ORIGINAL ARTICLE

EFFECT OF THE FLAVONOID 6-AMINOFLAVONE IN ASPIRIN-INDUCED GASTRIC ULCER IN SPRAGUE-DAWELY RATS: A HISTOMORPHOLOGICAL STUDY

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Background: The analgesic drugs are the main cause of gastric ulcer. The objective of this study was to determine the gastroprotective ability of flavonoid, 6-aminoflavone in a rat pyloric ligation model of aspirin associated gastro-ulcerogenesis. **Methods:** A laboratory based experimental study was conducted in the animal house and research laboratory at Khyber Medical College, Peshawar from July to November 2019. A total of 42 adult male Spargue-Dawely rats were divided into seven groups. Flavonoid, 6-aminoflavone was administered orally in doses of 10, 25 and 100 mg/kg with misoprostol, as standard at 50 µg/kg orally for 4 days. On the last day aspirin was given orally at 200 mg/kg and the pyloric ligation surgery was performed. After 4 hours all animals were killed by cervical dislocation. The gastric tissues were collected for histomorphological study. The obtained data were expressed as mean±SEM. Analysis was carried out by using ANOVA. *p* value <0.05 was considered significant. **Results:** The animals treated with the different doses of 6-aminoflavone showed a marked protective effect in the histological observations. The 10 mg/kg dose had a mild protective effect as occasional ulcerative changes were observed. However, doses of 25 and 100 mg/kg significantly caused the reduction in the ulcer score. These effects produced were equipotent to the gastroprotective effectiveness inherent in the misoprostol. **Conclusion:** These findings conclude that 6-aminoflavone as like other flavonoids has a significant gastroprotective propensity with significant effect produced at doses of 25 and 100 mg/kg and can be used as a part of therapy management for the treatment of gastrointestinal disease particularly ulcerative condition.

Keywords: Gastric ulceration; Gastroulcerogenesis model; Flavonoids

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INTRODUCTION

Analgesic drugs including non-steroidal anti-inflammatory drugs (NSAIDs) are widely used for the treatment of different painful conditions as well as for the reduction of inflammation and high temperature. However, their use is associated with the occurrence of various adverse effects that include gastrointestinal ulceration, cardiovascular and renal complications.[1] Among these, the NSAIDs induced ulcers remain a major adverse effect with a high morbidity and mortality that greatly limit their overall clinical efficacy.[2] The management of NSAIDs induced gastro-ulcerogenesis involves the use of COX-2-selective NSAIDs, proton pump inhibitors including proton pump inhibitor, histamine H_2 receptor antagonists like ranitidine and prostaglandin, misoprostol.[3,4] Despite the use of these therapeutic strategies, the management of NSAIDs induced peptic ulcer disease and its underlying complications remain a challenge for both clinicians and patients.

Recently, there is an increased interest in the use of alternative therapies including flavonoids, as they are considered to have less adverse effects as compared to conventional therapies so these are considered as the major reservoir of potentially new drugs.[5,6] Flavonoids have shown immense therapeutic potential as they possess a wide range of beneficial biological effects and this has been observed in preclinical experimentations.[7] These chemical compounds protect the mucosal lining of the gastrointestinal tract from the lesions produced by toxicants in various experimental ulcerative models.[8] Flavonoids produce their beneficial effects in the ulcerative conditions by preventing the release of histamine, inhibit the activity of H^+/K^+ proton pump, increase the synthesis of protective prostaglandins and produce a local cytoprotective effect.[9]

The present study evaluated the flavonoid, 6-aminoflavone for its effectiveness in NSAIDs induced gastric ulcer in particular. Previously, it was observed that 6-aminoflavone has potent antibacterial effect against pathogenic bacteria of

gastrointestinal tract and possess strong anticancer and anti-inflammatory properties.[10] The rationale of the study is that the patients who need regular NSAID can be given this compound before starting drugs shots to prevent the gastric ulcer induced by NSAID.

MATERIAL AND METHODS

An experimental study involving the use of live animals was conducted in the animal house and research laboratory at Khyber Medical College, Peshawar. For the induction of gastric ulceration, the well-developed rat model of aspirin associated gastric ulcerogenesis was used as previously reported[11]. A total of 42 adult male Spargue-Dawely rats were divided into seven groups with each group consisting of six animals (n = 6).

The animals were randomly divided into the following groups.

Group 1: Vehicle only as negative control

Group 2: Aspirin (200 mg/kg) only as ulcer control

Group 3: Misoprostol (50 µg/kg) as positive control for consecutive 4 days plus aspirin (200 mg/kg) on the last day

Group 4: 6-aminoflavone (10 mg/kg) for consecutive 4 days plus aspirin (200 mg/kg) on the last day

Group 5: 6-aminofalvone (25 mg/kg) for consecutive 4 days plus aspirin (200 mg/kg) on the last day

Group 6: 6-aminoflavone (100 mg/kg) for consecutive 4 days plus aspirin (200 mg/kg) on the last day

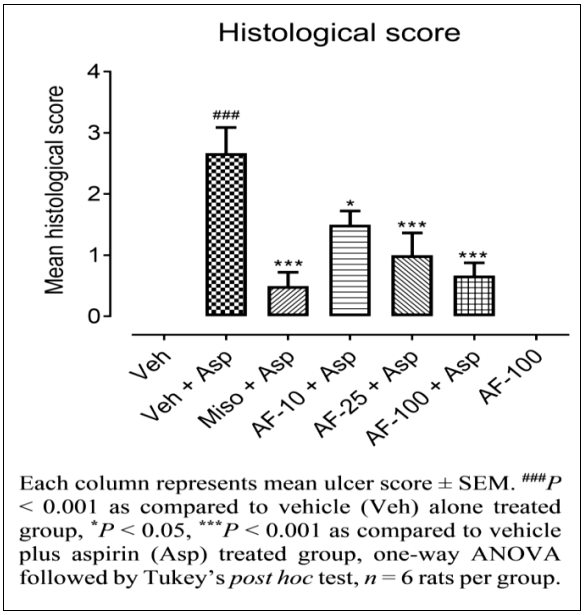
Group 7**:** 6-aminoflavone (100 mg/kg) only for consecutive 4 days

The animals were fasted for 18 hours and were then anesthetized. The pyloric ligation surgery was performed and the animals were allowed to recover. After 4 hours, all the animals were euthanized by cervical dislocation and the gastric tissues were collected for histomorphological study. Scoring and grading system was applied in order to evaluate the degree of gastric ulceration[12]. Changes were marked as no change, mild, moderate and severe.

Histopathological changes in the various layers including mucosa, submucosa, muscularis externa and serosa of the stomach and evaluation of gastric mucosa for ulcerative changes were variables under study. 6-aminoflavone is bioactive synthetic flavonoid compound were obtained from Sigma-Aldrich Co, St. Louis, Mo USA. Data were expressed as mean±SEM. Analysis was carried out by using ANOVA. *p* value ≤0.05 was considered significant.

RESULTS

When the animals were treated with the different doses of 6-aminoflavone, a marked protective effect was observed in the histological observations. The 10 mg/kg dose had a mild protective effect on the mucosa as occasional ulcerative changes were observed. However, when the animals were treated with the 25 mg/kg dose, the mucosal lining showed a complete histological appearance of mucosa, muscularis mucosa, and sub-mucosa. Similarly, the higher dose of 6-aminoflavone, i.e., 100 mg/kg was also much more effective in protecting the mucosa of the stomach from the deteriorating effects of aspirin. Likewise, the administration of the standard, misoprostol also had a beneficial protective propensity against the ulcerative nature of aspirin, as the histological features showed no considerable aberrations in the morphological characteristics of mucosa and sub-mucosa, when compared to the group of vehicles plus aspirin administrated animals.



Each column represents mean ulcer score ± SEM. $^{###}P < 0.001$ as compared to vehicle (Veh) alone treated group, $^{*}P < 0.05$, $^{***}P < 0.001$ as compared to vehicle plus aspirin (Asp) treated group, one-way ANOVA followed by Tukey's *post hoc* test, $n = 6$ rats per group.

Figure-1: Effect of 6-aminoflavone on histological score in the aspirin induced gastro-ulcerogenesis.

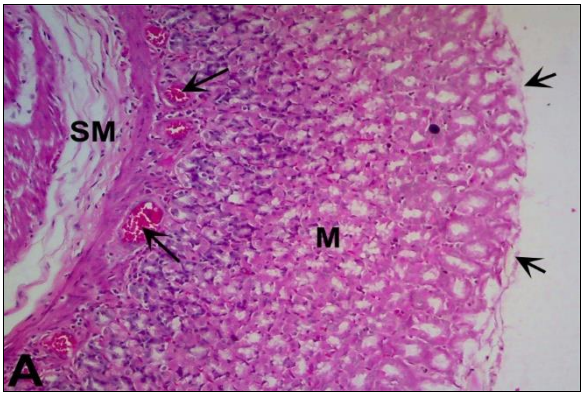


Figure-2: Photomicrograph showing the effect of ingestion of vehicle on the histological features of gastric mucosal tissue after H&E staining. The gastric tissue appeared normal.

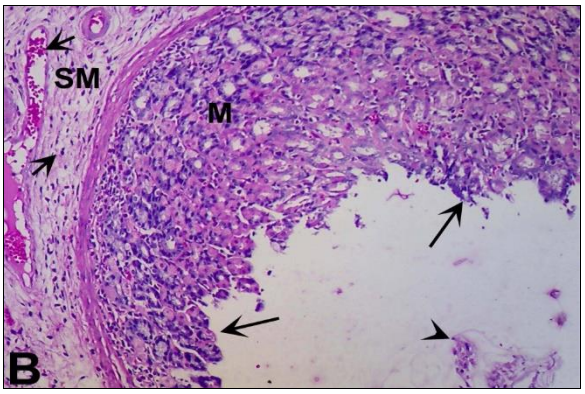


Figure-3: Showing aspirin associated histopathological changes in the gastric mucosal layer after H&E staining. The extensive exfoliation of the superficial layer of mucosa (large arrows) with cellular cast (arrow head) visible in the lumen. The ulcer extended towards the base of the mucosal layer. The submucosal layer (SM) showed edematous changes. The blood vessels were congested with red blood cells (small arrow). There was extensive infiltration of lymphocytes not only in the submucosa (small arrow) but also in the mucosal layer of the gastric tissue.

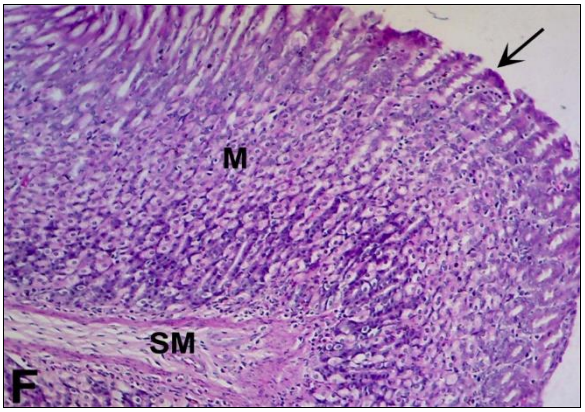


Figure-4: Photomicrograph showing effect of 6-aminoflavone at 100 mg/kg on the aspirin associated histopathological changes. The mucosal layer (M) showed complete integrity of the superficial mucus producing cells (large arrow) along with the other important cell layers. The submucosal layer (SM) also appeared normal with blood vessels congestion or inflammatory changes.

DISCUSSION

The present study utilized the pylorus ligation ulcerogenesis rat model. The ligation of the pylorus of stomach is a well-established rat model of induction of gastric ulceration having similar features as that observed clinically in patients diagnosed with ulcer. In this model, the ulceration is caused by the gastric mucosal auto-digestion and damage to the mucosal barrier after ingestion of ulcerative substances.[13] The degree of ulcerative changes produced by the pylorus model in this study affirmed the previous studies regarding the utilization of this model for the assessment of gastroportective substances.[13,14]

In the present study, the aspirin was used for inducing the characteristic features of gastric ulceration. When the aspirin was administered to the vehicle control animals, marked histological changes were observed. The most prominent histopathological changes in the gastric mucosa include extensive superficial mucosal damage, ulcerative erosion not only confined to mucus neck cells but also extending to the parietal cells area and muscularis mucosa. The degree and severity of aspirin induced gastric ulcer in this study was similar to the previously reported study in 1987 by PH Guth.[15]

In the present study, the flavonoid, 6-aminoflavone produced a marked protective effect (more significantly in dose of 25 and100mg/kg body weight) against aspirin induced gastric ulceration. Various flavonoids have been shown to produce strong anti-ulcerogenic activity that is comparative to the clinically used anti-ulcer medications. The flavonoid naringin, when administered at a dose of 200 mg/kg significantly decreased the ulcer index induced by acetylsalicyclic acid.[16] The flavonoids, quercetin, rutin and kaempferol produced a dose-dependent inhibition of gastric ulcerogenicity at a dose range of 25–100 mg/kg and the protection has been shown to be mediated through strong inhibition of platelet activating factor.[17] Flavonoids including quercetin and naringenin are able to decrease acute gastric ulceration by decreasing the secretion of histamine[18]. A similar protective activity has also been observed with hesperidin and neohesperidin dihydrochalcone in a model of cold-restraint induced ulcer.[19] The flavonoids, meciadanol has shown increased anti-ulcerogenic effectiveness in both preclinical and clinical trials.[20]

CONCLUSION

It is concluded that 6-aminoflavone as like other flavonoids has a significant gastroprotective propensity with significant effect produced at doses of 25 and 100 mg/kg and can be used as a part of therapy management for the treatment of gastrointestinal disease particularly ulcerative condition.

AUTHORS' CONTRIBUTION

TM: Concept of main theme, Study design. SA: literature search and write up. MS & NAS, MR: Data

collection, analysis and interpretation. IJ, FR& AR: Proof reading and minimizing plagiarism.

REFERENCES

1. Nalamachu S. An overview of pain management: the clinical efficacy and value of treatment. Am J Manag Care 2013;19(14 Suppl):261–6.
2. Drini M. Peptic ulcer disease and non-steroidal anti-inflammatory drugs. Aust Prescr 2017;40(3):91–3.
3. Kuna L, Jakab J, Smolic R, Raguz-Lucic N, Vcev A, Smolic M. Peptic ulcer disease: a brief review of conventional therapy and herbal treatment options. J Clin Med 2019;8(2):179.
4. Melcarne L, García-Iglesias P, Calvet X. Management of NSAID-associated peptic ulcer disease. Expert Rev Gastroenterol Hopatol 2016;10(6):723–33.
5. Yuan H, Ma Q, Ye L, Piao G. The traditional medicine and modern medicine from natural products. Molecules 2016;21(5):559.
6. Cragg GM, Newman DJ. Natural products: A continuing source of novel drug leads. Biochim Biophys Acta 2013;1830(6):3670–95.
7. Jucá MM, Cysne Filho FM, de Almeida JC, Mesquita DD, Barriga JR, Dias KC, Barbosa TM, *et al*. Flavonoids: biological activities and therapeutic potential. Nat Prod Res 2020;34(5):692–705.
8. Parmar NS, Parmar S. Anti-ulcer potential of flavonoids. Indian J Physiol Pharmacol 1998;42(3):343–51.
9. Mota KS, Dias GE, Pinto ME, Luiz-Ferreira A, Souza-Brito AR, Hiruma-Lima CA, *et al*. Flavonoids with gastroprotective activity. Molecules 2009;14(3):979–1012.
10. Moorkoth S. Synthesis and anti-cancer activity of novel thiazolidinone analogs of 6-aminoflavone. Chem Pharm Bull (Tokyo) 2015;63(12):974–85.
11. Umamaheswari M, Asokkumar K, Rathidevi R, Sivashanmugam AT, Subhadradevi V, Ravi TK. Antiulcer and in vitro antioxidant activities of Jasminum grandiflorum L. J Ethnopharmacol 2007;110(3):464–70.
12. Imaoka H, Ishihara S, Kazumori H, Kadowaki Y, Aziz MM, Rahman FB, *et al*. Exacerbation of indomethacin-induced small intestinal injuries in Reg I-knockout mice. Am J Physiol Gastrointest Liver Physiol 2010;299(2):G311–9.
13. Rastogi L, Patnaik GK, Dikshit M. Free radicals and antioxidant status following pylorus ligation induced gastric mucosal injury in rats. Pharmacol Res 1998;38(2):125–32.
14. Wagner KA, Nandi J, King RL, Levine RA. Effects of nonsteroidal antiinflammatory drugs on ulcerogenesis and gastric secretion in pylorus-ligated rat. Dig Dis Sci 1995;40(1):134–40.
15. Guth PH, Paulsen G. Aspirin-induced gastric injury in the rat: histologic changes and sucralfate cytoprotection. Pro Soc Exp Bio Med 1987;184(4):423–8.
16. Galati EM, Monforte MT, d'Aquino A, Miceli N, Di Mauro D, Sanogo R. Effects of naringin on experimental ulcer in rats. Phytomedicine 1998;5(5):361–6.
17. Izzo AA, Carlo GD, Mascolo N, Capasso F, Autore G. Antiulcer effect of flavonoids. Role of endogenous PAF. Phytother Res 1994;8(3):179–81.
18. Martin MJ, Motilva V, ÓN de la Lastra CA. Quercetin and naringenin; effects on ulcer formation and gastric secretion in rats. Phytother Res 1999;7(2):150–3.
19. Suarez J, Herrera MD, Marhuenda E. Hesperidin and neohesperidin dihydrochalcone on different experimental models of induced gastric ulcer. Phytother Res 1996;10(7):616–8.
20. Jayaraj AP, Lewin MR, Tovey FI, Kitler ME, Clark CG. The protective effect of Meciadanol (O-methyl-3 (+)-catechin) on experimental ulceration. Eur J Pharmacol 1988;147(2):265–71.

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