Effect of *Acacia Catechu* on Aspirin Induced Gastric Ulcers in Albino Rats

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ABSTRACT

Background and Objectives*: Aspirin, one of the most widely used drugs, causes deleterious effects on gastric mucosa. Anti-inflammatory properties of Acacia catechu have already been established. This study is unique as it evaluated the histopathological changes induced by aspirin in the stomach of albino rats and assessed the protective effect of different doses of Acacia catechu.*

Methods: *Forty eight adult albino rats, both males and female, were divided randomly into four groups A, B, C and D; each comprising of 12 rats. Group A, (control) was given chow and water ad libitum. Group B was treated with aspirin 100 mg/kg. Group C and D were given aspirin 100 mg/kg along with Acacia catechu 250 mg/kg 500 mg/kg respectively by oral route. Half of the rats from individual group were sacrificed on 3rd day and the rest on 7th day. Stomach was examined for macroscopic (ulcer index) and microscopic (inflammatory cells) parameters.*

Results: *Gross and microscopic findings on days 3 and 7 were similar. Control groups A1 and A2 showed normal healthy gastric mucosa and the least number of inflammatory cells. In group B, aspirin produced ulcerations and linear breaks; with highest ulcer index. On microscopic examination, numerous inflammatory cells were noted. Group C and D rats had minimum ulcer index and fewer inflammatory cells.*

Conclusion: *Aqueous solution of Acacia catechu has protective role against gastric ulcers by decreasing ulcers, and inflammation.*

KEYWORDS: *Aspirin, Acacia catechu, Gastric mucosa, Non-Steroidal Anti-inflammatory Drugs, Cyclooxygenase.*

INTRODUCTION

Aspirin has been prescribed globally for its efficacy as an antipyretic, anti-inflammatory and anti-platelet drug.[1] It is also used to treat rheumatoid arthritis, and vascular thrombosis.[2] Aspirin causes harmful side effects on gastrointestinal tract such as gastrointestinal bleeding and perforation together with exacerbation of stress-induced gastric injury and meddling with the recovery process of gastric ulcers.[3,4] Aspirin inhibits the synthesis of prostaglandins derived cyclooxygenase-1 and cyclooxygenase-2 at the inflammatory site, which results in decreased blood flow of gastric mucosa and increased leucocyte adhesion to endothelium in the gastric mucosal vessels.[3,5]It has been documented that a dose of 100mg/kg causes gastric ulcers in rats.[6]

Acacia catechu is a medium sized thorny tree, native to Pakistan, India, Myanmar, Nepal and Thailand.[7] Active ingredients of the plant include catechin, tannin gummy matter, and moisture.[8]

Acacia catechu has antipyretic, antidiarrheal, hypoglycemic and hepatoprotective role.[8] Catechin protects gastric mucosa against gastric ulcers by inhibiting cyclooxygenase and 5-lipoxygenase to decrease inflammation.[9,10] Scientific reports have indicated that tannins have cytoprotective properties by increasing prostaglandin levels in gastric mucosa.[11] Hence it can be used as a naturally occurring substitute to conventional anti-ulcer drugs. This study aims to find out if *Acacia catechu* can prevent the formation of aspirin-induced gastric inflammation.

METHODS

This experimental study was carried out at animal house and histology laboratory of Postgraduate Medical Institute with 48 Wistar rats of both genders, having weight between 150-250g, and acquired from animal house of University of Veterinary and Animal Sciences Lahore. The study protocol was approved by the Advanced Studies and Research Board of University of Health Sciences, Lahore and Ethical

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Committee of Postgraduate Medical Institute, Lahore. Aspirin was purchased from Bayer Pharma Pakistan. *Acacia catechu* bark was obtained from department of Botany, Government College University Lahore. It was withe-red in shadow, crushed to powder form and stored in an air proof container.[12]

Rats were randomly divided into 4 groups A, B, C & D, each containing 12 animals. Each group was sub-divided into two groups (1 and 2); A1, A2, B1, B2 C1, C2, D1 and D2with 6 rats in each subgroup. Rats were fasted overnight before administration of the dose. Group A was given 4ml of distilled water only.[6] Group B was treated with aspirin at a dosage of 100 mg/kg body weight. Groups C and D were given aspirin 100 mg/kg body weight with a conjunctive dose of 250 mg/kg and 500 mg/kg body weight of *Acacia catechu* respectively (Table-1).[8]The doses were given by oral route after forming solution in 4 ml. distilled water. Subgroups A1, B1, C1 and D1 were sacrificed on day 3 and subgroups A2, B2, C2 and D2 were sacrificed on day 7, after drug treatment.

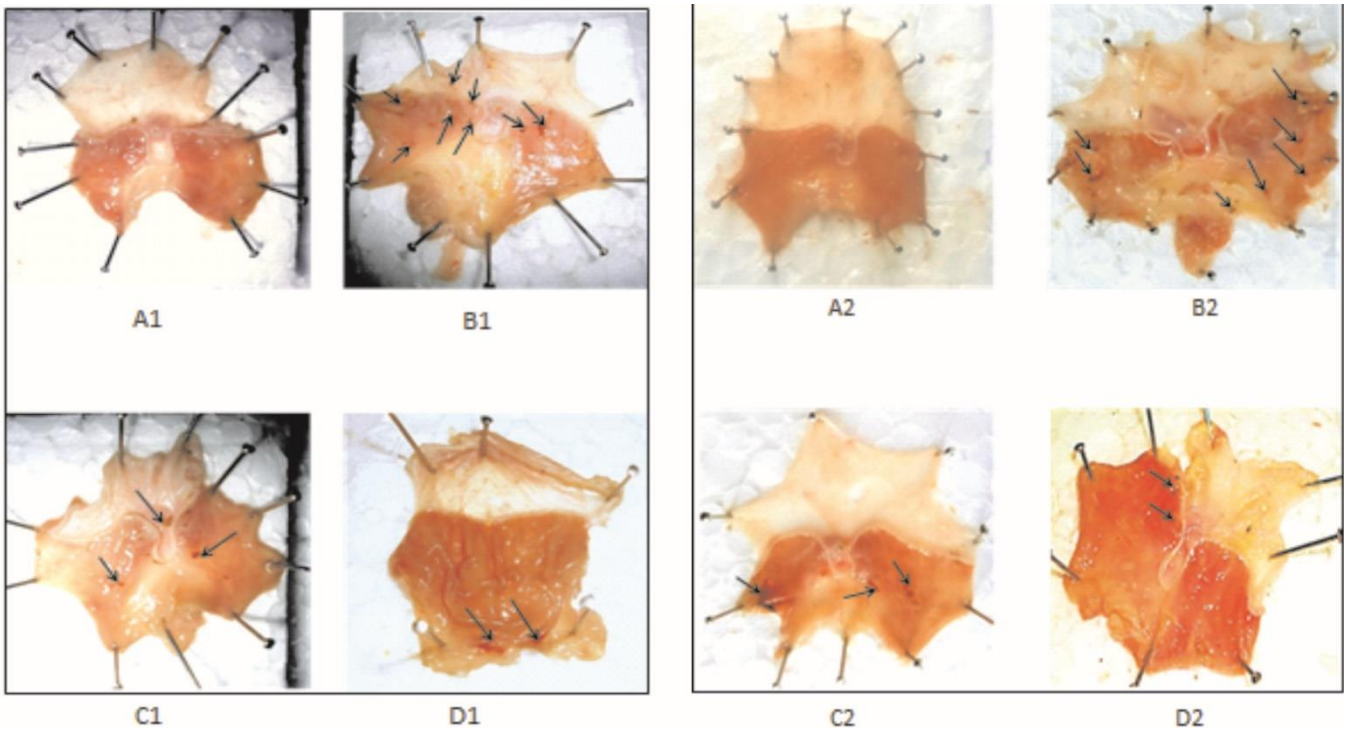
After abdominal dissection, stomach was incised along the greater curvature and secured on thermocol sheet. The gastric mucosa was observed under a dissecting microscope (X10 magnification). Measuring scale was used to calculate each lesion along its greatest length. In the case of petechiae, 4 lesions were taken equivalent to an ulcerated area of 1mm.[2] For determination of the ulcer index, the overall area of the ulcerated mucosa in each rat along with area of glandular part of stomach mucosa was measured.[6,14] Hematoxylin and eosin stained specimens were used for histological analysis. The number of inflammatory cells in mucosa and sub-mucosa of ulcerated area were counted by light microscopy under X100 magnification.

STATISTICAL ANALYSIS

The data was analyzed using Statistical Package for Social Sciences (SPSS) version 20. Mean and standard deviations were calculated for the continuous variables. Difference of means between the groups was assessed by one-way ANOVA was used to assess the mean. The difference of means between individual groups was assessed by using post-hoc Tukey test *P-value of ≤0.05* was considered as statistically significant.

RESULTS

On 3rd& 7th day, ulcer index in control groups was0. The mucosal surface was pink and healthy (Fig. 1). Oral administration of aspirin produced highly significant mucosal lesions, hemorrhage, ulcerations with superficial erosions and linear breaks were seen (Fig.1a).Decreased Ulcer index in groups C and D was decreased as compared to B group. Degenerative mucosal changes, petechiae and some linear breaks were noticed *(P < 0.001)* (Fig. 1b) (Graph 1).



(a) (b)

Fig. 1: *Photographs showing stomach ulcers in rats on day 3 (a) and day 7 (b).*

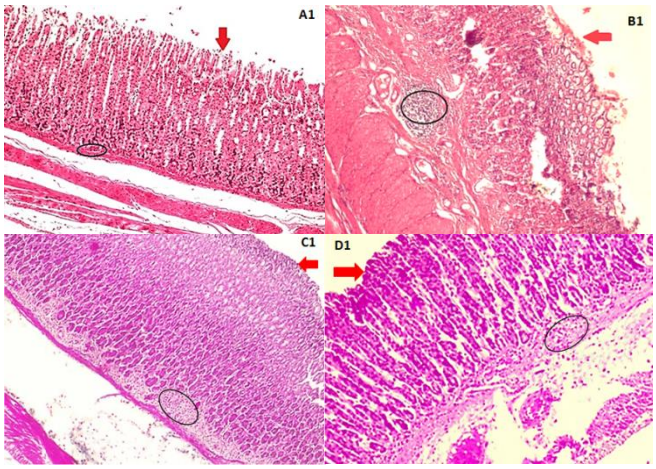


Fig. 2: *Day 3 images showing surface epithelium (red arrows), inflammatory cells (black circle)in control and experimental groups H&E X40.*

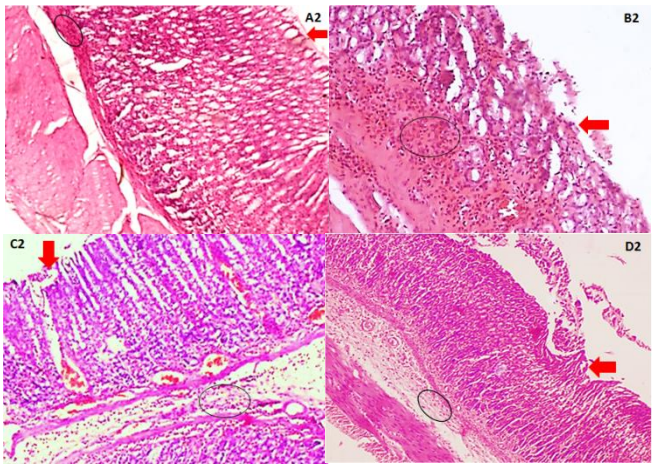


Fig. 3: *Day 7 images showing surface epithelium (red arrows), inflammatory cells (black circle)in control and experimental groups H&E X40.*

On 3rd day, highest numbers was seen in group B which further increased on day 7. Predominantly neutrophilic infiltration was seen on day 3 while on day 7 macrophages and lymphocytes were also increased *(P < 0.001)* (Fig.2).

On 7th day, there were a few inflammatory cells in control group A2 (Fig. 3). Substantial rise in inflammatory cells was observed in group B when compared to control and *Acacia catechu* treated groups. *Acacia catechu* reduced leucocytic infiltration, thus reducing the inflammatory process. A higher dosage of 500mg/kg however revealed satisfactory results in comparison 250 mg/kg dosage *(P < 0.001)*.

Table-1: *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*

**P-value ≤ 0.05 is statistically significant.*

DISCUSSION

In the present study, the protective effects of aqueous solution of bark of acacia catechu were evaluated against aspirin induced gastric mucosal lesions in rats. Increased gastric ulcers were observed in aspirin treated groups. Superficial gastric mucosal damage after administration of 100 mg/kg aspirin have been previously reported.[15] Aspirin causes gastric mucosal damage by COX inhibition; this was proved by in a study conducted on rats.[16] COX inhibition prompts suppression of prostaglandin which leads to decrease in secretion of mucus. As a consequence, mucosa is exposed to acidic gastric secretions resulting in mucosal cell injury leading to enhanced production of ROS (reactive oxygen species). This incapacitates mucosal barrier and leads to cellular necrosis also found that equal doses of vitamin C and ASA decreased the extent of stomach damage in healthy subjects as compared to ASA alone.[16,17]

In the current study, ulcer formation was decreased in both experimental groups receiving a combination of aspirin and acacia catechu which is attributed to increased secretion of COX 1 prostaglandins by *Acacia catechu*. The antioxidant role of flavonoids is mediated by decreased generation and increased scavenging of free radicals by gastric mu-

cosal cells.[18] Catechin and rutin play vital role as free radical scavengers of *Acacia catechu*.[10] Aqueous solution of *Acacia catechu* bark exhibits antioxidant activity through its anti-lipid per oxidation ability.[19]

Statistically significant increase in number of inflammatory cells was noticed amongst experimental groups when compared with control group. Conversely, the inflammatory cell count in group C and D was considerably lesser than group B. This finding can be attributed to ability of platelets to liberate interleukin 1, a pro-inflammatory cytokine, in normal wound healing, that acts as chemo-attractant for neutrophils to the wound site.[20,21] Abundant inflammatory cells were seen in group B. Neutrophils` role in the pathogenesis of NSAID induced ulcer formation was investigated in indomethacin treated animal study on rat stomach. It was observed that a rise in the neutrophilic infiltration succeeded severe and intense ulceration into the gastric antrum leading to ulceration.[22]

Flavocoxid present in *Acacia catechu* prevents pro inflammatory cytokines formationsuch as tumor necrosis factor-α, hence forth hindering reactive oxidative species synthesis resulting in reduced leukocyte infiltration.[23]Flavocoxid contains catechin which reduces neutrophil invasion, thus inflammation. This outcome was supported in an experiment on carrageen induced paw edemamodel.[24]

Table- 2: *Ulcer Index: 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	-16.250	0.000*
		Group-C	-3.562	0.053
		Group-D	-1.025	0.997
	Group-B	Group-C	12.687	0.000*
		Group-D	15.225	0.000*
	Group-C	Group-D	2,537	0.389
7th day	Group-A	Group-B	-14.500	0.000*
		Group-C	-2.750	0.277
		Group-D	-0.875	0.999
	Group-B	Group-C	11.750	0.000*
		Group-D	13.625	0.000*
	Group-C	Group-D	1.875	0.794

*p value ≤ 0.05 is statistically significant.

CONCLUSION

The present study sheds light on preliminary data on the anti-ulcer property of *Acacia catechu* bark and justifies its orthodox usage. Acacia catechu has a protective effect on gastric mucosal injury by increasing mucus secretion and by enhancing secretion of prostaglandins.

LIMITATIONS OF STUDY

This study does not clarify whether aqueous solution of bark of *Acacia catechu* is effective in prolonged treatment with acetylsalicylic acid and on healing process of preexisting gastric ulcers. Further research with extracts prepared with ethyl acetate, ethanol, and methanol on gastric mucosa should be evaluated.

ACKNOWLEDGEMENT

Authors wish to thank Dr. Rozina Jaffar, Professor of Histopathology, Rahbar Medical and Dental College, Dr. Sharjeel Ilyas and Dr. Mariam Ashraf of Anatomy Department, Postgraduate Medical Institute, and Dr. Ashraf Choudhary, Head of department, Community Medicine, CMH Medical and Dental College.

AUTHOR'S CONTRIBUTION

UW: Conception, design and interpretation of data
MS: Acquisition of data.
AG, MBB: Analysis & interpretation of the data.
FQ: Critical revision of the article for intellectual content.
QM: Analysis of data.
UW: Final approval of the article.

CONFLICT OF INTEREST

None to declare.

GRANT SUPPORT AND FINANCIAL DISCLOSURE

None to disclose.

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- Received for Publication: 08-04-2019
- First revision received: 20-05-2019
- Second Revision received: 02-07-2019
- Accepted for publication: 05-09-2019