These reported properties position atorvastatin as a promising candidate in the treatment of conditions like gastric ulcers, where inflammation and oxidative stress are key contributors to pathogenesis. Thus, we speculate that the antioxidant properties of atorvastatin enhanced its possible moping or detoxification of the free radicals produced by continuous release of superoxide anion and hydroperoxy free radicals during metabolism of indomethacin and even those released via the distortion of the balance between the aggressive and defensive factors. The sharp decline in the severity of the red discoloration, hemorrhagic streaks, and perforations in the artovostatin-pretreated gastric mucosa greatly showed the cytoprotective potential of atorvastatin in a dose dependent manner. NSAIDs have actually been reported as an excellent agent in investigating the potential usefulness of anti-secretory and cytoprotective agents since its fundamental pathophysiology involves gastric acid secretion and mucosal prostaglandin synthesis.(11) It is obvious that atorvastatin inhibited the gastric acid secretion and boosted the mucosal defense mechanisms by likely increasing mucosal production, stabilizing the surface epithelial cells, and upregulating the synthesis of prostaglandin which has been reported to be inhibited by indomethacin.

The ulcer index, a measure of ulcerated area in the gastric mucosa was significantly reduced upon increasing dose of atorvastatin. A better reduction was observed in the atorvastatin-pretreated groups. We observed no significant difference between the atorvastatin pretreated group and the rats treated with cimetidine. This revealed a restoration of the indomethacin damaged gastric mucosa by atorvastatin. Infact a study by Lin et al., 2017(18) reported the effect of statin use and the incidence of peptic ulcer disease in Taiwanese population. Their results showed that statin therapy reduced the risk of peptic ulcer and their observed reduction was associated with the high cumulative defined daily dose of prescribed statins. (18) From their reports, active use of statins is associated with decreased risk for peptic ulcer.

The improved gastric mucosa and diminishing ulcer index observed upon atorvastatin treatment culminated to an admirable gastroprotection. Again, the pretreated rats had better protection that was very close to the gastric mucosa of healthy rats. This also re-emphasized the cytoprotective potential of atorvastatin observed in this study. In a systematic and meta-analysis review by Lin *et al* (2017),(18) the protective effect of statins against PUD was assessed. They found that the risk of PUD was numerically lower among statins-users compared with non-users. However, the difference did not achieve statistical significance. There are several potential mechanisms that could theoretically give rise to the protective effect of atorvastatin shown in this study as well as its impressive anti-ulcer activity. A synergy between atorvastatin’s anti-oxidant and anti-inflammatory potential may have reduced inflammatory burden in the gastric mucosa and protected the gastric mucosa of the pretreated rats against the development of ulcer induced by the indomethacin. The possibility of atorvastatin enhancing the expression of prostaglandin synthetase cannot be refuted, hence, we recommend that these analyses be carried out to ascertain the mechanism with which atorvastatin prevented and even treated indomethacin-induced ulcer as seen in this study.

5. Conclusion

Gastric ulcer complications pose significant and life-threatening risks, often progressing beyond initial expectations. Prolonged administration of NSAIDs is well-documented to impair prostaglandin-mediated gastric mucosal protection, resulting in the formation of lesions on the gastric epithelium. In this study, atorvastatin demonstrated a notable protective effect against such gastric injury, as evidenced by improved gastric mucosal integrity, a reduced ulcer index, and enhanced gastroprotection. These effects were particularly pronounced at higher doses of atorvastatin and in the pretreated groups, highlighting its potential as a therapeutic intervention for NSAID-induced gastric ulceration.

Ethical Approval

Animal Ethic committee approval has been collected and preserved by the author(s)

Competing interests

The authors have declared that no competing interests exist.

Imaobong Etti designed the study, performed statistical analysis and wrote the manuscript;

Victor Neeka conducted the experiment and analyzed the data while Uduak Inwang contributed in the literature review.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

**Disclaimer (Artificial intelligence)**

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

References

1. Ahmed MA, Elsayed ET, Samy W. Gastric ulcer disease: Pathogenesis, management, and challenges. . International Journal of Gastroenterology. 2022;32(4):124–35.

2. Ugwah-Oguejiofor C, Ugwah OM. Gastroprotective activity of ethyl acetate fraction obtained from the stem bark extract of desert date (balanites aegyptiaca). Fudma journal of sciences. 2023 Jun 30;7(3):131–6.

3. Malfertheiner P, Chan FK, McColl KE. Peptic ulcer disease. The Lancet,  . The Lancet. 2017;390(10094):613–24.

4.Etti IC, Unoh EE, Akpan MR, Umanah UU, Agbonika RE, Kadir AA, et al. Attenuation of testosterone-induced benign prostatic hyperplasia with Andrographis paniculata (burm.f.) leaf extract in Wistar rats. Nat Prod Res. 2024

5. Moayyedi P, Leontiadis GI. The treatment of Helicobacter pylori infection and its impact on gastric ulcer disease. . The Lancet Gastroenterology & Hepatology. 2021;6(7):529–42.

6. Joshi RK. , & ., Rao S, Kumar A. The pleiotropic effects of statins: A review on their role in non-cardiovascular conditions. . Curr Pharm Des. 2021;27(34):4465–76.

7. Kavalukas SL, Barbul A, Mathews DD. Role of oxidative stress in the pathophysiology of peptic ulcer disease. American Journal of Gastroenterology. 2013;108(3):399–408.

8.Yin MJ, Chen YT, Chiang MT. Atorvastatin ameliorates inflammation and oxidative stress in experimentally induced gastric ulcers. Free Radical Research. 2019;53(12):1223–34.

9.Ahmed O, Nedi T, Yimer EM. Evaluation of anti-gastric ulcer activity of aqueous and 80% methanol leaf extracts of Urtica simensis in rats. Metabol Open. 2022 Jun;14:100172.

10. Singh SK, Pandey R, Gupta S. Advances in animal models for anti-ulcer drug discovery. Pharmacological Reports. 2021;73(5):1193–203.

11. Ulucan K, Karagoz A. NSAID-induced peptic ulcers: Mechanisms and potential therapies. Drug Development Research. 2020;81(5):540–51.

12. Abebaw M, Mishra B, Gelayee DA. Evaluation of anti-ulcer activity of the leaf extract of Osyris quadripartita Decne. (Santalaceae) in rats. J Exp Pharmacol. 2017 Jan 16;9:1–11.

13. Shristi JB, Neha J, Indu BP, Rajesh G. A review on some Indian medicinal plants for antiulcer activity. Journal of Scientific Research in Pharmacy . 2012;1:6–9.

14. Sabiu S, Garuba T, Sunmonu T, Ajani E, Sulyman A, Nurain I, et al. Indomethacin-induced gastric ulceration in rats: Protective roles of Spondias mombin and Ficus exasperata. Toxicol Rep. 2015 Jan 8;2:261–7.

15. Koushki K, Shahbaz SK, Mashayekhi K, Sadeghi M, Zayeri ZD, Taba MY, et al. Anti-inflammatory Action of Statins in Cardiovascular Disease: the Role of Inflammasome and Toll-Like Receptor Pathways. Vol. 60, Clinical Reviews in Allergy and Immunology. Springer; 2021. p. 175–99.

16. Trujillo-Rangel WA, Castillo-Romero A, Totsuka-Sutto SE, Rodríguez-Lara SQ, García-Cobián TA, Campos-Bayardo TI, et al. Effect of atorvastatin on oxidative damage and inflammation in experimental hindlimb ischemia–reperfusion model. Vol. 16, European Journal of Inflammation. SAGE Publications Inc.; 2018.

17. Mansouri A, Reiner Ž, Ruscica M, Tedeschi-Reiner E, Radbakhsh S, Ekta MB, et al. Antioxidant Effects of Statins by Modulating Nrf2 and Nrf2/HO-1 Signaling in Different Diseases. Vol. 11, Journal of Clinical Medicine. MDPI; 2022.

18. Lin CJ, Liao WC, Chen YA, Lin HJ, Feng CL, Lin CL, et al. Statin therapy is associated with reduced risk of peptic ulcer disease in the Taiwanese population. Front Pharmacol. 2017 Apr 28;8(APR).