ORIGINAL ARTICLE

Gastroprotective Effect of Sagu Pearls on Diclofenac Sodium Induced Gastric Ulcer

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ABSTRACT

Objective: To see the possible gastro protective effect of Sagu pearls on Diclofenac Sodium (NSAID) induced gastric ulcer by enhancing mucosal barrier.

Study Design: Randomized control trial.

Place and Duration of Study: The study was carried out in the department of Anatomy, Islamic International Medical College, Rawalpindi, in collaboration with National Institute of Health, Islamabad. It was conducted for a period of six months, from 15th September 2014 till 30th March, 2015.

Materials and Methods: Fifty adult rats of both sexes of Sprague Drawly strain were divided into three groups: Group I (control); Group II (ulcer group) given Diclofenac sodium orally at the dose of 50mg/kg body weight, daily for 2 weeks and Group III given Sagu pearls daily at the dose of 200mg/kg body weight for 2 weeks along with Diclofenac Sodium. Animals of all the groups were sacrificed on day 16 and their stomachs were studied macro and microscopically. Statistical analysis was done to see any significant difference between the groups. Anti-ulcer effects were assessed on the qualitative and quantitative parameters like ulcer size and index, histological determination of depth of the mucosal lesion and mucus thickness.

Results: Results highlighted the probable protective effect of Sagu pearls by exhibiting almost 90% decrease in ulcer index in group III accompanied by a continuous thick mucus layer on the surface of mucosal cells confirmed by Periodic Acid Schiff (PAS) stain.

Conclusion: Sagudana can provide protection against NSAID induced gastric ulcer by strengthening mucus barrier and thus can be used as an adjunct along with NSAIDs.

Keywords: *Gastric Ulcer, Starch (Sagu), Non-Steroidal Anti-Inflammatory Drugs, Anti-Ulcer Agents.*

Introduction

Peptic ulcer, an interruption in the continuity of gastrointestinal mucosal lining occurs most commonly in patients aged 30 to 50 years but above 60 years account only for 15% of cases.[1] Aggressive factors leading to imbalance in gastric mucosal offensive and defensive factors are generally accepted as the cause of gastric ulcer.[2] Untreated ulcers can increase morbidity by anemia, haematemesis or perforations.[3] Such ulcers are a cause of economic burden as their treatment imposes at least 10% of the total cost of treatment of digestive disorders.[4]

NSAIDs, commonly prescribed medicine for general to chronic ailments in Pakistan[5], are one of the aggressive factors for ulcerogenesis.[6] Those who receive high dosage of NSAIDs even for shorter span of time or those using moderate dosage but for a long period have shown large number of ulcerative lesions.[7] Statistical data collected from randomized control trials conducted in hospitals infers that about 1 in 10 NSAID induced ulcer bleeds.[8] Numbers of adjunct therapies are prescribed along with the usage of NSAIDs to reduce their ulcerative effects but some are not cost effective or have side effects of their own along with noncompliance of patient.[7] There is a need to find some natural, cost effective prophylaxis along with NSAIDs to minimize patient's agony. Sagu starch, an inexpensive natural polysaccharide, marketed in the form of small globules known as sagu pearl or Sagudana, has been used traditionally for 70 years during sickness.[9] Both homeopaths and allopaths consider it as best food during fever as it is a promising natural tonic and easily digested, non-irritating food in inflammatory cases.[10] It is a very rich source of carbohydrates.[11] Number of ulcer protective natural herbs, has been found by different researchers, with different parts of plant being used, after removing their unwanted chemicals.[12] Sagu starch being a pure polysaccharide

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is totally inert and highly viscous which can be used easily.

Effect on quality of life, time lost from work due to ulceratic pain, expense of hospitalization and expensive adjuvant therapy along with NSAIDs, having their own side effects, made us find cost effective, nontoxic and easily available prophylaxis. The idea that the surface-active agents would be preferable to acid inhibitors as they do not alter the bactericidal activity of the stomach along with low cost and high yield as compared to other sources of starches and traditional belief of people made sagu pearls as the choice of prophylactic substance.

Materials and Methods

It was a randomized control trial conducted in Anatomy Department IIMC in collaboration with NIH after the approval from Institutional Review Committee, for a period of six months, from 15th September 2014 till 30th March, 2015. Fifty adult Sprague Drawly rats of both sexes, more than 3 months old, weighing approximately 180-250g, were selected by balloting method and purchased from animal house of NIH, Islamabad. Rats with any obvious physical pathology were excluded. Twenty five male and twenty five female rats were divided in 3 groups and kept in separate cages, under standard laboratory conditions in NIH. They were acclimatized for one week at a room temperature of 23-25°C with a 12 hour dark/light cycle and were allowed to feed and drink ad libitum on standard pellet diet and tap water.

Sagu pearls, 200gms/pack, of local brand were purchased from market. Diclofenac sodium (50 mg) (Voltral) of Novartis Pharma (Pakistan) Limited was purchased from local pharmacy.

Group I (control) where n =10 was divided into sub groups: IA having 5 male rats and IB having 5 female rats. They had free access to feed and water. Group II (ulcer group) where n =20 were divided into subgroups: II A having 10 male rats and II B having 10 female rats. They were given diclofenac sodium at a dose of 50 mg/kg body weight[13], once daily for 15 days, orally mixed in water so as to reduce the bias of stress as ulcerative factor. Group III (Test group) where n = 20; was divided into sub groups III A having 10 male rats and III B having 10 female rats. They were given Sagu pearls at the dose of 200 mg/kg body weight[14], once daily along with diclofenac sodium as above, for 15 days in the form of gruel. Gruel was made by boiling 100 granules in 700ml of water to form thin paste. At the end of 2 weeks the animals of all groups were anesthetized and sacrificed. The stomachs were isolated, opened along the greater curvature, washed gently with saline and were examined by hand lens for change of color, hemorrhagic area or presence of crater. Presence of ulcer was confirmed by measuring ulcer size[15] and ulcer index.[16] The samples were then placed in10% neutral-buffered formalin for 24 hours. 2 mm wide parallel strips from glandular portion were dissected and embedded in Paraffin. The prepared serial sections were then stained with Haematoxylin and Eosin (H&E) for detecting the depth of lesion and Periodic Acid Schiff (PAS) stain for detection of neutral mucins on the mucosal surface. The depth of lesion was graded as type 1, 2 and 3 according to criteria laid by Natalie.[17] Ulcer index was calculated according to the formula notified by Sharma[16]:

UI=UN +US +UP × 10-1

Where UI= ulcer index, UN= average number of ulcers per animal in each group

US= ulcer score per group and UP= percentage of animal with ulcers in each group.

The mucus thickness was measured as the vertical distance between cell surface and luminal mucus surface with linear eyepiece micrometer at 40 magnification of objective. The mean value of 4 different measurements was taken.[18] The data was entered and analyzed using SPSS 20.0. One Way Analysis of Variance (ANOVA) was applied to compare the mean differences among groups. A p–value of <0.05 was considered as statistically significant.

Results

Macroscopic examination of opened stomach in control group exhibited pink colored glandular part with prominent rugae. Mucosal damage of varying severity ranging from pin point erosions to dark brown lesions were seen in both Groups II and III .

Ulcer index was higher in group II as compared to group III.

H & E stained slides of gastric mucosa of Group I (control) revealed gastric glands having columnar surface mucous secreting cells with basal oval nuclei followed by predominant mucous neck cells and

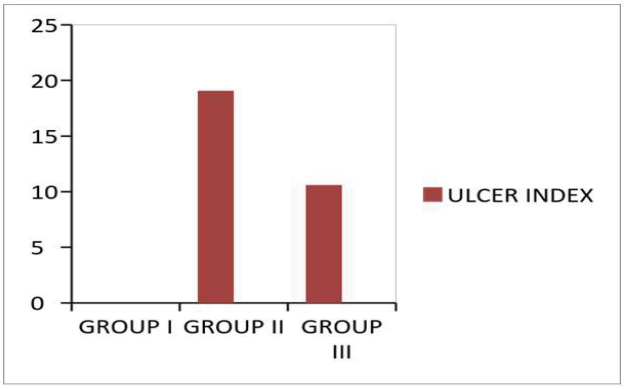

Fig 1: Comparison of Ulcer Index in stomachs between the three groups

parietal cells. The parietal cells appeared as deeply eosinophilic rounded to ovoid cells with central rounded nuclei. At the base of the gland abundant chief cells with few parietal cells were observed. The glands fully occupied the thickness of lamina propria along with blood vessels and few dispersed lymphocytes. Smooth muscle layer, muscularis mucosae, limited the mucosal layer from submucosa. The submucosa was evident as loose connective tissue with blood vessels. The depth of lesion in Group II and III was determined on the basis of extent of destruction of cells which was graded as type 1, 2 and 3 according to the involvement of upper or lower part of lamina propria respectively.17 In Group II the damaged cells appeared as shrunken with pyknotic nuclei extending from neck till base of gland. Submucosal edema with congested blood vessels was prominent (Fig 3). Group III had predominance of proliferating mucous cells extending along the neck of the gland. Glands with dilated lumen were prominent. No submucosal odema was detectable as compare to Group II.

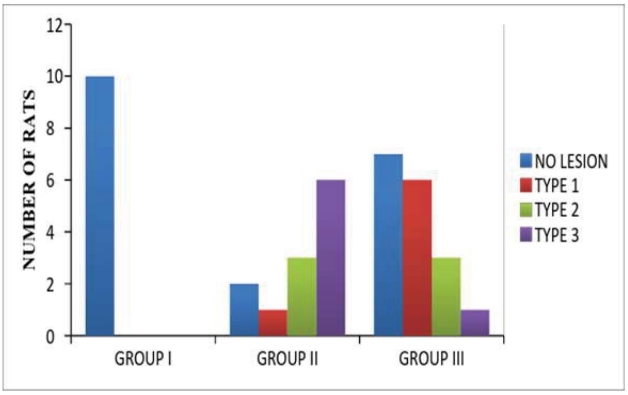

Fig 2: Comparison of depth of lesion between the three groups

Table I: Comparison of depth of ulcerative lesions between the three groups

Type of Lesion	Group I (n = 10)	Group II (n = 12)	Group II (n = 17)	P-value
No Lesion	10 (100%)	2 (16.7%)	7 (41.2%)	1*
Type 1 Lesions	0 (0%)	1 (8.3%)	6 (35.3%)	
Type 2 Lesions	0 (0%)	3 (25%)	3 (17.6%)	
Type 3 Lesions	0 (0%)	6 (50%)	1 (5.9%)	

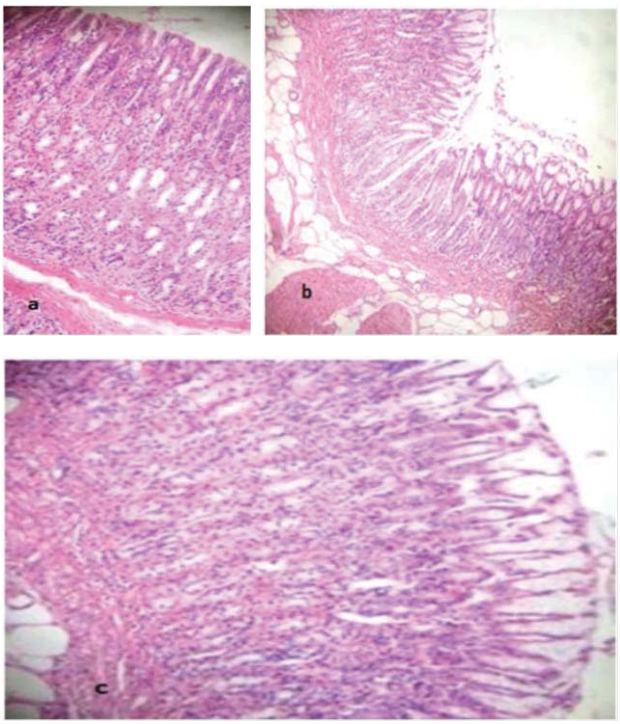

Fig 3: Photomicrograph of glandular mucosa of (a) control Group showing normal glandular architecture with intact surface mucous cells and normal submucosal thickness(b) Group II showing sloughed cells with ulcer and sub mucosaloedema (c) Group III with intact mucus layer and normal architecture H&E 100x

In Group I a continuous magenta colored layer and a positive reaction was observed in surface and mucous neck cells while interrupted mucosal layer and weak PAS reaction was observed in mucous neck cells in Group II. Group III exhibited a continuous, thick magenta color at the surface and strong PAS positive reaction extending to the pits of the glands. (Fig 4).

Discussion

In our study a variety of macroscopic mucosal gastric lesions ranging from mere color change to hyperemia to gross lesions were noticed in group II. This is in accordance with the changes reported by all studies conducted on gastric ulcers.[19,20] There was significant increase in the ulcer index in group II as

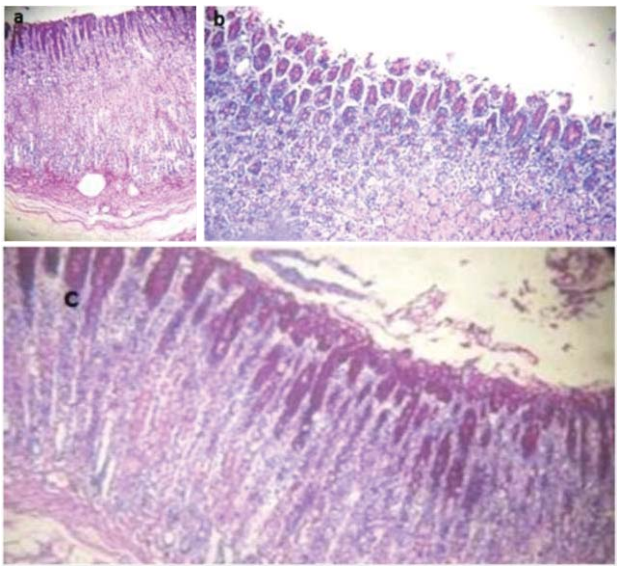

Fig 4: Photomicrograph (a) showing continuous mucus layer in Control (b) showing interrupted mucus layer and weak PAS reaction in Group II (c) showing thick continuous mucus layer and strong PAS reaction extending into pits in Group III. PAS stain 100x

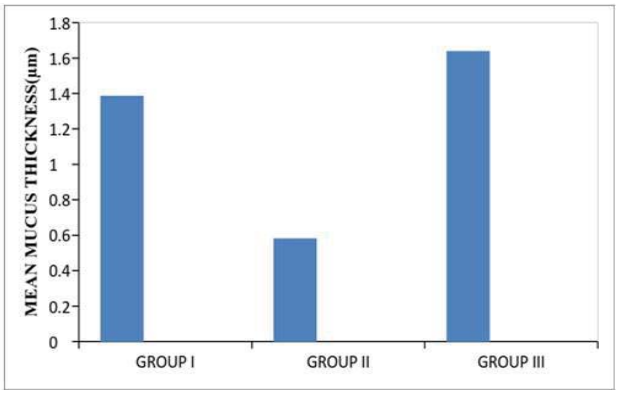

Fig 5: Comparison of mean mucus thickness (µm) on the luminal surface of mucosal cells of stomach, between the three groups.

Table II: Post-hoc comparison of Mean Mucus thickness (µm) between the three groups

Group Comparisons	Mean mucus thickness	
	Mean difference	p-value
Group I vs. Group II	2.009	0.003 *
Group I vs. Group III	- 0.652	0.425
Group II vs.Group III	- 2.661	0.001 *

* p< 0.05 = Significant

compared to Group I. Moreover there was a statistically significant decrease in the ulcer index in group III compared to group II. Regarding the depth of lesion, group II showed all three types of lesions. Microscopically sloughed off surface mucous cells and mucus neck cells were evident forming erosions. Cells with highly eosinophilic cytoplasm and pyknotic nuclei extended from the neck to the base of group II whereas they were restricted to basal parts only in group III. In agreement with above results it's seen that diclofenac sodium produced reactive gastropathy. This damage may be due to excessive hydrogen ion movement from the lumen to inside when diclofenac is given in therapeutic doses for a longer period of time as is the case with aspirin.[20]

Regarding group III examination of H&E stained slides showed an intact mucus layer with hyperplastic mucous cells extending deep down the length of gland, depth of lesion was significantly reduced as compared to the other group.

In this study significant difference was observed in mucus thickness between group II and group III. PAS stained slides revealed attenuation of mucus layer in group II given only diclofenac as has been observed by Singh et al[21] in an aspirin induced gastic ulcer. Thick mucus layer and intense reaction (magenta color) extending into gastric pits, the lumen of the gland and neck region was seen in group III. This is in agreement with Mohammad[22]who also measured the mean optical density of magenta color. Flemstorm and Isenberg[23] have highlighted that gastric mucosal barrier plays a vital role in the protection of gastric wall from aggressive factors responsible for damage. Silva[24] while explaining mucus gel layer emphasized that it provides a diffusion barrier against aggressive factors, entraps microorganisms and holds bicarbonate ions thus controlling intraluminal pH. Jainu[20] has proved that depletion of sulphated mucin glycoprotein leads to small erosions in stomach.

Available literature has shown multiple mechanisms of action of NSAIDs in inducing gastropathy.[2,25,26] Most commonly accepted mechanism is interruption of mucosal layer which in turn is due to depletion of prostaglandin. Cyclooxygenases are the key enzymes in prostaglandin biosynthesis and the target enzymes for the widely used NSAIDs.[27]

PAS stain highlighted deep magenta color on the surface as well as lower down the pits. It can be due to mucilaginous polysaccharides in sagu starch. Same effects are seen by Maria et al[28] who worked on rhamnogalacturonan, a polysaccharide. Prabha et al

has seen the effect of plaintain[29] which stimulates the growth of gastric mucosa because of its water soluble polysaccharides. Wei, Hui and Mao[30] work on finding safe herbal medicines in treating gastric ulcer notified the work of Bhattacharya and Banerjee who had proved local mucus enhancement by plants like Piper Betel extract.

The prophylactic gastro protective mechanism is based on the ability to strengthen defensive factors like prostaglandin synthesis in addition to other factors. Prostaglandins can provide gastric cytoprotection without reducing gastric acid secretion[31] but by enhancing mucosal barrier and its blood flow. Sagu pearls cytoprotective role may be attributed to the polysaccharides which stimulated prostaglandin synthesis leading to mucosal regeneration.

Non availability of electron microscope has limited the study to know the exact histological changes occuring in the gastric mucosal cells after administration of sagu pearls.

Conclusion

This study has shown the prophylactic effect of sagu pearls, as has been confirmed by morphological and histological parameters. Lesser depths of lesions along with thick mucus layer generation on the luminal surface of mucosal cells, in rats given sagu pearls, are significant enough to prove our alternative hypothesis. Effect of sagu pearls on peptic ulcers induced by physical and chemical agents should also be seen to find its effect on acid secretion.

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