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ORIGINAL ARTICLE

Role of Acacia Catechu in Regulating Connective Tissue Components during Experimental Gastric Ulcer Healing

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ABSTRACT

Aim: To determine the healing properties of different doses of Acacia catechu on histopathological changes induced by aspirin in the stomach of adult albino rats.
Methods: In an experimental study which was carried out at Postgraduate Medical Institute for a period of 21 days, forty-eight adult albino rats were included. The animals were divided into four groups by ballottement. Every group was further subdivided into three groups based on the day of sacrifice. BDH (British drug houses) provided Aspirin in powder form, and the bark of *Acacia catechu* was obtained from Government College University (Botany Department). The study was approved by institutional Ethical Review Committee.
Results: Increased number of fibroblasts were seen in the groups taking acacia catechu, signifying healing, compared to aspirin only group. After applying one-way ANOVA, a statistically significant difference was observed among the groups on day 7 and day 14 (P-value <0.01*). However, on day 3, an insignificant difference was seen among the groups (P-value = 0.97).
Conclusion: Acacia catechu has a protective role against aspirin-induced gastric injury, by inhibiting inflammation.
Keywords: Fibroblast, Acacia catechu, ulcer, wound healing.

INTRODUCTION

Gastric ulcer is a common gastrointestinal disorder owing to changes in dietary habits and stressful lifestyles.[1] Gastric ulcer develops due to the disproportion between the protective and destructive factors, which can lead to a breach in the lining epithelium of the stomach[2].

Doctors prescribe non-steroidal anti-inflammatory drugs such as aspirin (acetylsalicylic acid) due to their antipyretic, anti-inflammatory, analgesic, and anti-platelet properties. Aspirin inhibits both isoforms of Cyclooxygenase (COX-1 and COX-2), which prevents the synthesis of prostaglandins generated by COX-2 that mediate inflammation at sites of injury[3], However, there is also decreased formation of COX-1-derived prostaglandins involved in protecting the gastrointestinal mucosa, which leaves it vulnerable to injury due to decreased resistance to ulcer formation, and increased exposure to gastric acid secretions. Ischemia and reactive oxygen species (ROS) formation cause aspirin-induced tissue necrosis resulting in oxidative injury to mucosa[4]. Aspirin administration inhibits the maturation of granulation tissue and delays ulcer healing[5], hence, aspirin has an established role in gastric ulcer formation, being one of the most common causal agents of medication side-effects leading to hospitalization.

Acacia catechu is a plant with origins mainly lying in Pakistan, India, Thailand, and Bangladesh. Historically, herbal medicine practitioners used Acacia catechu due to its therapeutic role in asthma, bronchitis, and cough[6,7]. Its constituents include polyphenols, tannins, flavonoids, alkaloids, and carbohydrates[8].

Fibroblasts are cells of lamina propria which stimulate tissue repair by epithelial regeneration, collagen synthesis, and other components of the extracellular matrix that provide support to the epithelial cells[9]. An in-vitro study documented that acute hypoxia was a direct stimulus for the growth of the fibroblast population in rodent and human cell lines[10]. About two or three days after the injury, fibroblasts enter the damaged tissue and reside in the granulation tissue of the healing ulcer by the third day[9]. During the 3rd and 4th days post-injury, macrophages start to secrete cytokines and growth factors; this induces chemotaxis and migration of fibroblasts towards the site of injury[11].

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The current study was designed to discern the modulating effect of acacia catechu healing properties on gastric ulcers by observing the changes in the fibroblast population of lamina propria of gastric mucosa, and by measurement of collagen content during wound induction and healing.

MATERIALS AND METHODS

This experimental study was carried out at Postgraduate Medical Institute, Lahore, Pakistan. Adult Albino rats were used in the study. Sample size was calculated by the following formula:

$$n = \frac{(Z_{1-\beta} + Z_1 - {}^{\alpha}/_{2})^2\,(\sigma_1^2 + \sigma_2^2)}{(\mu_1 - \mu_2)^2}$$

Value of standard deviation was taken from a previous study[12].

Aspirin in powder form was obtained from BDH (British drug houses) Limited Poole England. The bark of *Acacia catechu* was obtained from the Botany Department of Government College University, Lahore, dried and saved according to protocol mentioned in a previous study[13]. We divided forty-eight albino rats into four groups by ballottement. Every group was subdivided into three groups based on the day of sacrifice; Group A (Control) was given 4ml of distilled water orally for 3, 7 and 14 days, respectively[14]. To induce gastric ulcer formation, 100mg/ kg body weight of aspirin, dissolved in 4ml of distilled water was administered orally to animals of group B for 3, 7 and 14 days, respectively[15]. To observe effects of administration of Acacia catechu, 100mg/kg body weight of aspirin, was given orally to rats of group C along with 250mg/kg body weight of *Acacia catechu*, dissolved in 4ml of distilled water for 3, 7 and 14 days, respectively. For group D, 100mg/kg body weight of aspirin plus 500mg/kg body weight of *Acacia catechu*, dissolved in 4ml of distilled water, was administered orally for 3, 7 and 14 days, respectively[16]. On the 3rd day of the experiment, all the animals of groups A1, B1, C1, and D1 were sacrificed two hours after doses were administered. Likewise, on the 7th day, rats of groups A2, B2, C2, and D2 were sacrificed, and animals of groups A3, B3, C3, and D3 were sacrificed on the 14th day of the experiment. The data obtained from all the groups were entered into and analyzed using SPSS 20. For quantitative variables (fibroblasts and collagen content in the mucosa and submucosa of the stomach), Mean±SD was calculated. One-way ANOVA was used to compare means of histological quantitative variables among the control and experimental groups. Post hoc Tukey test was applied for multiple comparisons. A P value of < 0.05 was considered statistically significant.

RESULTS

We observed the changes in the fibroblast count in the mucosa and submucosa of the ulcer base of the stomach. A statistically significant difference (P value < 0.01) was noted among the groups after applying one-way ANOVA.

On day 3, few fibroblasts were observed in the control subgroup A1 (16±3.16). The fibroblast count of subgroup B1 (15.75 ± 4.35) was less than that of subgroups C1 (33.75±3.5) and D1 (22.75 ± 2.75).

On day 7, a marked rise in fibroblast count was observed in subgroup B2 (41±2.99) as compared to subgroup A2 (15±2.45). However, the number in subgroups C2 (22.5±2.08) and D2 (16.5±2.38) remained close to that in the control group.

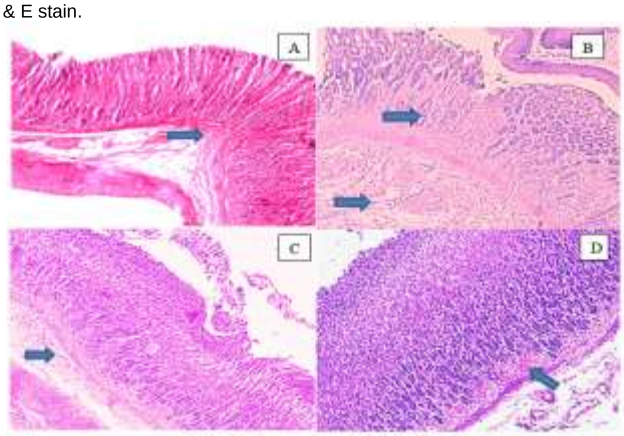
On day 14, comparatively fewer fibroblasts were seen in subgroup A3 (12.75±3.6). Subgroup B3 (43.25±2.36) manifested a marked increase in fibroblast count. The number of fibroblasts in subgroup group C3 (19.5±1.29) and subgroup D3 (11.25±0.96) remained closed to the control group.

Post hoc Tukey test revealed that the difference between subgroups C1 and D1 with subgroup A1 was statistically significant (P value < 0.01) on the third day. Differences between fibroblast count in subgroups B1 and C1 and subgroups C1 and D1 were statistically significant (P value < 0.01 for both comparisons).

On day 7, the group differences of subgroup B2 as compared to subgroups A2, C2, and D2 (P value < 0.01) were statistically significant. Difference between subgroup A2 (P value < 0.01) and subgroup C2 was significant (P value < 0.02). However, subgroups A2 and D2 did not show any significant difference (P value = 1.00). The variation in fibroblast numbers between subgroups C2 and D2 (P value = 0.15) was insignificant. Fibroblast count in subgroup B2 was significantly elevated on day 7 as compared to day 3 (P value < 0.01). However, fibroblast count in *Acacia catechu* treated subgroups C1 and D1 declined on day 7.

On day 14, the difference between subgroup B3 with subgroups A3, C3, and D3 (P < 0.01) was statistically significant. Mean fibroblast count in control and experimental groups is shown in figure 1.

Figure 1: Photomicrograph of histologic section of stomach on day-14 of group A, B, C and D, showing fibroblasts and collagen (blue arrows) X40, H & E stain.



Treatment with Masson's Trichrome stain showed that collagen appeared green in colour. Collagen was loosely distributed in between glands of mucosa while it was densely arranged in the submucosa of group A (control).

On day 3, there was no significant change in the collagen content of all the experimental subgroups (A=0.11±0.08, B=0.21±0.03, C= 0.15 ± 0.05, D= 0.13 ± 0.05, P value = 0.97).

However, **on 7th and 14th days,** the highest collagen content was observed in aspirin-treated subgroups B2 (0.31±0.06) and B3 (0.57±0.06). Lamina propria and submucosa showed increased deposition of connective tissue. The collagen content of subgroups C2 (0.14±0.04) and C3 (0.18±0.06) was less as compared to subgroups B2 and B3. The collagen content of subgroups D2 (0.09±0.02) and D3 (0.07± 0.07) remained close to control subgroups A2 (0.07±0.15) and A3 (0.09±0.03) (P <0.01). The collagen content of B2 was significantly higher than A2, C2, and D2. A2 had an insignificant difference with C2 and D2. Collagen content in group B3, receiving aspirin for 14 days, was observed to be significantly higher than in groups A3, C3, and D3. The difference between collagen content in C2 and D2 was also not significant. Collagen content in groups C3 and D3 remained insignificant with control group A3. The difference between subgroups C3 and D3 was statistically insignificant.

Fig. 2: Mean collagen content in control and experimental groups

DISCUSSION

In addition to secreting growth factors, fibroblasts also produce collagen, which is responsible for wound healing[17]. As early as 10 hours after injury, fibroblasts start to produce Type-III collagen and fibronectin[18]. Researchers in a study observed an increase in procollagen1 in the acetic acid-induced gastric ulcer model 3 days after ulcer induction. A study observed that the pro-collagen gene showed increased expression for up to 15 days and then the expression declined to the original levels by day 30[19], thus there is an attempt to restore connective tissue and epithelial components in this phase. Fibroblasts proliferate and hypertrophy in response to hypoxia[20] and produce collagen in the ulcer during the healing phase[21]. Simultaneously, epithelial cells, fibroblasts and other connective tissue cells begin to synthesize collagenases[9]. Initially, the collagen production exceeds degradation for the formation of the granulation tissue; after healing occurs, production and breakdown of collagen molecules become equal, and the maturation phase of the wound starts[21]. The number of fibroblasts decreases, and they start undergoing apoptosis while collagenases cleave the fibrillar collagens[9,21].

Researchers recommend incorporating Acacia catechu into traditional medicine for treating gastric ulcers[13]. Quercetin, catechin and rutin are the main components of *Acacia catechu*; catechins and flavonoids in Acacia catechu are responsible for the plant's antioxidant properties as these chemical constituents act as free radical scavengers[8]. Quercetin reduces the levels of pro-inflammatory markers and raises anti-inflammatory markers[4].

A previous study reported that the proliferation of fibroblasts lasted for a longer duration in animal models of acute hypoxia.[22] In the current study, an initial significantly higher fibroblast count in groups receiving aspirin and different doses of Acacia catechu indicated an early healing process. However, with an increase in the duration of exposure to aspirin in experimental rats, fibroblast count was significantly higher in the aspirin-only group compared to the aspirin + Acacia groups. Fibroblast count in both groups receiving aspirin plus Acacia catechu remained nearly similar to control group A3 indicating maximum ulcer healing in the Acacia-treated groups.

Pathogenesis of fibrosis is associated with decreased apoptosis of fibroblasts and fibro myoblasts[23]. Riddel et al. 1992 conducted a case-control study and documented the presence of a thickened sub-epithelial collagen plate in response to NSAIDs[24]. Schmassmann et al. documented that granulation tissue in the ulcer base matured into a thin scar within two weeks after the formation of the ulcer; however, continued treatment with NSAIDs led to thickened granulation tissue, indicating that NSAIDs inhibited maturation of the granulation tissue, and thus led to delayed healing of the ulcer[25]. This delayed ulcer healing was also observed in the current study that showed that the aspirin-only group had markedly increased fibroblast population and collagen content in an attempt to heal the ulcer.

On the other hand, groups receiving aspirin with Acacia catechu showed comparatively decreased collagen content with a mild rise in fibroblast population; in addition, the group receiving aspirin + acacia catechu for 14 consecutive days showed a collagen content and fibroblast population which was close to that seen in the control group. Hence, extended exposure to acacia led to a reduced number of fibroblasts and decreased collagen in lamina propria; this was probably because quercetin, one of the major chemical constituents of acacia catechu, inhibited the proliferation of cardiac fibroblasts and also decreased collagen synthesis[26]. Another study also documented the inhibitory action of quercetin on the biosynthesis of pro-collagen, the precursor collagen molecule in fibroblasts[27].

CONCLUSION

The major chemical constituents of ***Acacia catechu*** possess medicinal properties, and the protective effects of ***Acacia catechu*** can be attributed to the antioxidant properties of the plant.

Limitations: The current study was conducted for a short duration of two weeks only and thus provides some preliminary data on the antiulcer activity of *Acacia catechu* bark. Whether an aqueous solution of the bark of *Acacia catechu* will be an effective remedy for long term treatment with acetylsalicylic acid induced ulcers remains unclear. Further research, with extracts prepared with ethyl acetate, ethanol, and methanol, on gastric mucosa is recommended.

Recommendations: Future researches should focus on biochemical and genetic assays to assess whether there is reduced oxidative stress in Acacia catechu-treated animals and if these animals show more rapid healing due to a modified response due to up-regulation of genes regulating formation of anti-oxidants Although *Acacia catechu* has long been used in traditional medicine, further animals and human studies must be conducted to evaluate its safety and efficacy.

Declaration of Interest: The authors report no declarations of interest.

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