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The Effect of Silver Water (Ag) Administration on NF-κB Expression and Histopathology of Gastric in Rats Model of Inflammatory Bowel Disease

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Abstract: This study investigated the therapeutic effects of silver water on indomethacin-induced Inflammatory Bowel Disease (IBD) in rats. Male white rats (12 weeks old, ~250g) were divided into five groups: negative control (untreated), positive control (indomethacin-induced), and three treatment groups (T1, T2, T3) receiving 1, 2, and 3 ml of 25 ppm silver water, respectively for 14 days following indomethacin induction (15 mg/kg BW). The study evaluated NF-κB expression via immunohistochemistry and gastric histopathological changes using hematoxylin-eosin staining. Data were analyzed using ANOVA followed by the Tukey test (α = 0.05). Results demonstrated that silver water therapy at all tested volumes significantly reduced NF-κB expression, inflammatory cell infiltration, and epithelial erosion in gastric tissues compared to the positive control. The 3 ml dose showed the most pronounced therapeutic effect. These findings suggest that silver water possesses anti-inflammatory properties that suppress the NF-κB inflammatory pathway and promote gastric tissue repair in indomethacin-induced IBD, potentially offering a novel therapeutic approach for IBD management.
Keywords: Anti-inflammatory, Antioxidant, IBD, Indomethacin, Silver Water, Pathology.

1. Introduction

Inflammatory Bowel Disease (IBD) is a chronic inflammation of the digestive tract characterized by relapses, with the exact cause still unknown. Generally, IBD is classified into Crohn's disease (CD) and ulcerative colitis (UC) (Ghattamaneni et al, 2019). Crohn's disease (CD) can affect any part of the gastrointestinal tract from the mouth to the anus (Pariente et al, 2018). Ulcerative colitis (UC) is a nonspecific inflammatory disorder of the large intestine, often starting in the rectum and extending proximally with varying degrees of involvement (Taku et al., 2020).

The prevalence of IBD in dogs is 36% presenting with vomiting and 48% being asymptomatic (Craven et al., 2004). Based on research by Wagner et al (2018), there were 21 cases of Inflammatory Bowel Disease reported at the Clinic for Small Animals, Faculty of Veterinary Medicine, Hannover University, Germany. The pathomechanism of IBD involves inflammation triggered by increased pro-inflammatory cytokine expression, followed by NF-κB activation, which leads to tissue damage, including gastric mucosal inflammation and ulceration (Yao et al., 2019). Clinical manifestations of tissue damage include melena, diarrhea, and damage to the digestive organs (Ananthakrishnan and Xavier, 2020).

According to Sairenji and Evans (2017), IBD induction uses a non-steroidal anti-inflammatory drug (NSAID) such as indomethacin. Indomethacin can inhibit cyclooxygenase (COX), thereby preventing the conversion of arachidonic acid into prostanoids (Abdellatif et al., 2021). Prostanoids regulate mucus production and gastric acidity. Reduced mucus in the stomach reduces gastric protective barriers and increases gastric acid production, leading to irritation and damage to the gastric mucosa (Gyires, 2005).

While many studies have investigated the therapeutic effects of natural substances, the use of metal materials for therapeutic purposes remains relatively rare. Metals, such as silver, can be used for various medical purposes due to their physicochemical properties as anti-inflammatory and antimicrobial agents (Park et al., 2011). Silver has the potential to manage inflammation and exert antibacterial and antifungal effects (Burdusel et al., 2018). This potential suggests a tendency to influence inflammatory processes.

However, the molecular mechanisms underlying the therapeutic effects of silver water on IBD, particularly in the stomach, remain unclear. One of the key signaling pathways in IBD pathogenesis is Nuclear Factor-kappa B (NF-κB) (Papoutsopoulou & Campbell, 2021). NF-κB is a transcription factor that plays a central role in regulating inflammatory and immune responses (Bhatt & Ghosh, 2014). Excessive NF-κB activation has been associated with increased production of pro-inflammatory cytokines and tissue damage in patients with IBD (Neurath, 2014). Therefore, modulation of NF-κB expression is a potential therapeutic target for the management of IBD (Szatkowski et al., 2020).

Although several studies have investigated the effects of silver water on various inflammatory conditions, a knowledge gap remains regarding its influence on NF-κB expression and gastric histopathology in IBD. A better understanding of these molecular mechanisms could pave the way for the development of new, effective, and safe treatment strategies for IBD. This study aims to evaluate the effect of silver water (Ag) administration on NF-κB expression and gastric histopathology in a rat model of Inflammatory Bowel Disease.

2. Materials and Methods

Experimental Animal and Treatment

This study involved 25 male white rats, aged 12 weeks and weighing approximately 250 g, divided into five treatment groups. Group 1 consisted of untreated rats (C-), group 2 consisted of rats induced with IBD using indomethacin 15 mg/kg BW once (C+), group 3 consisted of IBD-induced rats using indomethacin 15 mg/kg BW once treated with 25 ppm silver water at a dose of 1 ml (T1), group 4 consisted of IBD-induced rats using indomethacin 15 mg/kg BW once treated with 25 ppm silver water at a dose of 2 ml (T2), and group 5 consisted of IBD-induced rats using indomethacin 15 mg/kg BW once treated with 25 ppm silver water at a dose of 3 ml (T3). The indomethacin induction dose was based on the study by Waranmaselembun et al. (2023). The study was conducted over 14 days; on the 15th day, the rats were euthanized by cervical dislocation. A laparotomy was performed to extract the Gastric organ, which was then placed in a 10% formaldehyde solution to prepare histopathology and immunohistochemistry slides.

Histological examination and grading

Gastric tissue was preserved in 10% neutral buffered formalin solution for 24 h and washed with 70% ethanol. Tissues were then placed in small metal caskets, stirred with a magnetic stirrer, dehydrated with an alcohol series ranging from 70% to 100%, and embedded in paraffin using an embedding machine. Paraffin blocks were sectioned using a rotary ultramicrotome, mounted on glass slides, and dried overnight. Slides were observed under a light microscope after being stained with hematoxylin and eosin (H&E) dyes and mounted. The five fields of view were assessed for each slide through a scoring system. Histopathological grading is performed using a semiquantitative scale: standard = 0, mild = <25%, moderate = 25–50%, and severe = >50% of the affected area (Palipoch and Punsawad, 2013) (Table 1).

Score	Erosion of the mucosal epithelium	Inflammatory cells infiltration
0 points	Normal, 0% of mucosal epithelium erosion	Normal, 0% of inflammatory cells infiltration
2 points	<25% of mucosal epithelium erosion	<25% of inflammatory cells infiltration
4 points	25-50% of mucosal epithelium erosion	25-50% of inflammatory cells infiltration
6 points	>50% of mucosal epithelium erosion	>50% of inflammatory cells infiltration

Table 1 – Semiquantitative scale for histopathological grading.

IHC methods for NF-κB analysis

IHC analysis was performed to assess NF-κB expression (Liu et al., 2017). First, an incision was made transversely through the ovarian tissues using paraffin blocks. IHC techniques were performed using a monoclonal antibody against NF-κB. NF-κB expression analyses were performed using a light microscope with a magnification of 400×. NF-κB expressions were indicated by the number of cells with brownish discoloration due to DAB-chromogen in each incision (Crosby et al., 2016). The five fields of view were assessed for each slide through a scoring system. The following IHC scoring system was used: IHC score = A × B, where A denotes the percentage of wide expression and B denotes the chromogen intensity (Nowak et al., 2007) (Table 2).

A	B
0 points no cells with positive reaction	0 points no color reaction
1 point to 10% cells with positive reaction	1 point low intensity of color reaction
2 points 11%−50% cells with positive reaction	2 points moderate intensity of color reaction
3 points 51%−80% cells with positive reaction	3 points intense color reaction
4 points >80% cells with positive reaction	

Table 2 – Semiquantitative IHC scale taking into account both percentage of positive cells (A) and intensity of reaction color (B), with the final score representing the product of the two variables (A x B).

Statistical analysis

The obtained data were expressed as the mean ± standard deviation (SD) and analyzed using a One-way analysis of variance (ANOVA) followed by a Duncan test to determine significance ($p < 0.05$) between treatment groups.

3. Results

The administration of indomethacin to rats inhibits COX-1 activity, thereby reducing the production of prostaglandin H2 (PGH) and thromboxane A2 (TXA), leading to decreased mucus secretion. The reduction in mucus secretion allows Gastric acid to irritate the Gastric lining, causing inflammation, as indicated by increased NF-κB expression (Table 3 and Figure 1), a key regulator of inflammation that triggers pro-inflammatory cytokines. Decreased mucus secretion irritates the gastric mucosa, leading to erosion of the gastric mucosal epithelium. When the gastric mucosa becomes inflamed, there is an increase in leukocytes on the surface of the endothelial postcapillary venules, characterized by the migration of macrophages and polymorphonuclear cells (an increase in the filtration of inflammatory cells) (Table 3 and Figure 2).

Group	Expresion of NF-κB	Erosion of the mucosal epithelium	Inflammatory cells infiltration
C-	2,04^{a}±0,30	0,56^{a}±0,36	0,80^{a}±0,49
C+	10,84^{e}±0,65	5.20^{e}±0,63	5,52^{e}±0,72
T1	8,16^{d}±0,57	4,40^{d}±0,28	4,16^{d}±0,36
T2	5,16^{c}±0,68	3,52^{c}±0,33	2,72^{c}+0,52
T3	3,60^{b}±0,37	1,52^{b}±0,44	1,92^{b}±0,18

Table 3 – Expression of NF-κB, Erosion of the mucosal epithelium, and infiltration of Inflammatory cells in the rat's gastric. Different superscripts (a,b,c,d,e) in the same column showed significant differences ($p < 0.05$). Group C- received no treatment (normal); Group C+ induced with 15 mg/kg BW indomethacin; group T1 induced with 15 mg/kg BW indomethacin and supplemented with 25 ppm silver water at a dose of 1 ml; group T2 induced with 15 mg/kg BW indomethacin and supplemented with 25 ppm silver water at a dose of 2 ml; group T3 induced with 15 mg/kg BW indomethacin and supplemented with 25 ppm silver water at a dose of 3 ml.

Based on Table 5.1, the expression of NF-κB, erosion of the mucosal epithelium, and infiltration of inflammatory cells in the Gastric organ of IBD model rats showed significant differences between the groups. The C+ group exhibited the highest expression of NF-κB, the most critical erosion of the gastric mucosal epithelium, and the most extensive infiltration of inflammatory cells in the gastric mucosa. Increasing the dosage of silver water treatment reduced NF-κB expression, mucosal epithelial erosion, and inflammatory cell infiltration, with results approaching normal values (C-).

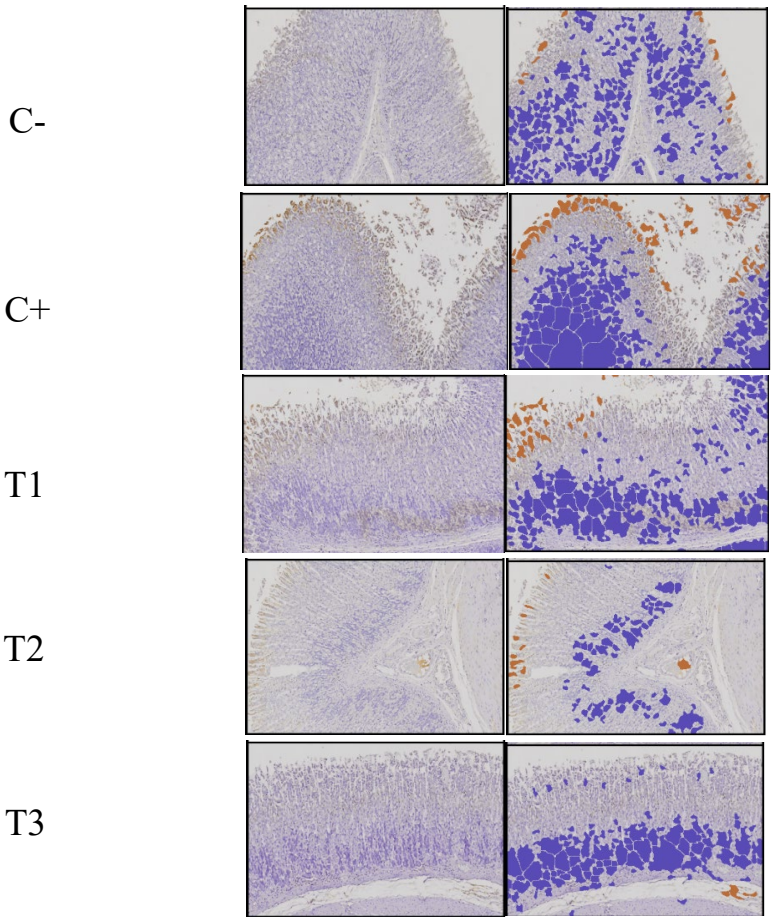

Figure 1 – Expression of NF-κB in the Gastric organs of IBD model rats. NF-κB expression is marked by brown coloration. Group C- received no treatment (control); Group C+ was induced with 15 mg/kg BW indomethacin; group T1 was induced with 15 mg/kg BW indomethacin and supplemented with 25 ppm silver water at a dose of 1 ml; group T2 was induced with 15 mg/kg BW indomethacin and supplemented with 25 ppm silver water at a dose of 2 ml; and group T3 was induced with 15 mg/kg BW indomethacin and supplemented with 25 ppm silver water at a dose of 3 ml.

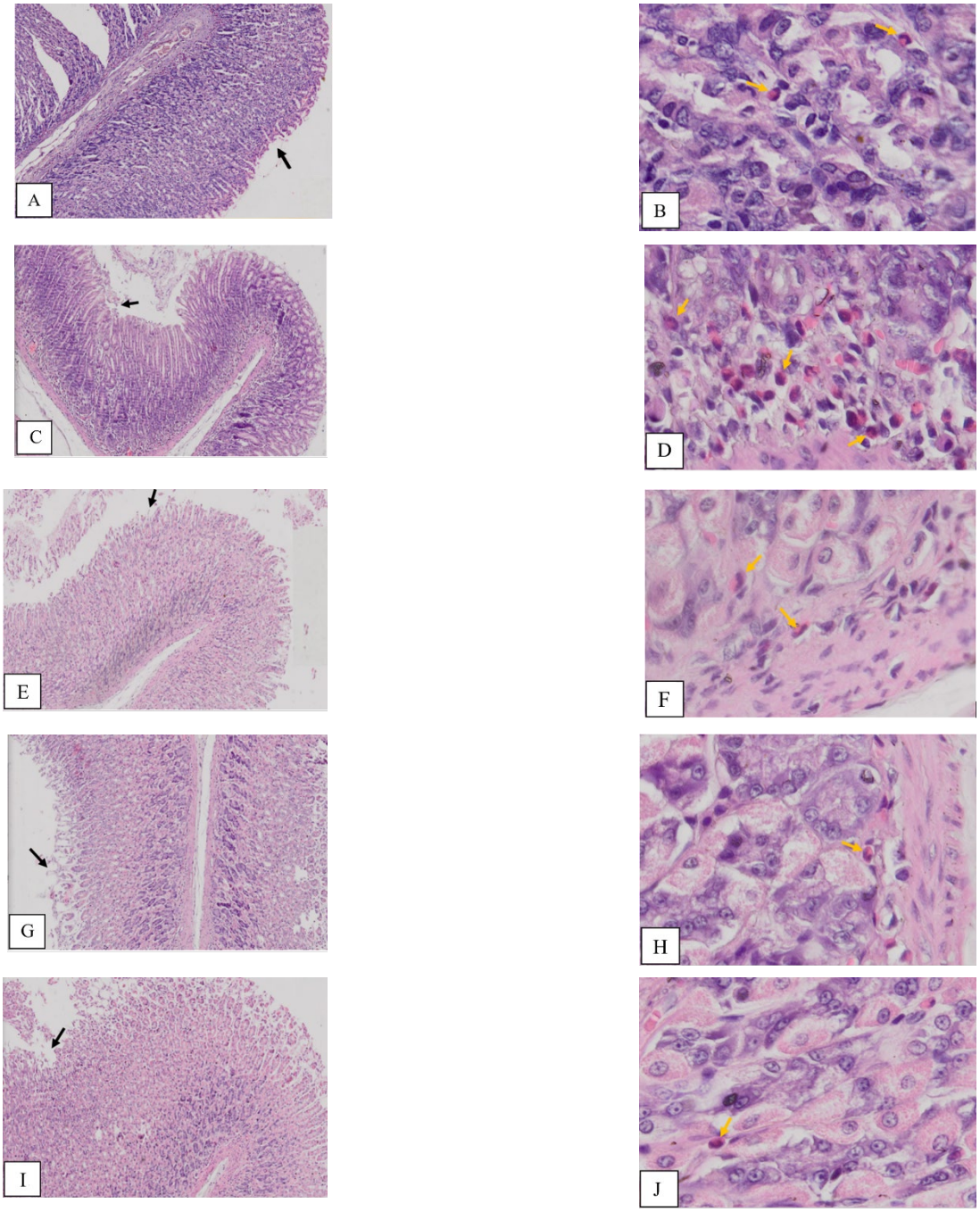

Figure 2 – Histology of Rat Gastric model IBD given silver water. A and B: Gastric histology in group C- which received no treatment (control). Erosion of gastric mucosal epithelium (black arrow), infiltration of inflammatory cells (yellow arrow); (C) and (D): Gastric histology in group C+ induced with 15 mg/kg BW indomethacin. Erosion of gastric mucosal epithelium (black arrow), infiltration of inflammatory cells (yellow arrow); (E) and (F): Gastric histology in group T1 induced with 15 mg/kg BW indomethacin and supplemented with 25 ppm silver water at a dose of 1 ml. Erosion of gastric mucosal epithelium (black arrow), infiltration of inflammatory cells (yellow arrow); (G) and (H): Gastric histology in group T2 induced with 15 mg/kg BW indomethacin and supplemented with 25 ppm silver water at a dose of 2 ml. Erosion of gastric mucosal epithelium (black arrow), infiltration of inflammatory cells (yellow arrow); (I) and (J): Gastric histology in group T3 induced with 15 mg/kg BW indomethacin and supplemented with 25 ppm silver water at a dose of 3 ml. Erosion of gastric mucosal epithelium (black arrow), infiltration of inflammatory cells (yellow arrow).

4. Discussion

NF-κB Expression

Usually, Nuclear Factor-kappa β (NF-κB) exists in the body in an inactive state, bound to Inhibitor kappa B (IkB) (Gu et al., 2018). Physiologically, NF-κB is produced in response to stimuli, such as oxidative stress (Mitchell and Carmody, 2018). NF-κB functions to induce cell defense, proliferation, immune system regulation, inflammatory responses, and apoptosis under certain

conditions (Lingappan, 2018). Therefore, NF-κB remains expressed under normal physiological conditions, as shown in the untreated group.

NF-κB expression in the Gastric organs of IBD model rats was highest in the group treated only with indomethacin, compared with the other treatment groups. This is consistent with the study by Bures et al. (2011), which found that administering indomethacin at 15 mg/kg BW caused tissue damage characterized by inflammation, irritation, and gastric mucosal injury. This leads to NF-κB activation, which serves as an inflammation regulator and triggers the production of proinflammatory cytokines. The increased NF-κB expression suggests that indomethacin may inhibit COX1 and COX2, thereby reducing prostaglandin (PGE2) production in the gastric mucosa (Cao et al., 2018).

Decreased PGE2 production prevents binding to G protein receptors at the intramembrane phospholipid, thereby inhibiting adenylate cyclase activity (Cheng et al., 2021). This inhibition disrupts the permeability of the parietal cell membrane, allowing H+ ions to exit into the Gastric lumen, making the cells more acidic (Okamoto et al., 2018). These ions then bind to Cl–, reducing trans-epithelial resistance and leading to damage to the gastric mucosa. This mucosal damage triggers an inflammatory reaction (Galura et al., 2019). When the gastric mucosa becomes inflamed, leukocytes adhere to the surface of postcapillary venous endothelial cells, with macrophages and polymorphonuclear cells migrating (Siczek et al., 2017).

Migrating macrophages release proinflammatory cytokines and attract neutrophils to the damaged tissue. Neutrophils then release proteases, causing oxidative stress (Delgado-Rizo et al., 2017). Indomethacin induction in experimental animals can increase ROS activity (Turkyilmaz et al., 2019). Excessive ROS production activates the NF-κB transcription factor in the nucleus and phosphorylates IkB (adding phosphate to Inhibitor kappa B). The NF-κB–IκB bond then breaks, freeing the NF-κB heterodimer (p50 and p65) (Andrade et al., 2022). This release automatically causes NF-κB to translocate into the nucleus. NF-κB activation induces the transcription of inflammatory genes, including proinflammatory cytokines (Wang et al., 2017).

The decreased NF-κB expression after administering silver water at increasing doses, compared with inducing inflammation with indometacin alone, indicates that silver water reduces inflammation in IBD. Silver water has potential as an anti-inflammatory by suppressing pro-inflammatory cytokines (Sousa et al., 2022). Anti-inflammatory agents are defined as drugs that reduce or inhibit the inflammatory process (Nunes et al., 2020). Inhibition of inflammation decreases ROS levels in inflamed cells, directly reducing damage to gastric mucosal epithelial cell membranes, infiltration of inflammatory cells, and mitochondrial dysfunction, thereby reducing apoptosis (Gu et al., 2018).

According to research by Nadworny et al. (2010), silver water has an anti-inflammatory role by inhibiting the production of inflammatory mediators via the ROS pathway, thereby reducing ROS levels in tissues. Lower ROS levels can no longer activate the NF-κB pathway, leading to reduced NF-κB activation due to inhibition of IkB degradation. As a result, the NF-κB dimer (p50 and p65) remains bound, unable to signal the nucleus to translate proinflammatory cytokines (Singh & Singh, 2020). Silver water nanoparticles then suppress proinflammatory cytokine activity by releasing signals that induce apoptosis in inflammatory cells in the tissue. Consequently, the infiltration of inflammatory cells from blood vessels into the tissue is reduced (Salama et al., 2023).

Histopathology of Gastric

Histopathology of the rat gastric mucosa in the untreated group shows a normal state, with no erosion observed on the surface epithelium, submucosa, or muscularis mucosa. The gastric mucosal layer consists of epithelium, lamina propria, and muscularis mucosa (Chandan, 2019). The luminal surface of the mucosal layer is covered by simple columnar epithelium (single-layered cylindrical epithelium), which extends into and lines the gastric foveola (invaginations of the epithelia). At the bottom of the epithelial layer lies a layer of loose connective tissue known as lamina propria, which fills the spaces between gastric glands. The outer layer of the mucosa is bounded by a thin layer of smooth muscle known as the muscularis mucosa. Bundles of smooth muscle and the muscularis mucosa extend and protrude into the lamina propria, leading towards the epithelial layer (Wen et al., 2016).

The histopathological appearance of the Gastric organ in the group treated with 15 mg/kg BW indomethacin shows damage to the gastric mucosal layer. This damage includes erosion of the gastric mucosa's surface epithelium and infiltration of inflammatory cells. Liu et al. (2017) stated that administering indomethacin at a dose of 15 mg/kg body weight (BW) orally in rats can cause irritation and damage to the mucosal layer of the Gastric organ. This damage can manifest as epithelial erosion and infiltration of blood vessels. Epithelial erosion is indicated by black arrows, and infiltration of inflammatory cells such as neutrophils is indicated by yellow arrows. Neutrophils are polymorphonuclear leukocytes with segmented nuclei (2-5), act as active phagocytes against bacteria, and are the first leukocytes to be activated when damage or infection occurs in the area (Bastaki et al., 2016).

The mechanism of action of indomethacin involves inhibition of cyclooxygenase isoforms. Cyclooxygenase produces prostaglandins in the gastric mucosa, which function as a form of cytoprotection (Głowacka et al., 2023). Inhibiting cyclooxygenase leads to decreased gastric mucosal protection, resulting in reduced mucus production and increased gastric HCl production (Takeuchi & Amagase, 2018), thereby causing gastric mucosal damage due to the acidic environment in the stomach along with a decrease in the gastric mucosal barrier, leading to inflammation (Ghattamaneni et al., 2019). The inflammatory response triggered by indomethacin-induced inflammation in the body can damage gastric mucosal epithelial cells, activate macrophages to phagocytose these cells, and release ROS such as ROO-, H2O2, O2·, and OH· (Dang et al., 2015). An imbalance between free radicals and antioxidants leads to the release of reactive oxygen species, resulting in oxidative stress and activation of the NF-κB pathway (Chaudhary et al., 2023). Activation of NF-κB can initiate neutrophil migration from blood vessels into inflamed tissues (Sokolova and Naumann, 2017). Damage to the gastric mucosal epithelial membrane in the inflamed area leads to increased free radicals (O'Reilly et al., 2018).

Histopathology of the Gastric organ in the treatment group induced with 15 mg/kgBW indomethacin and given graded therapy with 25 ppm silver water at doses of 1 ml, 2 ml, and 3 ml, shows tissue repair in the gastric mucosal layer. This includes the repair of damaged gastric mucosal epithelium, which shows erosion and decreased infiltration of inflammatory cells with increasing doses of silver water. Administration of 3 mL of 25 ppm silver water therapy is the best therapy in reducing histopathological damage in the white rat model of IBD. Silver ions have strong anti-inflammatory properties. When silver water enters the stomach, silver ions interact with the mucosal epithelial cell surface (Medici et al., 2019). Silver ions can modulate the local immune response by suppressing the release of pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6 from activated immune cells in the eroded area (Rao et al., 2018). This helps reduce the infiltration of inflammatory cells into the damaged gastric tissue.

At the cellular level, silver water can stimulate the proliferation and migration of healthy mucosal epithelial cells in the area surrounding the erosion. Silver ions are believed to activate cellular signaling pathways involved in tissue repair, including the Epidermal Growth Factor Receptor (EGFR) and Mitogen-Activated Protein Kinase (MAPK) pathways (Fuster et al., 2020). Activation of these pathways increases the production of growth factors and extracellular matrix proteins, which are essential for epithelial regeneration (Hassanaein et al., 2021). Silver water can also increase mucus production by goblet cells in the gastric mucosa. This mucus forms a protective layer that helps shield the newly formed epithelium from gastric acid and digestive enzymes, allowing sufficient time for healing (Bi et al., 2020).

Furthermore, the antioxidant properties of silver ions help neutralize free radicals produced during the inflammatory process, reducing oxidative stress on the mucosal epithelial cells (Morozova, 2021). This prevents further DNA and cell membrane damage, supporting the survival of healthy epithelial cells. Through its combination of anti-inflammatory, pro-regenerative, and antioxidant effects, silver water synergistically accelerates the repair of mucosal epithelial erosion. It reduces inflammatory cell infiltration, thereby helping restore the gastric histopathology of Wistar rats induced by indomethacin.

5. Conclusion

Silver water at 25 ppm demonstrated significant therapeutic potential in ameliorating indomethacin-induced Inflammatory Bowel Disease (IBD) in rats, with effectiveness increasing dose-dependently. Administration of 3 ml silver water substantially reduced NF-κB expression, mitigated gastric mucosal epithelial erosion, and decreased inflammatory cell infiltration compared to untreated controls. The therapeutic mechanism involves suppression of proinflammatory cytokines, reduction of reactive oxygen species, inhibition of NF-κB signaling, stimulation of epithelial regeneration, enhancement of protective mucus production, and neutralization of inflammatory free radicals. These findings suggest silver water represents a promising novel therapeutic approach for IBD management, offering anti-inflammatory, antioxidant, and tissue-regenerative properties that effectively counteract pathophysiological changes induced by COX inhibition.

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