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# EFFECT OF PRUNUS DULCIS ON EPITHELIAL DAMAGE CAUSED BY NONSTEROIDAL ANTI-INFLAMMATORY DRUGS

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## ABSTRACT

***Objective***: To assess the effects of Prunus dulcis on gastric epithelium affected by nonsteroidal anti-inflammatory drugs.

***Study Design***: Laboratory based experimental study.

***Place and Duration of Study***: Anatomy Department, Army Medical College Rawalpindi, in collaboration with National Institute of Health (NIH), Islamabad, from Jan 2015 to Dec 2015.

***Methodology***: Forty healthy adult male and female (BALB/c strain) mice, 9-11 weeks age, weighting 30-40gm were used in the experiment and were kept in standard conditions in animal house. Animals were divided into four equal groups. Group A served as control, whereas group B, C and D served as experimental groups. Animals in all groups were given standard lab diet for 60 days. In addition to standard lab diet, experimental groups C and D were given almond oil (extracted without peel of almonds) and finely ground almonds (with peel), respectively, via oral gavage once a day for 60 days. At 60th day, 400 mg/kg of ASA solution was given, via oral gavage needle, to induce gastric ulcers in animals of experimental groups B, C and D. Type of epithelium and height of epithelium were assessed under Light Microscope.

***Results***: Height and type of epithelium improved in experimental groups C and D on intergroup comparison. Whereas group D showed improved protection as compared to group C.

***Conclusion***: Prunus dulcis provides protection, to gastric epithelium, against gastric ulcers induced by non-steroidal anti inflammatory drugs. In addition, finely granular almonds provided better protective outcome in comparison with almond oil.

**Keywords**: Acetylsalicylic acid, Nonsteroidal anti-inflammatory drugs, Prunus dulcis.



## INTRODUCTION

Nonsteroidal antiinflammatory drugs (NSAIDs) are one of the most commonly used over-the-counter drugs[1]. NSAIDs include drugs like piroxicam, acetylsalicylic acid (ASA), ibuprofen, indomethacin and meloxicam[2]. They are used as analgesics, anti-inflammatory, and antipyretic drugs. They inhibit platelet aggregation, prolong bleeding time, used to close patent ductus arteriosus (PDA), relief of dysmenorrhea and inhibition of premature labor and are sometimes used for bronchoconstriction or bronchodilation[3]. ASA was chosen to induce acute gastric injury to gastric epithelium in this experiment.

One of the serious side effects of NSAIDs is gastrointestinal mucosal injury. Many effective strategies are being tirelessly sought to avoid the gastrointestinal mucosal damage. NSAIDs may cause gastric lesions by one of the following mechanisms: by inhibiting Prostaglandin (PG) production, by inhibiting cyclooxygenase (COX) production[4,5] or by direct cytotoxic effect on gastric mucosa[6]. WHO reported that large proportion of the population of developing countries depends on natural product drugs for health care[7,8].

Prunus dulcis (Almond) has high content of dietary fiber, proteins, flavonoid antioxidants, vitamin E and essential minerals like magnesium, copper, manganese, calcium, and potassium. These, and other, properties of almonds might be responsible for gastroprotective effect. Plants with traditional ethnomedicinal uses in peptic ulcer management thus need to be screened for

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potential antiulcer activity and as sources of anti-culcer lead compounds. It is, therefore, necessary to have credible experimental models that can be used to screen such phytomedicines with potential anti-gastroduodenal ulcer activity. The objective of current study was to evaluate the protective role of two forms of Prunus dulcis on gastric epithelium, damaged by NSAIDs.

## METHODOLOGY

The study was carried out in the Department of Anatomy, Army Medical College (AMC), Rawalpindi, in collaboration with National Institute of Health (NIH) Islamabad and Armed Force Institute of Pathology (AFIP), Rawalpindi. It was a laboratory based experimental study that spanned from January 2015 to December 2015.

Forty healthy adult male and female BALB/c mice of age 9-11 weeks with weight range of 30-40gm were used in the experiment and were housed in controlled environment of animal house of NIH, Islamabad. Male and female mice were housed separately and were kept in a well-ventilated room and under a temperature range of 20-26°C with the help of central temperature regulating system with a 12 hours dark-light sleep cycle throughout the duration of experiment. Animals were grouped into four equal groups by using random number table method and sampling was done using non-probability consecutive sampling technique.

Group A served as control, whereas groups B, C and D served as experimental groups. Animals in all groups were given standard lab diet for 60 days. In addition to standard lab diet, control group A and experimental group B were given water via oral gavage (to ensure ingestion of Aspirin), once a day, for 60 days. Whereas experimental groups C and D were given almond oil (oil extracted after removing peel of almonds) and finely ground almonds (with peel), respectively, via oral gavage, once a day, for 60 days. Finely granular almonds were prepared by gradually grinding raw almonds in electric grinder and were administered via oral gavage, by diluting it in water[9]. Almond oil was extracted using commercially available oil press machine[10]. ASA solution was prepared such that 0.83ml of ASA solution contained dose equaling 400 mg/kg body weight[11]. Prior to the administration of ASA on 60th day, the experimental groups were deprived of food for 24 hours but were given free access to water. At 60th day, 0.83ml of ASA solution was given, via oral gavage, to induce gastric ulcers in animals of experimental groups B, C and D. Type of epithelium, apical erosion and height of epithelium were assessed under light microscope.

At X40 (High Power Field-HPF), type of epithelium of stomach of mice was recorded as simple columnar or apical erosion or simple cuboidal. Apical erosion was defined as loss of surface integrity.

Epithelial height was measured by observing three high power fields (HPF), by considering three equally spaced fields from periphery to the center of slide, per specimen. In each HPF, ten adjacent epithelial cells were measured for epithelial height. The height of each cell was measured from the basement membrane until the apex of the cell facing the lumen at X40. Images were taken from each slide with the help of Olympus digital camera (10-mega pixel), stylus 1010 were used through the ocular of the Olympus DP21 light microscope. The images were then transferred in the computer. Each image was opened in Image J v1.48[12]. In Imagej, scale was set to measure the height in micrometer and epithelial height was calculated at X40 (HPF). Measurement tool 'Straight' was selected and the height to be measured was calculated by drawing a straight line from thin basement membrane of epithelium to apex of cell, the measurement was then analyzed and recorded. Result was expressed as mean for each specimen in micrometers[13].

SPSS-21 was used for data analysis. ANOVA test was applied for intergroup comparison of quantitative variable (height of epithelium) followed by post-hoc Tukey's HSD that was taken as mean and standard deviations (Mean ± SD). Qualitative variable (height of epithelium) was

presented by frequency and percentages. Chi square test was applied for comparison of qualitative variables (type of epithelium and presence of apical erosion). The *p*-value ≤0.05 was considered to be indicative of statistical significant.

## RESULTS

Type of epithelium of stomach of mice was recorded as tall columnar epithelium in all ten specimens of control group A. In experimental group B, 8 out of 10 (80%) specimens had apical erosion and rest of the two (20%) showed simple columnar epithelium. On intergroup comparison it was statistically significant with control group A (*p*=0.001), and experimental groups C (*p*=0.025) and D (*p*=0.007). In group C, epithelium was recorded as simple columnar in 7 out of 10 (70%) cases whereas three (30%) showed apical erosion. On intergroup comparison it was statistically insignificant with groups A (*p*=0.060) and D (*p*= 0.606) and statistically significant with experimental group B (*p*=0.025). Whereas in experimental group D, epithelium was simple columnar in 8 out of 10 (80%) cases and rest of the two (20%) had apical erosion of mucosal epithelium Figure-1 & 2. On intergroup comparison it was found to be statistically significant with group B (*p*=0.007) and statistically insignificant with groups A (*p*=0.136) and C (*p*=0.606).

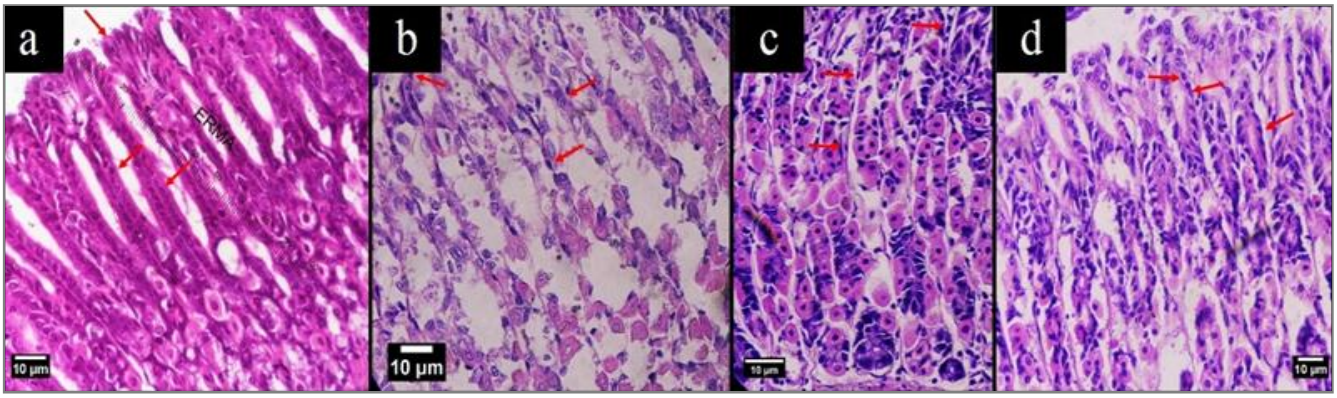


**Figure-1: Photomicrographs at x400 (H & E) showing type of epithelium (Red arrow heads) in control group A and experimental groups B, C & D. (a-columnar epithelium, b-distorted epithelium, c-columnar epithelium, d-columnar epithelium).**

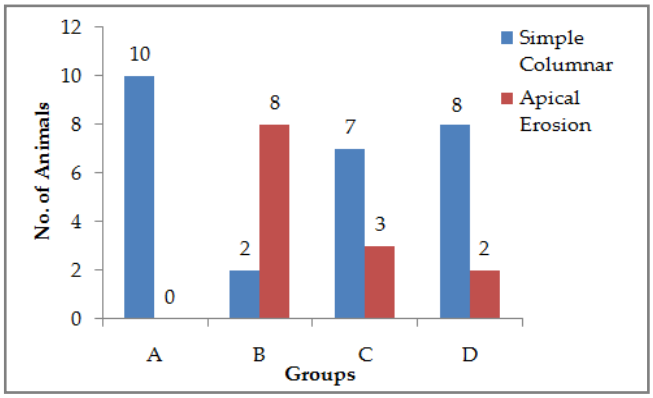


**Figure-2: Clustered column chart showing comparison of frequency of type of epithelium in control group A and experimental groups B, C and D.**

**Table-I: Mean ± SD of height of epithelium of control and experimental groups.**

| Groups | Height of Epithelium (Mean ± SD) |
|---|---|
| **A (Control group)** | 4.02 ± 0.69 |
| **B (Aspirin group)** | 1.40 ± 1.13 |
| **C (Almond oil plus Aspirin group)** | 2.27 ± 0.51 |
| **D (Finely granular almonds and Aspirin group)** | 3.47 ± 1.07 |

In control group A, mean and SD of epithelial height was 4.02 ± 0.69µm. Mean and SD of epithelial height of specimens of group B was 1.40 ± 1.13µm. On comparison with group A, epithelial height was significantly reduced in group B (*p*<0.001). Mean and SD of epithelial height of experimental group C was 2.27 ± 0.51µm and was statistically significant on comparison with groups A (*p*=0.001) and D (*p*=0.025). Whereas in

experimental group D, mean and SD of epithelial height was 3.47 ± 1.07µm. On intergroup comparison, it was statistically significant with group B ($p$<0.001) and group C ($p$=0.025) Table-I & II. The height of epithelium was improved when

**Table-II: One-way ANOVA with post-hoc Tukey's HSD showing comparison of mean difference and *p*-value of height of epithelium in control group A and experimental groups B, C and D.**

| Groups | Groups | Mean Difference | *p*-value (Significance)* |
|---|---|---|---|
| A | B | 2.624000 | 0.001 |
| | C | 1.748000 | 0.001 |
| | D | 0.555000 | 0.515 |
| B | A | -2.624000 | 0.001 |
| | C | -0.876000 | 0.145 |
| | D | -2.069000 | 0.001 |
| C | A | -1.748000 | 0.001 |
| | B | 0.876000 | 0.145 |
| | D | -1.193000 | 0.025 |
| D | A | -0.555000 | 0.515 |
| | B | 2.069000 | 0.001 |
| | C | 1.193000 | 0.025 |

*$p$-value <0.05 is statistically significant.

comparison of group B was done with groups D ($p$=0.001) and A ($p$<0.001). When groups C and D were compared with each other, group D showed improved protection as compared to group C ($p$=0.025) Table-II.

## DISCUSSION

On histomorphological examination, type of epithelium of stomach, experimental group B had apical erosion which was statistically significant with control group A and experimental group D. Whereas there was no statistically significant difference in comparison with experimental group C which contained almonds oil extracted after removing skin/peel of almonds. While the experimental group D was statistically significant with unprotected group B. This shows that Prunus dulcis offered protective effect in group D by preserving the epithelium due to additional protection offered by prebiotic properties of almond skin/peel which was present in finely granular almonds and was absent in almond oil. Similar findings were observed in in-vivo study conducted on intestines as seen in previous study[14,15].

Epithelium height considerably reduced in unprotected group B, while the mean epithelial height in protected groups C and D increased when compared with unprotected group B. On comparing $p$-values of experimental groups, it was observed that group D had more protection as compared to groups C ($p$=0.025) and B ($p$<0.001), indicating trend of epithelial restoration and regeneration. The underlying factor responsible for more protection to group D can be due to Transforming Growth Factor-α (TGF-α). TGF-α is a ligand for epidermal growth factor receptor, activates a signaling pathway for cell proliferation, differentiation and development. In stomach, it is produced in normal gastric mucosa and has shown to inhibit gastric acid secretion[16]. Biochemical analysis of almond skin in previous studies, show presence of nine phenolic compounds[17,18] imparting it the anti-oxidative properties[19].

## CONCLUSION

Prunus dulcis provides protection, to gastric epithelium, against gastric ulcers induced by non-steroidal anti inflammatory drugs. In addition, finely granular almonds provided better protective outcome in comparison with almond oil.

## CONFLICT OF INTEREST

This study has no conflict of interest to be declared by any author.

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