ERURJ **2025**, *4*, 2, 2454-2470

(Article)

The gastroprotective effects of dapagliflozin against NSAID-induced ulcerated rats

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Received: 19th March 2024, Revised 13th May 2024, Accepted 14th June 2024

DOI: 10.21608/erurj.2025.272088.1127

ABSTRACT

Peptic ulcer (PU) is a condition that is commonly linked to prolonged non-steroidal anti-inflammatory drugs (NSAIDs) use. This study aimed to examine the possible gastroprotective outcome of dapagliflozin (DAPA) on indomethacin (INDO)-provoked PU. Three rat groups were utilized, group I (control); group II (PU group) administered single oral dose (50 mg/kg) of INDO on the 14th day; group III (treatment group) administered daily oral dose (3 mg/kg) of DAPA for 14 days and PU was induced by INDO on the last day. The gastric mucosa was examined microscopically. Moreover, malonaldehyde (MDA), glutathione reductase (GSH), tumor necrosis factor-alpha (TNF-α), nuclear factor kappa B (NF-κB), interleukin 10 (IL-10), cyclooxygenase-II (COX-II), prostaglandin E2 (PGE2) and pepsin levels were assessed using ELISA. Our findings revealed that PU group displayed a significant rise in MDA, TNF-α, NF-κB and pepsin levels. Pre-treatment with DAPA attenuated these changes. Also, PU group exhibited reduced GSH, IL-10, COX-II and PGE2 levels which was reversed in the DAPA group. Microscopically, ulcerated and hemorrhagic areas were revealed in the INDO group which was mitigated by DAPA. In conclusion, DAPA showed gastroprotective effects against INDO-induced ulcerated rats through its anti-oxidant, anti-inflammatory actions and mucosal protective effect by increasing PGE2 and reducing pepsin levels.

Keywords: Dapagliflozin; Indomethacin; Inflammation; Oxidative stress; Ulcer.

1. Introduction

Peptic ulcer (PU) is outlined as mucosal abrasion that originate in mucosal lining of the stomach and proximal intestine, creating a hollow associated with both acute and chronic inflammation caused by gastric acid [1,2]. Numerous etiological factors, including alcohol intake, extended use of non-steroidal anti-inflammatory drugs (NSAIDs), stress, and H. pylori infection, have been connected to an elevated risk of PU [3]. One significant public health issue is peptic ulcer that is linked to non-steroidal anti-inflammatory drugs use, and the majority of its consequences have been the main contributors to morbidity and mortality [4]. Five to ten percent of people worldwide will experience stomach ulcers at some point in their lives [5].

Among the NSAIDs which induce peptic ulcer is indomethacin (INDO), a non-selective cyclooxygenase inhibitor. Existing studies suggest that indomethacin results in an imbalance between endogenous anti-oxidants and reactive oxygen species (ROS) provoking oxidative stress as shown by a decline in the quantities of reduced glutathione (GSH), glutathione peroxidase, and superoxide dismutase associated with the elevation of lipid peroxidation measured by the level of malonaldehyde (MDA) [6]. Furthermore, it has been observed that INDO exerts inhibitory effects on both types of cyclooxygenases (COX-I and COX-II), resulting in a reduction in the synthesis of endogenous prostaglandin E2 (PGE2). Prostaglandin E2 has a vital role as a gastroprotective mediator by preserving the integrity of the mucosal layer of the stomach. It exerts its gastric defense mechanism by stimulating bicarbonate release and mucus secretion, enhancing blood perfusion to the mucosa, and stimulating epithelium proliferation and repair [7]. Additionally, it prevents parietal cells from secreting acid and attenuates the inflammatory response by modulating leukocyte adhesion and cytokine production [8]. The reduction in PGE2 synthesis subsequently leads to the activation of tumor necrosis factor-alpha (TNF-α) and an elevation in pepsin levels [9,10]. Pepsin is the principal proteolytic stomach enzyme released by chief cells in the mucosal lining primarily as pro-enzyme known as pepsinogen which is activated by gastric acid to produce the active pepsin which contributes to the progression of peptic ulcer [11,12]. In addition to the previous mechanisms, the elevation in oxidative stress results in a subsequent rise in the level of nuclear factor kappa B (NF-κB), a transcription factor that mediates the expression of inflammatory genes. Consequently, it leads to the synthesis of pro-inflammatory cytokines [13]. Noteworthy, routes outside of COX, which

involve the formation of free radicals, are widely recognized as significant contributions to gastric injury [14].

Although studies on pathogenesis of peptic ulcer have revealed significant progress, its management is still a major health issue. Dapagliflozin (DAPA) is a selective inhibitor of sodium-glucose co-transporter 2 (SGLT2) approved for management of type 2 diabetes mellitus and is currently recommended for patients with heart failure regardless of the diabetes [15]. The mode of action of DAPA involves inhibiting SGLT2 in the kidney and thus blocks glucose resorption [16–18]. The earlier studies reported that DAPA possess anti-inflammatory effect mediated through modulating NF-κB/ TNF-α pathway. In addition, it exerts anti-oxidant effect by raising the levels of the anti-oxidant enzymes [19,20]. Thus, the current study's objective was to evaluate the possible gastroprotective role of Dapagliflozin in INDO-induced peptic ulcer and investigate its mode of action.

2. Experimental

2.1. Materials

Dapagliflozin was supplied by AstraZeneca (New Cairo, Cairo, Egypt) and INDO was supplied by Nile Pharmaceutical company (Cairo, Egypt). The highest analytical grade of chemicals was obtained for all other compounds.

2.2. Animals

Adult male Wistar rats (150–200 g body weight) were acquired from the animal facility in the faculty of pharmacy, Egyptian Russian University. Rats totally were held in a carefully restricted environment (22°C ± 2°C & constant humidity 50-70%) under a 12-hour cycles of light and dark. Animals were kept together for acclimatization seven days prior to the experiment's commencement. There was free water and standard diet pellets provided.

2.3. Ethics approval statement

The research methods adhered to the National Institutes of Health's standards for laboratory animal care and use [21]. The present animal experiment followed the ARRIVE standards [22] and approved by Ethics Committee of the Faculty of Pharmacy, Egyptian Russian University, Egypt (approval number is: ERUFP-PO-23-007).

2.4. Experimental design

Rats were randomly alienated into three groups (n = 6). The study was conducted for 2 weeks. The groups were as follow: the first group administered oral saline. The second group was given a single oral gavage of INDO (50 mg/kg/day; p.o) the last day 6 hours before scarification [23]. The third group administered DAPA (3 mg/kg/day; p.o) for 14 days as well as a single oral gavage of INDO (50 mg/kg/day; p.o) the last day 6 hours before scarification [24,25]. All rats were fasted for 24 hours before INDO administration, at that time, rats were kept in bottom-wired rodent cages to prevent coprophagia. Moreover, water was deprived two hours before INDO dosing.

Thiopental sodium (EIPICO, Tenth of Ramadan City, Egypt) (30 mg/kg; i.p) was used to induce anesthesia [26]. Rats were then sacrificed by decapitation; stomach were incised along the greater curvature and gastric content were garnered and gastric mucosal tissues were quickly dissected out. Tissues were separated into two fragments. One of them was fixed in formalin solution (10%, neutral buffered) for microscopic inspections. The other one was employed for biochemical examinations. Furthermore, the gastric contents were preserved for evaluation of pepsin level.

2.5. Biochemical examinations

2.5.1. Assessment of pepsin content in gastric tissue

The content of pepsin in gastric tissue was quantified by ELISA kit (cat#: MBS2507364) obtained from MyBioSource Inc. (San Diego, USA). The procedures were operated according to the manufacturer protocols.

2.5.2. Determination of PGE2 in gastric mucosa

The PGE2 level was determined using ELISA assay. Rat PGE2 kit (cat#: CSB-E07967r; CUSABIO, TX, USA) was used. The procedures were operated according to the manufacturer protocols.

2.5.3. Determination of inflammatory markers

The expression of inflammatory markers was assessed in gastric tissue using ELISA technique. This involved using CUSABIO (TX, USA) rat ELISA kits to quantify NF-κβ (cat#: CSB-E13148r) and COX-II (cat#: CSB-E13399r). ThermoFisher Scientific Inc. (MA, USA) rat

ELISA kit was used to quantify TNF-α (cat#: KRC3011). Moreover, MyBioSource Inc. (San Diego, USA) rat ELISA kit was used to quantify IL-10 (cat#: MBS355232). The procedures were operated according to the manufacturer protocols.

2.5.4. Determination of oxidative stress markers

Gastric tissue lipid peroxide level was assessed by measuring MDA level using rat ELISA kit (MyBioSource Inc., San Diego, USA, cat#: MBS268427). Whereas, the level of GSH was determined using MyBioSource Inc. (San Diego, USA) rat ELISA kit (cat#: MBS265966). The procedures were operated according to the manufacturer protocols.

2.6. Histopathological assessment of inflammation in gastric mucosal tissue

The fixed stomach samples (10% buffered formol-saline) were cut into 4 micrometer paraffin slices and stained with hematoxylin and eosin. Following that, the tissue slices were inspected using a light microscope (Leica Microsystems, Wetzlar, Germany). All morphometric data and light microscopic examinations were assessed using the Leica Application module, that was linked to a complete HD microscopic imaging system (Leica Microsystems, Germany) [27].

2.7. Statistical analysis

The mean of the group +/- standard deviation (SD) was used to represent data. One-way analysis of variance was performed for analysis of statistics and further supported by Tukey's post-hoc test for variables using GraphPad Prism Version 9. The cutoff point for statistical significance in each test was established as $P < 0.05$.

3. Results

3.1. Impact of DAPA on pepsin levels in INDO-induced ulcerated rats

The pepsin content is related to the degree of ulcer progression. Administration of INDO resulted in a significance elevation in pepsin content by 4.99-folds, in correlation to the control group. DAPA pre-treatment reversed this upsurge by 29.4%, compared to disease group (**Figure 1**).

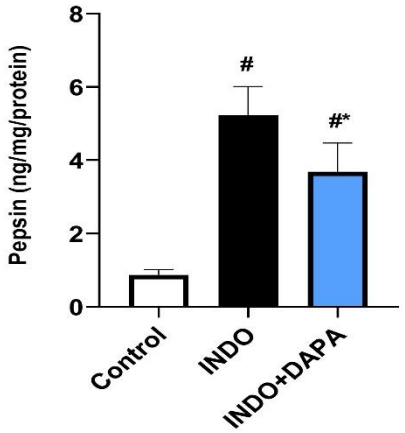

Figure 1: *shows the pepsin content in control, disease mode and treatment group, respectively. Data represented by (mean (n)=6 rats) ±SD. Statistical evaluation was done by one-way ANOVA succeeded by Tukey's test for multiple comparison. # Significant difference from normal control at P < 0.05; * Significant difference from indomethacin group at P < 0.05. INDO: indomethacin; DAPA: dapagliflozin; ANOVA: analysis of variance.*

3.2. Impact of DAPA on PGE2 level in gastric mucosa in INDO-induced ulcerated rats

Dosing of INDO resulted in a decline in mucosal PGE2 by 70%, compared to the control rats. DAPA pre-treatment reversed the reduction in PGE2 by 82% increase, compared to peptic ulcer rats (**Figure 2**).

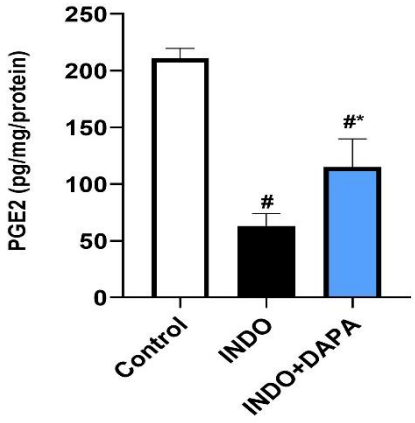

Figure 2: *Shows the mucosal content of PGE2 in the control, INDO-administered, and, DAPA-pretreated groups, respectively. Data represented by (mean (n)=6 rats) ±SD. Statistical evaluation was done by one-way ANOVA succeeded by Tukey's test for multiple comparison. # Significant difference from normal control at P < 0.05; * Significant difference from indomethacin group at P < 0.05. PGE2: Prostaglandin E2; INDO: indomethacin; DAPA: dapagliflozin; ANOVA: analysis of variance.*

3.3. Impact of DAPA on gastric inflammatory markers in INDO-induced ulcerated rats

Sole administration of INDO caused an exaggerated inflammatory response (**Figure 3**). Represented by significantly increased expression of TNF-α by 2-folds and NF-κB by 5.2-folds and reduced expression of IL-10 by 47.9% and COX-II by 82.8%, in comparison to ulcer group. These changes were reversed in the DAPA group as shown by a significant reduction in the expression level of TNF-α and NF-κB by 45.3% and 49.2%, respectively, as compared to INDO group. In addition, an elevation in the expression of IL-10 by 76% and COX-II by 2-folds was demonstrated, in comparison to INDO group.

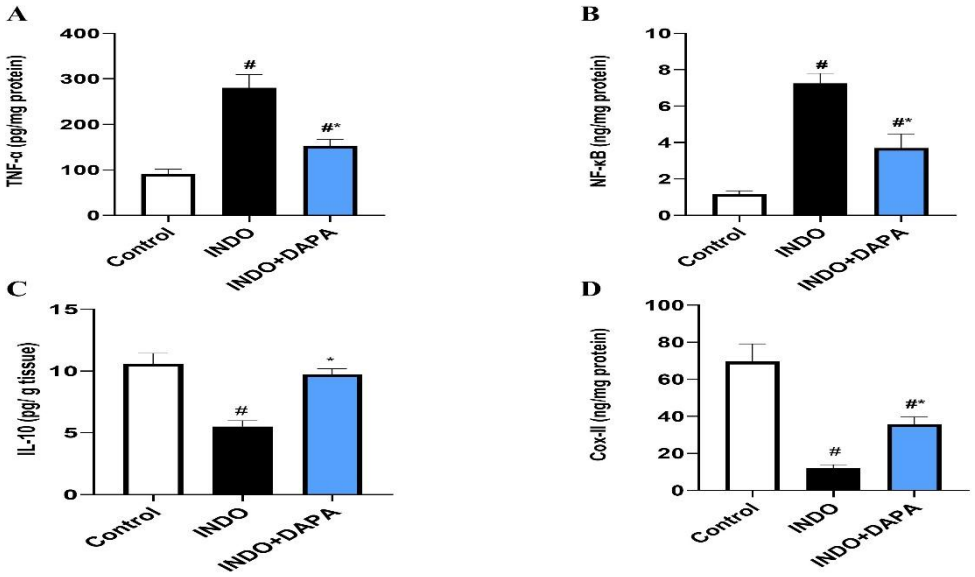

Figure 3: *represents the impact of DAPA on gastric (A) TNF-a, (B) NF-κB, (C) IL-10, and (D) COX-II expression levels in INDO-induced ulcerated rats. Data represented by (mean (n)=6 rats) ±SD. Statistical evaluation was done by one-way ANOVA succeeded by Tukey's test for multiple comparison. # Significant difference from normal control at P < 0.05; * Significant difference from indomethacin group at P < 0.05. COX-II: cyclooxygenase-II; IL-10: interleukin-10; TNF-a: tumor necrosis factor-alpha; NF-κB: nuclear factor kappa B; INDO: indomethacin; DAPA: dapagliflozin; ANOVA: analysis of variance.*

3.4. Impact of DAPA on oxidative stress markers in INDO-induced ulcerated rats

The levels of MDA and GSH in gastric tissue of control group, INDO group and DAPA-treated group are summarized in **Figure 4**. The administration of INDO resulted in an elevation in MDA level by 1.2-folds and a reduction in GSH level by 76.5%, as compared to control rats. On the other hand, DAPA pre-treatment significantly depressed the level of MDA by 65% and increased that of GSH by 2.6-folds, as compared to INDO group.

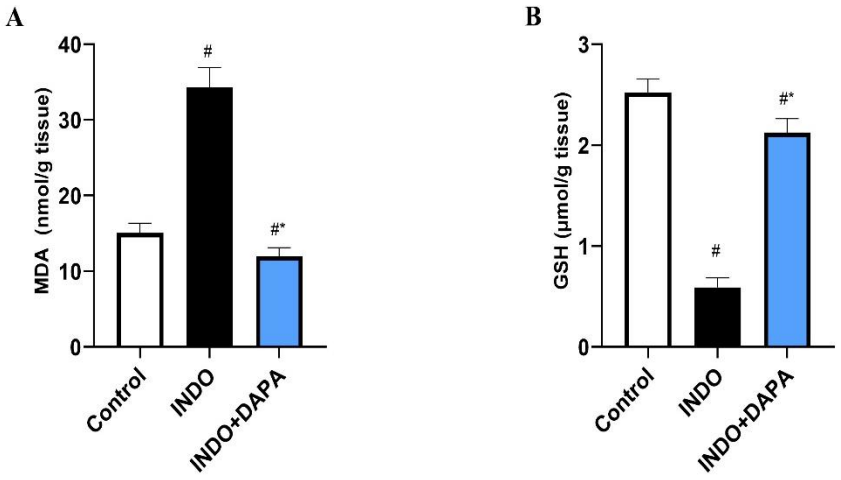

Figure 4*: represents the gastric levels of (A) MDA and (B) GSH in control group, INDO group and DAPA-pretreated group. Data represented by (mean (n)=6 rats) ±SD. Statistical evaluation was done by one-way ANOVA succeeded by Tukey's test for multiple comparison. # Significant difference from normal control at P < 0.05; * Significant difference from indomethacin group at P < 0.05. GSH: reduced glutathione; MDA: malondialdehyde; INDO: indomethacin; DAPA: dapagliflozin; ANOVA: analysis of variance.*

3.5.Microscopic examination

Histological examination of the control normal group showed normal organization of the glandular mucosa of the stomach and submucosal lining (**Figure 5**). Major histopathological abnormalities were spotted in indomethacin group. Multiple foci of ulcerated areas were spotted in the glandular mucosa of the stomach, which is described as epithelial lining desquamation associated with hemorrhages and buildup of eosinophilic and karyorrhectic necrotic tissue debris. Several cases showed excessive infiltration of inflammatory cells in the submucosa and mucosal layers. Increase vascular permeability was detected in the submucosal blood vessels with subsequent abundant edema (**Figure 6**). Marked protective effect was detected in DAPA group. The glandular mucosa revealed apparently normal gastric epithelial cells. Some examined sections showed submucosal edema and hyperemic blood vessels (**Figure 7**).

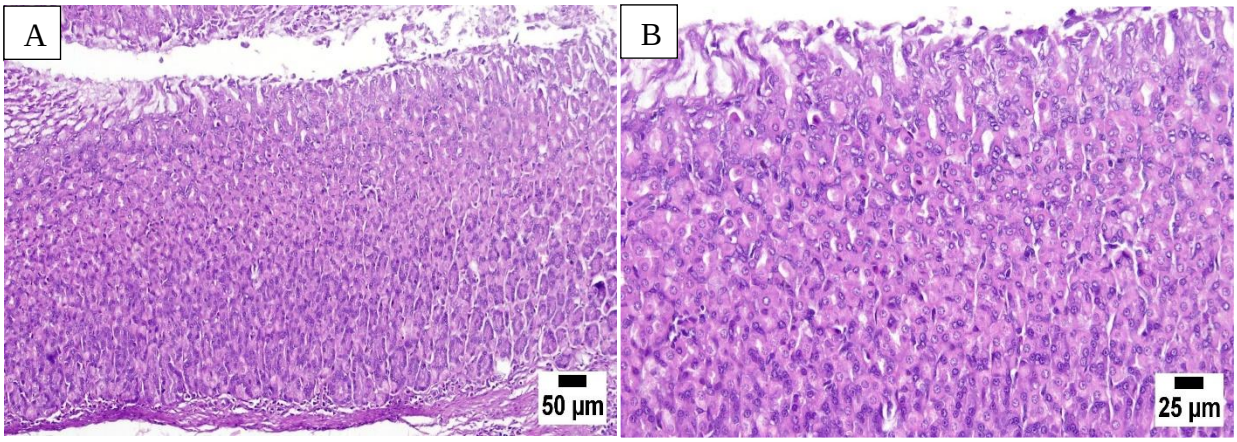

Figure 5*: represents H&E stain photomicrographs showing gastric tissue of normal control group with normal gastric mucosal and submucosal layers. Magnification: (A)50 µm and (B)200 µm.*

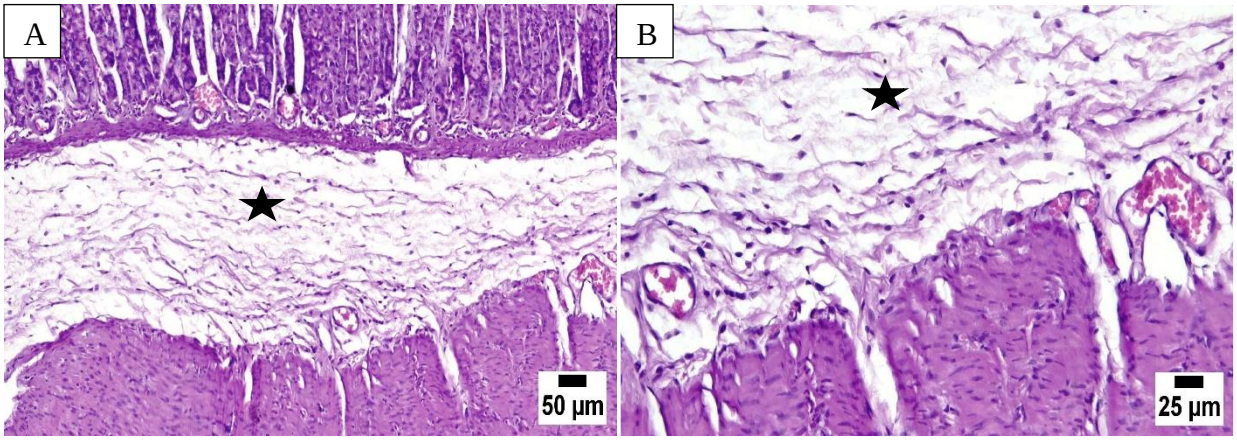

Figure 6*: represents H&E stain photomicrographs showing the stomach of indomethacin group with excessive edema in the submucosa (star) associated with blood vessels congestion. Magnification:(A)50 µm and (B)200 µm.*

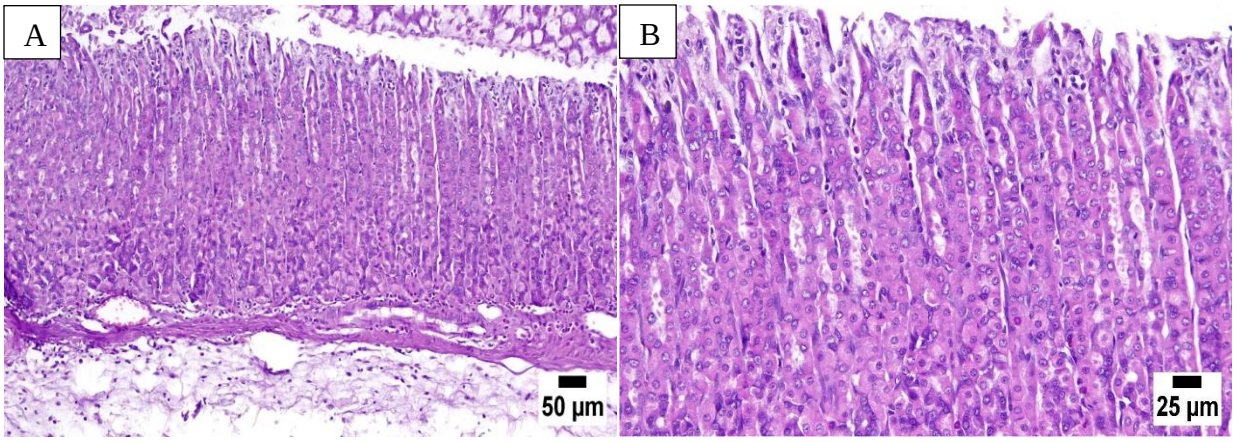

Figure 7: *represents H&E stain photomicrographs showing the stomach of DAPA pre-treated group with obviously normal mucosa accompanied by mild edema in the submucosa. Magnification:(A)50 µm and (B)200 µm.*

4. Discussion

In the present experiment, we studied the possible gastroprotective effects of DAPA in INDO-induced ulcer rat model, this investigation was established on the recently reported promising outcomes of Phloretin, as a SGLT1 and SGLT2 inhibitor, and its protective influence on model of INDO induced gastric ulcer in mice [28].

Our findings indicated that DAPA exhibited a protective effect on the stomach of rats. the mechanism underlying the gastroprotective action of DAPA involved anti-inflammatory and mucosal protective effect mediated through the interaction between TNF-α/ NF-κB/COX-2/PGE2 pathway.

The morphological examination of the stomach of rats administered INDO revealed visible abnormalities such as notable lesions and bleeding [26], these characteristics came in parallel with our histopathological analysis, which demonstrate multiple foci of ulcerated areas in the glandular mucosa of the stomach, epithelium desquamation associated with hemorrhages and buildup of necrotic tissue. Pre-treatment with DAPA markedly protected the stomach and ameliorated the histopathological abnormalities with some submucosal edema and hyper-emic blood vessels. Our results came across previous results that show DAPA produced intact mucosal layer with minimal edema and blood vessel dilation in gastric lesions induced by ethanol in rats [29].

Oxidative stress, indicated by an inequality between ROS and anti-oxidant enzymes, plays a central role in the development and progression of NSAID-induced ulcer [30]. The levels of MDA, an oxidative stress marker that is produced after lipid peroxidation, and GSH were used in this study to reflect the gastric oxidative stress condition. The administration of INDO was previously described to induce the level of ROS and ameliorate the anti-oxidant defence mechanism [31]. The production of ROS by INDO was attributed to an indirect effect that resulted from infiltration of inflammatory cells and mitochondrial dysfunction [31]. Our findings reveal inflammatory cells infiltration in the microscopic examination that could explain the increased level of ROS as shown by elevated MDA level. Of note, ROS promotes inflammatory cascades which in turn results in a cycle of increased ROS production and inflammation [32]. Pre-treatment with DAPA not only reduced the level of MDA but also elevated the antioxidant enzyme GSH, indicating that DAPA could ameliorate the pathogenesis of ulcer through its anti-

oxidant effect which will also regulate the inflammatory process. These outcomes corroborate earlier studies indicating an anti-oxidant effect for DAPA thus reinforcing the established understanding about the role of DAPA in oxidative stress-related conditions [33,34].

One pro-inflammatory cytokine that is quite important in the initiation and progression of inflammation is TNF-α [35]. Its overexpression is linked to the progression of gastric ulcers, as it induces the expression of various inflammatory mediators and activates downstream signalling pathways [36]. Similarly, our findings reveal significant rise in the levels of TNF-α after INDO administration to rats as described previously [37]. The significant reduction in TNF-α expression observed in the DAPA pre-treated group suggests that it may effectively suppress the generation of this cytokine, thereby mitigating the inflammation associated with gastric ulcer formation. The results of this study came in parallel with a prior study involved using canagliflozin as an SGLT-2 suppressor which suppressed the level of TNF-α in colitis in rats [38,39].

The transcription factor NF-κB controls the expression of genes related to immune responses and inflammation [40]. The intensification of NF-κB is tightly linked to the progression of gastric ulcers, as it promotes the transcription of pro-inflammatory genes, including COX [41]. COX, in turn, is an enzyme that is in charge of the production of prostaglandins that play a crucial part in either mediating inflammatory response and tissue damage or tissue healing depending on several factors such as the inflammatory stimuli and phase of inflammation [42]. Correspondingly, the administration of INDO to rats induced elevation in the level of NF-κB in this study which was reported previously [43]. The observed decrease in NF-κB levels in the DAPA-treated group suggests that DAPA may modulate the NF-κB signalling pathway, thereby decreasing the inflammation and mediating the healing process of tissue as evidenced by microscopic examination in the current experiment. This finding was in harmony with a recent study [44].

Interleukin-10 (IL-10) is a key anti-inflammatory cytokine that is essential for regulating the immune response and maintaining immune homeostasis [45]. In general, IL-10 reduces inflammation by inhibiting the synthesis of pro-inflammatory cytokines, such as tumor TNF-α, IL-1, and IL-6 [46]. The induction of ulcer with INDO is associated with reduced IL-10 expression and increased inflammatory cytokines [47], this came in accordance to our results. Despite that, DAPA showed upregulated IL-10 expression in the ulcer group. The ability of

DAPA to upregulate IL-10 and downregulate the inflammatory cytokines was previously described [48].

Interestingly, we also detected an upsurge in COX-II activity and PGE2 levels in the DAPA-treated group in relation to the disease model. COX-II is responsible for the production of various prostaglandins, including PGE2, which can exert both pro-inflammatory and protective effects [49]. Regarding our experiment, the administration of INDO produced a significant reduction in COX-II levels with subsequent reduction in PGE2 levels too. These findings were consistent with earlier studies [50,51]. The increased COX2 activity and PGE2 levels observed in the DAPA-treated group suggest a potential protective effect on the gastric mucosa. Although the specific mechanisms responsible for this observation remain incompletely understood, it is possible that DAPA's ability to induce COX-II which is able to act as a safeguard to the mucosa of the stomach when COX-I is inhibited [23]. The compensatory increase in COX-II favors the production of prostaglandins, promoting the production of PGE2, which may exert healing-boosting and gastroprotective effects through increased gastric mucus and bicarbonate secretion and inhibition of gastric contractions [23]. According to an earlier study, PGE2 synthesis was increased by SGLT-2 inhibitor in a model of obese diabetic mice which is similar to our result [52].

The current study also examined the effect of DAPA on pepsin level in INDO-induced ulcerated rats. Pepsin, an enzyme primarily produced in the stomach, plays a crucial function in protein digestion. It is typically present in higher concentrations in the gastric mucosa and gastric juice. INDO is recognized to result in injury to the gastric mucosa and increase pepsin secretion potentially contributing to the progression of gastric ulcer [53]. Similarly, our findings reveal a rise in pepsin levels following administration of INDO. The observed increase in pepsin content in the INDO group corroborates the well-established role of NSAIDs in disrupting gastric homeostasis and promoting mucosal damage. INDO-induced inhibition of COX enzymes leads to decreased prostaglandin synthesis, impairing the protective mechanisms of the gastric mucosa, including mucous secretion and bicarbonate production [54]. Consequently, the acidic environment and disrupted mucosal integrity facilitate pepsin leakage and activation, exacerbating tissue damage and ulcer formation. Notably, pre-treatment with DAPA lead to a marked decrease in pepsin content compared to the indomethacin group, which may be attributed

to increased PGE2 with subsequent central antisecretory action on pepsin as previously reported [55].

5. Conclusion

Overall, our findings propose that DAPA employs its effects on gastric ulcer development through multiple mechanisms. Restoring the balance between ROS and anti-oxidant system, thus, exerting anti-oxidant action, Also, the decrease in TNF-α and NF-κB levels and elevated IL-10 level indicates a suppression of the inflammatory response, while the increase in COX2 activity and PGE2 levels suggests a potential protective effect on the gastric mucosa and decrease in pepsin levels suggests a potential positive impact of DAPA on gastric function and pepsin production. This multi-faceted modulation of inflammatory parameters highlights the complex interplay between DAPA and the inflammatory pathways involved in gastric ulcer pathogenesis.

Conflict of Interest

There is no conflict of interest, according to the authors.

6. References

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