Evaluation of the Anti-ulcer Activity of Curcumin and Linseed Oil in an NSAID-induced Gastric Ulcer Model in Rats

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ABSTRACT
Background: Peptic Ulcer Disease is characterized by acid-induced ulcers in the stomach and duodenum. They are caused by both a decrease in mucosal defenses and a drop in the pH of gastric juice.1 Helicobacter pylori (H. pylori) infection, overuse of non-steroidal anti-inflammatory drugs (NSAIDs).2 external factors such as smoking and alcohol consumption,3,4 stress, and panic attacks5,6 are assumed to increase the incidence of peptic ulcer disease (PUD). Patients with neurosis and patients on polypharmacy also are prone to PUD.7 Conventional medicines, currently available for the treatment of ulcers exhibit various side effects depicted in (Figure 1). Herbal medicines are increasingly employed to address digestive issues as part of Complementary and Alternative Medicine (CAM) in developed as well as developing countries. In addition to being one of the most alluring sources of innovative pharmaceuticals, medicinal/herbal plants and their extracts also have the benefit of being inexpensive. Natural substances from therapeutic herbs include flavonoids, polyphenols, terpenoids, saponins, alkaloids, and mucilaginous polysaccharides, which have anti-inflammatory, antibacterial, antiulcer, antioxidant, and anticancer potential.7 Curcumin is a chemical compound derived from the roots of Zingiberaceae and Araceae plants. Curcumin has extensive pharmacological actions, low side effects, easy to procure, and is relatively inexpensive. It has been proven to be effective against H. pylori in vitro.8,9 Flaxseed, is obtained from Linum usitatissimum, (family Linaceae). Mucilage, soluble and insoluble fibers, protein, 30-40% oil, and alpha-linolenic acid are valuable constituents of flax seeds. The oil and mucilage from flaxseed have gastroprotective properties against gastric ulcers.10,11 Long-term use of aspirin reduces Prostaglandins (PG) levels, due to which the protective mechanism of the gastric layer gets affected and results in sore formation and cytokine infiltration at the site of damage along with the inflammation. Curcumin is well known for its anti-inflammatory and ulcer-protective activity whereas the oil of flaxseed has antihistaminic and anticholinergic properties.

INTRODUCTION
Peptic ulcers are acid-induced ulcers that can form in the stomach and duodenum. They are caused by both a decrease in mucosal defenses and a drop in the pH of gastric juice.1 Helicobacter pylori (H. pylori) infection, overuse of non-steroidal anti-inflammatory drugs (NSAIDs).2 external factors such as smoking and alcohol consumption,3,4 stress, and panic attacks5,6 are assumed to increase the incidence of peptic ulcer disease (PUD). Patients with neurosis and patients on polypharmacy also are prone to PUD.7 Conventional medicines, currently available for the treatment of ulcers exhibit various side effects depicted in (Figure 1). Herbal medicines are increasingly employed to address digestive issues as part of Complementary and Alternative Medicine (CAM) in developed as well as developing countries. In addition to being one of the most alluring sources of innovative pharmaceuticals, medicinal/herbal plants and their extracts also have the benefit of being inexpensive. Natural substances from therapeutic herbs include flavonoids, polyphenols, terpenoids, saponins, alkaloids, and mucilaginous polysaccharides, which have anti-inflammatory, antibacterial, antiulcer, antioxidant, and anticancer potential.7 Curcumin is a chemical compound derived from the roots of Zingiberaceae and Araceae plants. Curcumin has extensive pharmacological actions, low side effects, easy to procure, and is relatively inexpensive. It has been proven to be effective against H. pylori in vitro.8,9 Flaxseed, is obtained from Linum usitatissimum, (family Linaceae). Mucilage, soluble and insoluble fibers, protein, 30-40% oil, and alpha-linolenic acid are valuable constituents of flax seeds. The oil and mucilage from flaxseed have gastroprotective properties against gastric ulcers.10,11 Long-term use of aspirin reduces Prostaglandins (PG) levels, due to which the protective mechanism of the gastric layer gets affected and results in sore formation and cytokine infiltration at the site of damage along with the inflammation. Curcumin is well known for its anti-inflammatory and ulcer-protective activity whereas the oil of flaxseed has antihistaminic and anticholinergic properties.
The rationale of the current work (Figure 2) was to explore the possibility of synergistic effects of curcumin and oil of flaxseed.

**MATERIALS AND METHODS**

The Ranitidine hydrochloride (purity \( \geq 99.89\% \)) was procured from Zeta Scientific (Mumbai-India). The inducer Aspirin (purity \( \geq 99.82\% \)) and curcumin (purity \( \geq 95.12\% \)) were obtained from Alta Laboratories Ltd (Mumbai-India), and Sunpure Extracts Private Limited (Delhi-India) respectively. Edible-grade flaxseed oil was procured from BO International (Delhi-India). The chemicals used in studies were of standard grade. ELISA kits were purchased from Krishgen Biosystems, India.

**Animals and Experimental Design**

National Institute of Biosciences, (Pune-India) provided Male Wistar rats (150-200 g) for studies. 12:12 hr light/dark cycle at a temperature of 25ºC and relative humidity of 50-55% were maintained for rats in plastic Perspex cage. Water that had been filtered and chow pellets were readily available. Prior to the start of the experiments, rats were given a week to acclimate. The protocol was approved (CPCSEA/IAEC/BNC/46/2021). A total of 48 rats were segregated into 8 groups as follows:

- **Group I-Control group.**
- **Group II-Vehicle group (corn oil).**
- **Group III-[Aspirin (200 mg/kg)]-Negative control.**
- **Group IV-[Ranitidine (50 mg/kg)]-Standard group.**
- **Group V-Curcumin (40 mg/kg).**
- **Group VI-Flaxseed oil (5 mL/kg).**
- **Group VII Curcumin (20 mg/kg)+Flaxseed oil (2.5 mL/kg).**
- **Group VIII Curcumin (40 mg/kg)+Flaxseed oil (5 mL/kg).**

Group, I was normal control group while Group II received only corn oil for 10 days. Starting from day 1, Aspirin (200 mg/kg) suspended in CMC was given in groups III, IV, V, VI, VII, and VIII for 7 days. Curcumin homogeneously suspended in corn oil was given for 10 days in groups V, VII, and VIII respectively. Flaxseed oil was given for 10 days in groups VI, VII, and VIII respectively. In each group, after the aspirin administration animals fasted for 4-6 hr and the interval between each dose administered was 30 min. On day 11, animals were sacrificed, 24 hr after the last dose of the drug, their stomach was taken out and opened along the greater curvature and gastric juice was collected for measurement of gastric secretion parameters; whereas mucosal content was used for the determination of macroscopic ulcer scoring, ulcer index, and percentage ulcer inhibition. Each animal’s blood was collected into separate Eppendorf for hematology. Part of each rat’s stomach was fixed in 10% formalin for histopathological studies, while the other stomach tissues were used for histamine assay and determination of MDA and cytokines levels.

**Evaluation Parameters**

Each rat’s body weight was recorded on 0, 3rd, 7th, and 11th day. Evaluation of ulcer index and percentage ulcer inhibition was done as follows:

The total sum of ulcer scores assigned to gastric lesions was used to calculate the Mean Ulcer Index (MUI). Using the formula, the percentage of ulcer inhibition was calculated:

\[
\text{Inhibition} = \left( \frac{\text{MUI}_{\text{control}} - \text{MUI}_{\text{test}}}{\text{MUI}_{\text{control}}} \right) \times 100\% 
\]

Lastly, for the measurement of gastric juice content; rats were sacrificed, their stomach was removed and the gastric contents were collected. A cylinder was used to quantify the gastric volume and the contents were centrifuged at 1000 rpm for 10 min and the total volume was recorded. The pH meter was used to analyze the pH of gastric content after diluting it with 1 mL of distilled water. Free acidity and total acidity were estimated using the collected supernatant of gastric content after centrifugation. Using Topfer’s reagent and phenolphthalein as indicators, titration of collected gastric juice with 0.01 N NaOH (filled in burette) were used to calculate free and total acidity respectively. The gastric content was collected and poured into a beaker containing 50 mL of distilled water. 25 mL of gastric juice was pipetted out of this solution and two drops of Topfer’s reagent are added to estimate free acidity. Furthermore, gastric content was titrated till yellow coloration was observed. The total amount of NaOH used was recorded, and the free acidity was calculated. Similarly, two drops, of phenolphthalein were added to another 25 mL gastric juice to estimate total acidity.

![Figure 1: Graphical abstract.](image-url)
Hematological indices

Normal hematological measurements are very crucial factors of good health. Aspirin has been linked to digestive tract abrasion and occult hemorrhage; furthermore, it has been observed to decrease iron uptake from the stomach, resulting in iron-deficiency anemia. The HCT, Hb, RBC, MCV, MCHC, WBC, and PL counts were measured using a (Nihon Kohden) autoanalyzer.\textsuperscript{12}

Histamine content

Shore. et.al method was used to determine histamine content. Tissue was minced and homogenized in perchloric acid and centrifuged at 3000 rpm for 10 min then the supernatant was collected. The supernatant was mixed with n-butanol followed by the addition of HCl and n-heptane mixing along with vigorous shaking and centrifugation. The heptane layer was removed, and in an acidic layer o-phthal-aldehyde was added to produce a highly luminescent product that was measured with a spectrofluorimeter (450 nm emission and 360 nm activation).\textsuperscript{13}

Biochemical parameters

The stomach of rats was homogenized in a 50 mM Tris-HCl buffer (pH 7.4) and then cold centrifuged at 10,000 rpm for 15 min. The reduced glutathione (GSH), and Malondialdehde (MDA) were determined using collected supernatant. 0.5 mL (10 mM) Ellman’s reagent was added to 1 mL supernatant followed by the addition of 2 mL phosphate buffer which gives a yellow solution. Similarly, a set of standards and blank was read at 412 nm.\textsuperscript{14} Ohkawa et al. described a method for measuring lipid peroxidation as MDA. Each 0.5 mL supernatant received 0.5 mL phosphate buffer (0.1 M, pH 8.0) and 0.5 mL 24% TCA. The resulting mixture was incubated for 10 min at room temperature before being centrifuged at 2000 rpm for 20 min. An aliquot of 1 mL of the resulting supernatant was mixed with 0.25 mL of 0.33% TBA in 20% acetic acid and boiled for 1 hr at 95ºC. After cooling, the pink-colored compound was measured for absorbance at 532 nm.\textsuperscript{15}

Measurement of IL-1β- Interleukin (1β) and Tumor Necrosis Factor-alpha (TNF-α) levels

The amounts of IL-1β and TNF-a levels in stomach homogenate were determined using ELISA kits (Krishgen Biosystems). All dilution and sample preparation procedures were performed as per the standard technique described in the datasheet for the ELISA kits. The absorbance was measured at 450 nm using an Epoch-2 microplate reader (BioTek, Germany). The sandwich ELISA technique was used in this method.\textsuperscript{16,17} Before homogenization, stomach samples were washed in buffered saline (PBS) and preserved at -80ºC. Tissues were finely chopped and homogenized in PBS (pH 7.4) using an ice-cold glass homogenizer. They were thawed at 2-8ºC followed by centrifugation at 2000-3000 rpm for 20 min.

Histopathology and Macroscopic examination of ulcers

To examine the gastric ulcers macroscopically, the stomach’s gastric mucosal layer was magnified ten times using a magnifying lens. The Kulkarni method for ulcer scoring was used to score and count the ulcerated areas.\textsuperscript{18} For histological examination, the stomach was dissected, quickly cleansed with 0.9% w/V

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**Figure 2:** Pathophysiology of Peptic Ulcer Disease along with its conventional drug approach.
saline, preserved in neutral formalin (10%), and stained with Haematoxylin and Eosin (H & E).

**Statistical analysis**

The statistical analysis was done using GraphPad Prism 8.4.2 for 64-bit Windows. To determine statistical significance, study groups were compared using one-way ANOVA (Analysis of Variance) and Tukey’s multiple comparisons tests.

**RESULTS**

**Evaluation of body weight**

The negative control group showed markedly decreased body weight as compared to control group. Days 0 and 3 showed no significance with any of the study groups. Day 7 showed a decrease in body weight of the negative control group when compared with the control group. On day 11, before sacrifice; combination [curcumin (20 mg/Kg) and flaxseed oil (2.5 mL/Kg)] when compared with the negative control group showed significance (Figure 3).

**Evaluation of ulcer index and percentage ulcer inhibition**

Oral administration of a combination drug for 10 days showed an ulcer-protective effect against aspirin-induced gastric ulcers when compared with the normal control group. Combination and ranitidine pre-treated groups exhibited a significantly decrease in ulcer index than the negative control group. Ranitidine (50 mg/Kg) exhibited the highest inhibition followed by a combination dose, flaxseed oil (5 mL/Kg), and curcumin 40 mg/Kg depicted in (Table 1).

**Measurement of gastric juice content**

The estimation of gastric volume, pH, total, and free acidity is represented in (Figure 4). When compared to the normal control group, the negative control group showed rise in gastric volume content with pH being decreased. Combination pre-treated groups exhibited significantly decreased gastric volume and increased pH than the negative control group. Moreover, in comparison to the normal control group, negative group had significantly higher total acidity and free acidity. Combination

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**Table 1: Effect of different groups on ulcer index and percentage ulcer.**

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Ulcer Index</th>
<th>Percentage ulcer inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>0.00±0.00</td>
<td>100%</td>
</tr>
<tr>
<td>Vehicle control (Corn oil)</td>
<td>0.00±0.00</td>
<td>100%</td>
</tr>
<tr>
<td>Negative control</td>
<td>25.16±3.31***</td>
<td>-</td>
</tr>
<tr>
<td>Standard</td>
<td>4.5±0.23***</td>
<td>46.35%</td>
</tr>
<tr>
<td>Curcumin</td>
<td>16.25±0.83#</td>
<td>35.43%</td>
</tr>
<tr>
<td>Flaxseed oil</td>
<td>15.83±1.19**</td>
<td>37.08%</td>
</tr>
<tr>
<td>Combination [curcumin (20 mg/Kg) flaxseed oil (2.5 mL/Kg)]</td>
<td>14.83±1.38##</td>
<td>41.05%</td>
</tr>
<tr>
<td>Combination [curcumin (40 mg/Kg) flaxseed oil (5 mL/Kg)]</td>
<td>15.41±0.77##</td>
<td>38.74%</td>
</tr>
</tbody>
</table>

One-way ANOVA was used to determine statistical comparison, accompanied by Tukey’s multiple comparisons. When compared to the normal control group and the negative control group, the significant difference is shown by the notations *p 0.05, **p 0.01, ***p 0.001 and #p 0.05, ##p 0.01, ###p 0.001.

**Table 2: Effect of different groups on Malondialdehyde (MDA) and Glutathione (GSH) levels.**

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>GSH levels (nmol /g tissue)</th>
<th>MDA level (nmol/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>9.49±0.34</td>
<td>2.17±0.21</td>
</tr>
<tr>
<td>Vehicle</td>
<td>9.40±0.34</td>
<td>2.25±0.20</td>
</tr>
<tr>
<td>Negative control</td>
<td>4.74±0.20***</td>
<td>6.67±0.29***</td>
</tr>
<tr>
<td>Standard</td>
<td>9.34±0.23***</td>
<td>3.49±0.61**</td>
</tr>
<tr>
<td>Curcumin</td>
<td>8.88±0.30##</td>
<td>4.79±0.68</td>
</tr>
<tr>
<td>Flaxseed oil</td>
<td>8.73±0.43##</td>
<td>4.41±0.75</td>
</tr>
<tr>
<td>Combination [curcumin (20 mg/Kg) flaxseed oil (2.5 mL/Kg)]</td>
<td>9.14±0.28##</td>
<td>3.76±0.38##</td>
</tr>
<tr>
<td>Combination [curcumin (40 mg/Kg) flaxseed oil (5 mL/Kg)]</td>
<td>9.03±0.48##</td>
<td>3.87±0.67##</td>
</tr>
</tbody>
</table>

One-way ANOVA was used to determine statistical comparison, accompanied by Tukey’s multiple comparisons. When compared to the normal control group and the negative control group, the significant difference is shown by the notations *p 0.05, **p 0.01, ***p 0.001 and #p 0.05, ##p 0.01, ###p 0.001.
pre-treated groups exhibited decreased acidity than the negative control group.

**Effect of Combination on antioxidant enzyme and lipid peroxidation**

Tissue GSH levels in the negative group were found to be significantly lower than those in the normal control were significantly higher MDA levels. Combination dose pre-treated rats exhibited increased tissue GSH levels than the negative control group. Combination [Curcumin (20 mg/Kg) flaxseed oil (2.5 mL/Kg)] pre-treated rats and Combination [Curcumin (40 mg/Kg) flaxseed oil (5 mL/Kg)] pre-treated rats exhibited decreased MDA levels than the negative control group (Table 2).

**Measurement of tissue cytokine concentration**

The estimation of the IL-1β and TNF-α levels is represented in (Figure 5). For IL-1β, a significant relationship was observed with the negative control group for ranitidine (50 mg/Kg), combination [Curcumin (20 mg/Kg) flaxseed oil (2.5 mL/Kg)] and combination [Curcumin (40 mg/Kg) flaxseed oil (5 mL/Kg)]. For TNF-α, a significant relationship was observed with the negative control group for ranitidine and combination doses.

**Histamine content determination**

The estimation of Histamine content is represented in (Figure 6). A significant increase in histamine levels was observed in the negative control group. Combination [Curcumin (20 mg/Kg) flaxseed oil (2.5 mL/Kg)] pre-treated rats and Combination [Curcumin (40 mg/Kg) flaxseed oil (5 mL/Kg)] pre-treated rats exhibited decreased MDA levels than the negative control group (Table 2).
Figure 4: Effect of different study groups on gastric volume, pH, total and free acidity in aspirin-induced ulcerated rats. One-way ANOVA was used to determine statistical comparison, accompanied by Tukey's multiple comparisons. When compared to the normal control group and the negative control group, the significant difference is shown by the notations *p 0.05, **p 0.01, ***p 0.001 and #p 0.05, ##p 0.01, ###p 0.001.

Table 3: Effect of different groups in hematological parameters.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Normal control</th>
<th>Vehicle</th>
<th>Negative control</th>
<th>Standard</th>
<th>Curcumin (20 mg/Kg)</th>
<th>Flaxseed oil (2.5 mL/Kg)</th>
<th>Combination [curcumin (40 mg/Kg) flaxseed oil (5 mL/Kg)]</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC (×10^9/L)</td>
<td>4.35±0.11</td>
<td>4.40±0.06</td>
<td>7.50±0.18</td>
<td>5.86±0.29</td>
<td>6.86±0.10</td>
<td>6.50±0.18</td>
<td>6.30±0.18</td>
</tr>
<tr>
<td>RBC (×10^6/L)</td>
<td>7.49±0.11</td>
<td>7.46±0.12</td>
<td>5.55±0.13</td>
<td>7.26±0.09</td>
<td>6.16±0.19</td>
<td>6.77±0.20</td>
<td>7.05±0.01</td>
</tr>
<tr>
<td>HGB (g/dl)</td>
<td>14.7±0.09</td>
<td>14.36±0.17</td>
<td>11.66±0.27</td>
<td>13.93±0.16</td>
<td>12.20±0.33</td>
<td>12.03±0.18</td>
<td>13.13±0.22</td>
</tr>
<tr>
<td>HCT (%)</td>
<td>45.00±0.73</td>
<td>45.33±1.11</td>
<td>30.30±1.99</td>
<td>43.06±0.72</td>
<td>39.26±0.81</td>
<td>40.13±0.39</td>
<td>42.06±0.07</td>
</tr>
<tr>
<td>MCV (fL)</td>
<td>55.83±0.23</td>
<td>54.70±0.74</td>
<td>41.00±0.36</td>
<td>52.36±0.54</td>
<td>48.06±1.04</td>
<td>48.8±0.22</td>
<td>51.30±0.44</td>
</tr>
<tr>
<td>MCH (g/L)</td>
<td>18.5±0.15</td>
<td>18.33±0.18</td>
<td>13.83±0.38</td>
<td>18.10±0.03</td>
<td>18.36±0.05</td>
<td>18.36±0.61</td>
<td>18.23±0.18</td>
</tr>
<tr>
<td>MCHC (g/dl)</td>
<td>34.00±0.48</td>
<td>33.56±0.13</td>
<td>32.16±0.90</td>
<td>34.36±0.31</td>
<td>33.20±0.19</td>
<td>33.03±0.09</td>
<td>33.46±0.20</td>
</tr>
<tr>
<td>PLT (10^3/µL)</td>
<td>584±31.42</td>
<td>575±1.82</td>
<td>500±18.25</td>
<td>545±5.47</td>
<td>511±16.10</td>
<td>542±13.82</td>
<td>540±6.76</td>
</tr>
</tbody>
</table>
Kg) flaxseed oil (2.5 mL/Kg) and standard (ranitidine 50 mg/
Kg) pre-treated rats exhibited a significant reduction in histamine
levels than the negative control group. Combination [Curcumin
(40 mg/Kg) flaxseed oil (5 mL/Kg)] pre-treated rats also exhibited
a significant reduction.

**Hematological indices**
A significant decrease in RBCs and Hb was seen in the negative
control group when compared to the normal control group. Whereas,
significant rise in WBC count in the negative control
was noticed when compared with the normal control. An
insignificant change in the platelet count was observed (Table 3).

**Histopathological analysis**
No infiltration of inflammatory cells in mucosa and loss of
cellularity damage is seen in the normal control group and
lesions. Infiltration of inflammatory cells in mucosa and
moderate histopathological damage, as well as lesions, erosion,
arid hemorrhage, were noticed in the negative control group.
No infiltration of inflammatory cells in the mucosa and loss of
cellularity damage were observed in standard (Ranitidine 50
mg/Kg). On the contrary, combination half dose and full dose

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**Figure 5:** Represents the tissue cytokine levels of IL-1β and TNF-α levels of different study groups. One-way ANOVA was used to
determine statistical comparison, accompanied by Tukey’s multiple comparisons. When compared to the normal control group and
the negative control group, the significant difference is shown by the notations *p 0.05, **p 0.01, ***p 0.001 and #p 0.05, ##p 0.01, ###p
0.001.

**Figure 6:** Effect of combination on histamine content of different study
groups. One-way ANOVA was used to determine statistical comparison,
accompanied by Tukey’s multiple comparisons. When compared to the
normal control group and the negative control group, the significant
difference is shown by the notations *p 0.05, **p 0.01, ***p 0.001 and #p 0.05, ##p 0.01, ###p 0.001.
showed mild infiltration of inflammatory cells in the mucosa and slight invasion of inflammatory cells in the mucosa respectively. Histopathological analysis of the stomach of various groups is shown in (Figures 7 A and 7 B).

**DISCUSSION**

There is a growing emphasis on the systematic assessment of bioactive phytoconstituents from natural sources as an unconventional curative approach for the prevention and treatment of gastric ulcers. The current study used an aspirin-induced ulcer model to test the gastroprotective effect of a combination of curcumin and flaxseed oil. The results of the study revealed that when compared to the negative control group, the combination groups showed a significant decrease in acid output, total and free acidity but alleviated gastric juice volume, and followed by an increase in pH. Thus, combination groups reduced the gastric damaging effect of NSAIDs by safeguarding the epithelium, resulting in a remarkable reduction in ulcer index when compared to the negative control and treatment groups.

MDA, an oxidative stress metabolite, was used to determine the levels of lipid peroxidation in gastric tissue of rats. GSH, on the other hand, is an antioxidative defense system that can be used to scavenge excess free radicals of oxygen and allow them to maintain at normal physiological levels. Combination groups resulted in a significant decrease in lipid peroxidation and an increase in GSH when compared to the negative control group.

**Figure 7a:** Histopathological (100 X and 400 X) and macroscopic evaluation (10 X magnification) of the stomach (H & E staining) of different study groups. (A1,A2,A3): Normal control; (B1, B2, B3): Vehicle group; (C1, C2, C3): Negative control group; (D1, D2, D3). Yellow Arrow: Infiltration of inflammatory cells in mucosa and submucosa, Red Arrow: Muscle layer, Black arrow: Loss of cellularity due to damage to surface epithelial mucosal layer, Blue Arrow: Mucosa layer, Purple Arrow: Sub mucosal layer.
Thus, the combination groups provided greater gastric protection by lessening oxidative stress and obstructing histamine receptors when compared to individual drug treatment groups. Aspirin severely damages the gastric mucosa, resulting in increased neutrophil infiltration into the submucosa. Histopathological examination of the combination groups revealed that the gastric mucosa was preserved and leucocyte invasion into the submucosa was restrained. Leucocyte infiltration into the submucosa was inhibited, which suggests that the chosen combination may have anti-inflammatory qualities that could aid in preventing stomach ulcers. Aspirin causes significant decrease in RBCs and haemoglobin values and increased WBC count. Combination pre-treatment groups had higher RBC and haemoglobin counts than the negative control group. This study suggests that combination drugs have the potential to improve haemoglobin content by boosting RBC count thereby increasing iron absorption in the body and offering protection against NSAID-induced hepatotoxicity. It was observed that combination pre-treated groups showed decreased pro-inflammatory levels, viz IL-1 β, and TNF-α.

**Figure 7b:** Histopathological (100 X and 400 X) and macroscopic evaluation (10 X magnification) of the stomach (H & E staining) of various groups. Standard; (E1, E2, E3): Curcumin; (F1, F2, F3): Flaxseed oil; (G1, G2, G3): combination [curcumin (20 mg/Kg) flaxseed oil (2.5 mL/Kg)];(H1, H2, H3): combination [curcumin (40 mg/Kg) flaxseed oil (5 mL/Kg)]. Yellow Arrow: Infiltration of inflammatory cells in mucosa and submucosa, Red Arrow: Muscle layer, Black arrow: Loss of cellularity due to damage to surface epithelial mucosal layer, Blue Arrow: Mucosa layer, Purple Arrow: Sub mucosal layer.
CONCLUSION

The performed study shows that the selected combination of curcumin and flaxseed oil possesses ulcer protective and healing properties. Thus, the adverse effects of a synthetic conventional drug can be minimized by the usage of this natural drug combination. However, more pre-clinical and clinical research is needed to determine the detailed precise mechanism of action for establishing gastroprotective effect.

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CONFLICT OF INTEREST

The authors declare that there is no conflicts of interest.

ABBREVIATIONS

CAM: Complementary and alternative medicine; CMC: Carboxy Methyl Cellulose; ELISA: Enzyme-linked immunoassay; GSH: Glutathione; H. pylori: Helicobacter pylori; IL-1β: Interleukin 1β; IAEC: Institutional Animal Ethics Committee; NSAIDs: non-steroidal anti-inflammatory drugs; MDA: Malondialdehyde; PG: Prostaglandins; PUD: Peptic Ulcer Disease; TNF-α: Tumor Necrosis Factor-alpha.

SUMMARY

Dr Gaurav Doshi is working as Assistant Professor at SVKM’s Dr. Bhanuben Nanavati College of Pharmacy, Mumbai. He has 17 years of research experience. Alveera is M.Pham Research Scholar at VKM’s Dr. Bhanuben Nanavati College of Pharmacy, Mumbai.

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