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Original Research Article

Anti-inflammatory activity of acacia catechu-bark aqueous solution in aspirin induced gastric ulcer in rodents

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ABSTRACT

Background: Aspirin is amongst the most widely used drugs and has many adverse effects on gastric mucosa. Anti-inflammatory properties of *Acacia catechu* have been established already. The objective was to evaluate the histopathological changes induced by aspirin in the stomach of albino rats and to assess the protective effect of different doses of *Acacia catechu*.
Methods: An experimental study was carried out in Postgraduate Medical Institute, Lahore for 21 days. Forty-eight adult albino rats, both males and females, were divided into four groups A, B, C and D randomly; each comprising of 12 rats. Group A was control, group B was given aspirin 100 mg/kg and group C and D were given aspirin 100 mg/kg along with *Acacia catechu* 250 mg/kg and 500 mg/kg respectively by oral route. The rats from individual group were sacrificed on 3rd day, 7th day and 14th day and stomachs were examined under light microscope to observe the inflammatory cells infiltration.
Results: Gross and microscopic findings on days 3, 7 and 14 were similar. Control groups A_1, A_2 and A_3 showed normal healthy gastric mucosa and the least number of inflammatory cells. In group B, aspirin produced ulcerations and linear breaks; with highest inflammatory infiltrates. On microscopic examination, numerous inflammatory cells were noted. Group C and D rats had minimum ulcer index and fewer inflammatory cells.
Conclusions: *Acacia catechu* has protective role against gastric injury by inhibiting inflammation.

Keywords: *Acacia catechu*, Anti-inflammatory, Rodents, Ulcer

INTRODUCTION

In this modern era of science, focus has been shifted towards the search of medicine from the natural sources assuming that they will have good safety profile and tolerability. Nowadays, people prefer using medicines made from the plant resources because they consider them safer than the commercial pharmaceutical drugs. Antioxidants are the drugs which decrease the oxidative stress thus reducing the oxidative damage to the body. Plants are a rich source of antioxidants.[1,2]

Acacia catechu is widely available throughout the Indian subcontinent. Its main constituents are catechins, epicatechins, epigallocatechin, gallate, rocatechin, phologlucin and protocatechuic acid. Catechin has high content of antioxidants and a high antioxidant and anti-inflammatory potential. These are used to treat many inflammatory conditions like boils, ulcer, skin lesions, pharyngitis, leucorrhea, erysipelas, spongy gums, diarrhea and hypertrophy of gums.[2-6]

The uses of *Acacia catechu* can be found in the history from the date betel leaves were used. In 15th century, it

was exported to Europe and in 17th century it was named as *Acacia catechu*. During the 19th century it was commercially used in France. It's of two types, dark and pale catechu, which were used commercially.[4]

Antioxidant potential was studied by Priyadarsini et al. They studied its antioxidant potential as inhibition of lipid peroxidation by radiation in the rat liver. This antioxidant potential is not unique to the acacia catechu and many other plants have been studied for this. *Acacia catechu* is one of them.[4-12] Studies have shown inverse relationship between disease and food intake rich in antioxidants. Though use of commercially prepared antioxidants has resulted in liver damage and even cancer, efforts are being made to look for antioxidants from natural sources like plants.[14,15]

The aim of this study was to look for anti-inflammatory potential of *Acacia catechu* in albino rats. We assessed this by measuring inflammatory infiltrate in the gastric ulcers induced by aspirin in rodents.

METHODS

This research was carried out at Postgraduate Medical Institute, Lahore after taking ethical permission from ethical committee. 48 male and female albino rats, weighing 150-250 gm were purchased from the University of Veterinary and Animal Sciences Lahore. All the animals were examined thoroughly and weighed before the commencement of experiment. The rats were housed in experimental research laboratory of Postgraduate Medical Institute, Lahore, under controlled conditions of temperature 22±0.5°C, humidity (50±10%). Aspirin in powder form was taken from BDH (British drug houses) Limited, Poole England. Bark of *Acacia catechu* was obtained from Botany Department of Government College University, Lahore.

Rats were randomly divided using balloting method into four groups, comprising of twelve animals each. The animals in each group were numbered, using colours on their tails and these identification points were checked each time before intervention. Group A served as a control and was further subdivided into three groups A_1, A_2 and A_3 which were given 4 ml of distilled water orally for 3, 7 and 14 days. Group B was further subdivided into three groups B_1, B_2 and B_3 which were given aspirin at a dose of 100 mg/kg body weight, dissolved in 4 ml of distilled water orally for 3, 7 and 14 days, respectively.[16]

Group C was further subdivided into three groups C_1, C_2 and C_3 which were given 100 mg/kg body weight of aspirin along with 250 mg/kg body weight of *Acacia catechu*, dissolved in 4 ml of distilled water orally for 3, 7 and 14 days.

Group D was further subdivided into three groups D_1, D_2 and D_3 which were given 100 mg/kg body weight of aspirin along with 500 mg/kg body weight of *Acacia catechu*, dissolved in 4 ml of distilled water orally for 3, 7 and 14 days.[17]

Animals of group A_1, B_1, C_1 and D_1 were sacrificed on day three, animals of group A_2, B_2, C_2 and D_2 were sacrificed on day 7, similarly animals of A_3, B_3, C_3 and D_3 were sacrificed on day 14.

Statistical analysis

The data was added and analysed using SPSS 20 (Statistical Package for Social Sciences). Mean±SD was calculated for inflammatory cells, in the mucosa and submucosa of stomachs. One way ANOVA was applied to compare means of inflammatory cells among the control and experimental groups. Post hoc Tukey test was used for multiple comparisons.

P value of ≤0.05 was considered as statistically significant.

RESULTS

Inflammatory cells were counted in lamina propria and submucosa of the stomach in the ulcer base.

On day 3, few inflammatory cells were seen in subgroup A_1. Highest number was seen in subgroup B_1. Subgroup C_1 had milder inflammation as compared to subgroup D_1.

On day 7 very few inflammatory cells were observed in control subgroup A_2. Marked increase in inflammatory cells was seen in subgroup B_2. An increased inflammatory response in subgroup B_2 indicated a continued process of tissue injury. Inflammatory cells in subgroup C_2 and subgroup D_2 were less as compared to subgroup B_2.

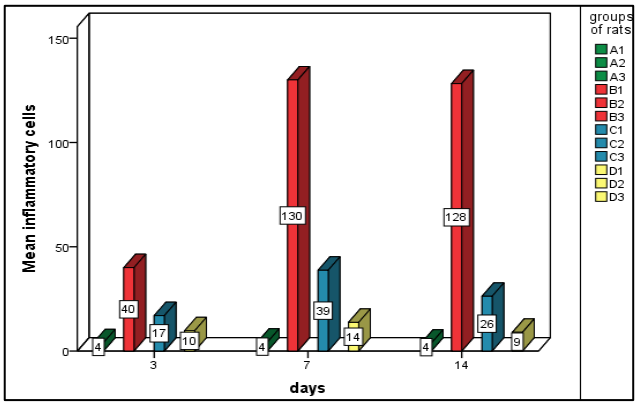

Figure 1: Bar chart showing comparison of number of inflammatory cells and comparison in different study groups at different days.

On day 14, very little number of inflammatory cells were observed in control subgroup A_3. Marked increase was seen in subgroup B_3. Inflammatory cells in subgroup C_3 and subgroup D_3 showed little increase as compared to

control group. *Acacia catechu* inhibited inflammatory cell infiltration hence inhibiting inflammation. A dose of 500 mg/kg however showed better results than a dose of 250 mg/kg.

Mean number of inflammatory cells in control and experimental groups is shown in Table 1 and Figure 1.

After applying post hoc Tukey test, it was seen that number of inflammatory cells was significantly less in subgroup A_1 as compared to subgroup B_1 (p=0.000) as well as subgroup C_1 (p=0.003) and subgroup D_1 (p<0.05). Statistically significant difference between inflammatory cell count in subgroup B_1 with subgroup C_1 and subgroup D_1 was noted (p=0.000). Difference between subgroup D_1 with subgroup A_1 (p=0.666) and subgroup C_1 (p=0.333) was also significant. (Figure 1, Table 2). Inflammatory cell count in B_2 was strikingly higher than subgroup A_2, subgroup C_2 and subgroup D_2 (p=0.000).

Statistically significant increase in inflammatory cells was also seen in subgroup C_2 as compared to subgroup A_2 and subgroup D_2 (0.000). The difference between inflammatory cell count in A subgroup 2 and subgroup D_2 was not statistically significant (p=0.067) (Table 2).

Statistically significant difference in subgroup B_3 as compared to subgroups A_3, C_3 and D_3 (p=0.000) was seen. Difference between inflammatory cells in subgroup A_3 and subgroup D_3 was (p=0.774) which was not statistically significant. The difference between inflammatory cell count in subgroups A_3, C_3 and D_3 was also statistically significant (p=0.000) (Table 2).

Table 1: Descriptive statistics of mean inflammatory cell count and comparison in different study groups.

No. of inflammatory cells	Days	Group A_1 Mean±SD (n=4)	Group B_1 Mean±SD (n=4)	Group C_1 Mean±SD (n=4)	Group D_1 Mean±S (n=4)	P value
	3rd day	4.0±0.82	40.0±4.08	17.0±2.45	9.75±3.86	<0.001*
	7th day	4.25±1.26	130.0±9.13	38.75±5.3	13.75±3.5	<0.001*
	14th day	3.75±0.96	128.25±3.95	26.25±1.89	9.00±2.58	<0.001*

*p value ≤0.05 is statistically significant.

Table 2: Inflammatory cells: multiple/paired wise comparison using post hoc Tukey test.

Time of sacrifice	(I) Study groups	(J) Study groups	Mean difference (I-J)	P value
3rd day	Group-A	Group-B	-36.000	0.000*
		Group-C	-13.000	0.000*
		Group-D	-5.7500	0.666
	Group-B	Group-C	23.000	0.000*
		Group-D	30.250	0.000*
	Group-C	Group-D	7.250	0.333
7th day	Group-A	Group-B	-125.750	0.000*
		Group-C	-34.500	0.000*
		Group-D	-9.500	0.067
	Group-B	Group-C	11.750	0.000*
		Group-D	13.625	0.000*
	Group-C	Group-D	25.000	0.000*
14th day	Group-A	Group-B	-124.500	0.000*
		Group-C	-22.500	0.000*
		Group-D	-5.250	0.774
	Group-B	Group-C	12.925	0.000*
		Group-D	14.950	0.000*
	Group-C	Group-D	17.250*	0.000

*p value ≤0.05 is statistically significant.

DISCUSSION

Significant increase in number of inflammatory cells in subgroups B_1, C_1 and D_1 as compared to subgroup A_1 was noticed. However, number of inflammatory cells in subgroups C_1 and D_1 was significantly less than subgroup B_1. In normal wound healing, platelets release various growth factors as well as proinflammatory cytokines like IL-1 that attract neutrophils to the wound site.[18] Neutrophils infiltrate the site of injury within an hour. Cellular debris is phagocytosed and bacteria are killed by free radicals generated by neutrophils.[19] Macrophages migrate to the site of injury at about 48 to 96 hours after tissue injury.[20] They phagocytose poly morphonuclear

leucocytes and become predominant cells in the wound by Day 2 after injury.[21] However abundant neutrophils along with macrophages were present in subgroup B_1 denoting ongoing inflammatory process. In a comparative study, effect of aspirin on pyloric ligated rat models of gastric ulcers was evaluated. Results showed ulcer crater containing distorted gastric glands, inflammatory exudates and cellular debris.[22]

Flavocoxid obtained from *Acacia catechu* decreases pro inflammatory cytokines as TNF-α, hence inhibiting ROS formation resulting in reduced leukocyte infiltration.[23]

Day 7

Significant increase in number of inflammatory cells in subgroup B_2 as compared to control group A_2 was noticed. Functional neutrophils have life-spans of around 2 days at the site of injury. After completing their task, they undergo apoptosis and get phagocytosed by the macrophages.[19] Macrophages are stimulated by hypoxia and induce angiogenesis in the granulation tissue.[24] As the inflammation resolves numbers of neutrophils and macrophages are reduced.[25] Number of inflammatory cells in subgroup B_2 on day 7 was significantly more as compared to subgroup B_1 on day 3. In a study, role of neutrophils in the pathogenesis of indomethacin induced ulceration was studied on gastric antrum of rat. Results revealed that there was a time dependent increase in the extent and severity of ulceration and neutrophil infiltration into the gastric antrum after treatment with indomethacin.[26] Flavocoxid containing catechin from *Acacia catechu* attenuates inflammation and neutrophil invasion in a carrageenan-induced paw edema model.[27] Number of inflammatory cells in subgroup B_2 was significantly greater than subgroup C1.Similarly subgroup D_1 showed significantly decrease in inflammatory cells as compared to subgroup C_1.

Day 14

Significant increase in number of inflammatory cells in subgroup B_3 as compared to control group A_3 was noticed. Inflammation lasts as long as there is debris in the wound. In an in vitro study on dermal equivalent model, it was found that that the presence of macrophages actually delays wound contraction. Hence the withdrawal of macrophages from the site of wound may be essential for subsequent healing.[28] Presence of macrophages in wound indicates ongoing leucocyte infiltration. Inflammation and neutrophil infiltration are vital in causing gastric damage induced by NSAIDs.[26] The inflammation results in increased TNF-α production, which enhances neutrophil-derived superoxide generation.[26] This stimulates IL-1 production resulting in neutrophil accumulation.[29] Inhibition of inflammation by *Acacia catechu* can be attributed to quercetin. In an experimental study it was found that quercetin had an inhibitory effect on the secretion of inflammatory factors TNF-α, IL-1β and IL-6 of cardiac fibroblasts hence inhibiting their inflammatory secretions.[30] Arjmandi et al, in 2014 conducted a 1-week clinical trial on osteoarthritic patients to examine the effectiveness of UP446 from *Acacia catechu* and *Scutellaria baicalensis* extracts (500 mg/day) to detect selected biomarkers of inflammation in comparison to naproxen (440 mg/day). Serum interleukins IL-1β, IL-6 and TNF-α were inhibited indicating a decrease in inflammation hence improving the symptoms of knee osteoarthritis.[31] Another component of *Acacia catechu* is tannin which is present abundantly in its bark. Anti-inflammatory effect of *Syzygium cumini* bark which is rich in tannins, was investigated in animal models of formaldehyde induced paw edema and cotton pellet granuloma in rats. The study proved that the extract has a potent anti-inflammatory action without any adverse effect on gastric mucosa.[32]

This was a short duration study, so its prolonged efficacy as antiulcer and anti-inflammatory effect is unclear.

CONCLUSION

In conclusion, the current study provides initial data on the inflammatory and antiulcer activity of *Acacia catechu* bark and justifies its uses. The main chemical constituents of *Acacia catechu* such as flavonoids, alkaloids and tannins have been shown to possess multiple medicinal properties. It is posited in this in this study that protective effects of *Acacia catechu* are due to its antioxidant properties.

Recommendations

Further research with extracts prepared with ethyl acetate, ethanol, and methanol on gastric mucosa should be evaluated. Future researches should focus on biochemical assays to show that there was reduced oxidative stress in *Acacia catechu* treated animals. Although *Acacia catechu* has been used for years, in traditional medicine there is still a need for well-controlled animals and human studies to evaluate its safety and efficacy.

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