JAMC, Vol. 5, No. 2, 1992

GASTRIC ULCEROGENICITY OF FENOPRON ANTAGONIZED BY PARACETAMOL IN ALBINO RATS

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ABSTRACT:

The ulcerogenic effect of fenopron and its protection by paracetamol was observed under dissecting as well as laboratory microscope. The protection was complete under dissecting microscope, in the dose of 250 mg/kg. body weight but there was significant decrease in erosion score with gum acacia followed by Fenopron.

Mucosal thickness was significantly increased in paracetamol followed by fenopron group as compared to other group. Increased secretory activity of mucous neck cells was observed in the same group which might have resulted as protection of rat stomach by the damaging effect of fenopron; due to increased production of mucous as well as prostaglandin.

INTRODUCTION

Acute gastric ulcers have been thought to be due to disruption of gastric mucosal barrier which appears as multiple erosions.[1] Aspirin and paracetamol are most commonly used as minor analgesic but their effect on gastrointestinal tract differ widely. Aspirin significantly associated with major upper intestinal haemorrhage, whereas paracetamol not.[2] In rats the combination of aspirin with caffeine significantly increased whereas aspirin with paracetamol decreases the incidence of gastric lesions compared with aspirin alone.[3] For further detailed study of this hypothesis the influence of paracetamol on the gastric activity of fenopron is being considered. Fenopron (fenopron) is a phenyl propionic acid derivative, which has analgesic, antipyretic action and it is used in the treatment of mild and moderate pain of rheumatic disorders.[4] However, causes gastric irritation and ulceration.[5]

MATERIAL AND METHODS:

Adult male albino rats weighing between 200 and 350 grams were used in the present study.

Fenopron (lilly) and paracetamol were administered as suspension in one percent gum acacia in distilled water by oral incubation in a volume of one milliliter per 100-gram body weight. The ulcerogenic doses of fenopron was 32.3 mg. per kg. body weight.[6]

A total of 40 animals used in the present study were divided into four groups A, B, C and D, each group had 10 animals. The animals from each group were kept on fasting for 24 hours, water however was available to them freely. The animals were sacrificed 6 hours after the treatment.

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GROUP A: These animals served as normal control. They received gum acacia in the same volume as used for the animals receiving fenopron.

GROUP B: These animals received fenopron only

GROUP C: These animals received gum acacia in the same volume as used for paracetamol followed by fenopron.

GROUP D: These animals received paracetamol followed by fenopron.

The erosions produced by the fenopron was observed under dissecting microscope and assessed according to the procedure adopted by Van Kolf Schoien et al., 1983, and score was given to the erosion according to arbitrary scale designed by Bonta.[7]

ARBITRARY SCALE OF BONTA

APPROXIMATE DIAMETER OF EROSION/ULCER IN MM		SCORE
Less than	1	0.5
" "	1—2	1.0
" "	2 — 3	2.0
" "	3—4	4.0
More than	4	8.0
Preformation		12.0

The cumulative score of one group was divided by the number of animals and expressed as median erosion score of the group. These erosions were subjected to detailed histological study with the help of laboratory microscope to observe the change in the thickness of mucosa, and the distribution and height of the surface epithelium, mucous neck, chief and parietal cells of the gastric gland to assess their secretory activity. The general morphology of the erosion was studied under 6-micron thick paraffin embedded H & E stained sections and the count of the secretory cells in PAS stained sections. The cell count was done under 40x objective and 8x ocular in a strip covering the whole field measuring 150m in width extending from surface to the base of the gland.

OBSERVATIONS AND RESULTS:

A) NORMAL CONTROL GROUP: Opening along the greater curvature, the stomach in half of the animals revealed food contents mixed with pale yellow fluid. However, in the remaining half, little muddy coloured food contents were observed under dissecting microscope. The internal surface of each stomach was clearly identified into two parts by raised ridge. The grayish part continuous with the oesophagus was squamous part (rumen) while the pink area raised into folds (rugae) continuous with the duodenum was identified as secretory or glandular part.

Under laboratory microscope, H & E stained section revealed all layers of the stomach (Fig 2). The surface epithelium composed of mucous secreting tall columnar cells with eosinophilic granular cytoplasm and basel location of rounded or oval nuclei. The gastric glands round to tubular in shape were line by three types of cells; mucous neck cells, chief and pariental cells. Mucous neck cells secrete mucous, showed pale vacuolated cytoplasm with basal nuclei perpendicular to the long axis of the cells.

The pyramidal shaped chief cells having rounded or oval nuclei, showing basophil granular cytoplasm and apical vaculations were located at the base of the gland.

The large avoid parietal cells with light pink to eosinophilic cyto; plasm and central nuclei, lies at the adluminal side between chief cells and the basement membrances at the base of the gland.

B) FENOPRON TREATED GROUP: In the fundic area 2 — 4 dark brown patches were observed which appeared circumscribed, elongated erosions under dissecting microscope (Fig 1). The median erosion score was 3.50 ± 0.44 which was significantly decreased when compared with group C (Table 1). Considerable number of spider like tortuous dilated blood vessels were seen on the rugae near the erosions on the anterior as well as posterior wall of the stomach.

Under light microscope the mean mucosal thickness was 400.8 ± 17.7 urn (Table 3) which was significantly decreased when compared with group A and D. The mucosa was infiltrated by the dark brown pigment covering 1/5 — 3/5 of the depth of the mucosa causing necrosis of the epithelium as well as tubules of the gland, lymphocyte, plasma cells neutrophils and parietal cells with pyknotic nuclei were observed within and around the erosion (Fig. 3).

The mean value of the surface mucous cell count 31.3 ± 1.43/unit area was significantly decreased as compared with group A and D but their mean height (12.32 urn ± 0.38) was decreased significantly when compared with group D only (Table 2 and 3).

A significant increase in the mean height of mucous neck cells (9.78 urn ± 0.22) was observed when compared with group A. However, their mean cell count 24.99 ± 2.45) was decreased significantly when compared with group D (Table 2 and 3).

The mean value of parietal cells counts (74.19 ± 5.36) was significantly increased when compared with group A and decreased with group D. The mean values of the chief cells count (75.46 ± 2.54) was significantly increased when compared with group C and decreased when compared with group D. (Table 3).

C) GUM ACACIA FOLLOWED BY FENOPRON GROUP: This group served as control group to compare the protective action of paracetamol. Gum acacia is an inert substance used for the preparation of suspension of fenopron and paracetamol, because have very little solubility in water. It is presumed that it may be having some protective effect on the gastric mucosa. Similar type of changes was observed as we have seen in group B with regard to the type of the erosion, the colour and histological characteristics but their intensity was decreased.

Under dissecting microscope, the median erosion score (2.30 ± 0.12) was significantly decreased when compared with group B (Table 1).

Under laboratory microscope, all the three layers of gastric mucosa showed similar type of changes as seen in group B except decreased inflammatory exudate. The mean mucosal thickness (360 um ± 15.86) was significantly decreased when compared with group A and D (Table 3).

The mean value of the surface mucous cell count (29.06 ± 1.20) was decreased significantly as compared with group A and D, whereas their mean height (11.23 urn ± 0.59) was significantly decreased as compared with group D (Table 2 and 3).

The mean value of the parietal cells count (75 ± 3.14) was significantly increased when compared with group A and decreased significantly when compares with group D.

The mean count of the chief cells (59.39 ± 5.58) was decreased significantly when compared with groups A, B and D Table 3).

In the submucosa there was inflammatory exudate as compared to group B. No abnormality was detected in muscle coat and serosa.

D) PARACETAMOL FOLLOWED BY FENOPRON: On naked eye examination, the stomach contained normal pale yellowish gastric secretions without any food content. Mucosal surface appeared normal under dissecting microscope, slight dilatation of blood vessels was observed in the fundic area of the stomach.

Under laboratory microscope, mucosa showed slight grade of pyknotic nuclei in the lining columnar epithelium. Glands and muscularis mucosa appeared normal, whereas mean mucosal thickness (493.6 urn ± 18.08) was significantly increased when compared with groups B and C (Fig 4).

The mean value of surface mucous cell count (53.16 ± 2.99) and their mean height (14.18 urn ± 0.21) were significantly increased when compared with group B and C (Table 2 and 3). The mean value of mucous neck cell count (34.36 ± 1.11) was significantly increased when compared with group A and B, however, their mean height (10.27 urn ± 0.25) was significantly increased when compared with group A (Table 2 and 3).

The mean value of the parietal cell count (87.99 ± 4.22) was significantly increased when compared with group A and C.

The mean value of the chief cell count (116.86 ± 3.97) was significantly increased when compared with all other groups, whereas their mean height (9.16 urn ± 0.14) was significantly muscle coat and serosa were normal.

DISCUSSION:

The hypothesis about the protection of gastric mucosa by paracetamol against ulcerogenic agents was studied cither biochemically or by dissecting microscope. By these methods microscopic picture of the gastric mucosa was not clear. As we know that an ulcer and erosion cannot be differentiated under dissecting microscope, so it was difficult to know about the actual damage to the gastric mucosa as well a secretory glands, the present study was designed to observe the protective effect by localizing the site of lesion under dissecting microscope, and there after subjecting these sites to the detailed morphological study with the help of laboratory microscope for change in the thickness of the mucosa, in the epithelium and gastric glands with reference to various secretory cells. To observe the effect of suspending material (gum acacia) another group (C) was included in this study.

As regard the protective dose of paracetamol the data available shows considerable variations presented by different workers. For example, one group of worker[5] observed 30 mg/kg body weight as decreasing the damaging effect of aspirin and 150 mg/kg body weight as complete protective while another group [8] found that 80 mg/kg body weight decreases ulcerogenic effect significantly. Similarly, findings of a group[15] reveal 500 mg/kg body weight as protective dose. While in our study 250 mg should complete protection and under dissecting microscope by administering different doses of paracetamol (ranging between 30 mg kg body weight and 300 mg/kg body weight) against fenopron. But we came to know that this is no complete protection as gum acacia (the suspending material) also showed significant decrease in the erosion score which did not catch the attention of these workers.

The mucous might have provided a protective shield or barrier between the damaging agent and the surface mucosa. This is in the agreement with the finding of a previous study[10] based on the culture of the fundic epithelial cells which showed significant increase of mucous by $P6E_2$ in the gastric mucosa of the rat. Probably this prostaglandin is responsible for increase mucous secretion because two different groups of workers[811] reported that no protection was observed when they administered indomethacin (Prostaglandin in hibitor) in their study against ulcerogenic agents. While prostaglandin itself have also been reported as protecting agents by group of worker 12 against different ulcerogenic, erosive agents and even against boiling water. Our study is in

agreement with these workers because we have found increase in number height of surface mucous cells as well as increase neck cells in group D as compare to group B and C which reveals their secretory activity, that mean increase secretion of mucous by those cells.

The decrease in the number of chief cells in group B and C as compared to group A may be explained by comparative decrease of mucous neck cells (from which the chief cell differentiates) in that region, however, their increase in height reveals the state of active secretion. The increase in number and height of cells in group D as compare with group B suggests an elevated secretory activity of these cells i.e., secretion of pepsin which may be responsible for the flattening of surface mucous cells, with slight exfoliation at certain places in group D. Whereas, the mucosal thickness was significantly increased in group D as compared with group B and C, but it was relatively increased when compared with group A, is a sign of partial protection.

The decrease in the size and significant increase in number per unit area of parietal cells in fenopron treated group when compared with normal group A reflect the suppression in activity of these cells. Our this finding could be correlated to the study[12] which states that the protection of gastric mucosa is neither by decreasing the gastric secretion nor by the amount of hydro chloric acid.

In the end we have concluded that paracetamol provides partial protection to the gastric mucosa in the dose of 250 mg/kg body weight. The variations in the effect of fenopron and the previous studies can be presumed to be cither due to difference in the cellular response or their mode of action on different type of cells. So the present study may act as a base line for the extension of the project to the human beings which may bring fruitful results in minimizing the danger of erosion when ulcerogence drugs are used in combination with paracetamol, even if are used with empty stomach.

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TABLE I

* MEDIAN EROSION SCORE OF RACTIFIED SPIRIT

GROUPS	MEDIAN EROSION SCORE
A	0
B	2.50 ± 0.44
C	2.30 ±0.21
D	O

* Median erosion score ± standard error

STATISTICAL COMPARISON BETWEEN

B & C	P< 0.02

TABLE 2

MEAN VALUES OF MUCOSAL THICKNESS AND COUNT

GROUPS	MEAN MUCOSAL THICKNESS (UM	SURFACE MUCOUS CELL COUNT	MUCOUS NECK CELL COUNT	PARIETAL CELL COUNT	CHIEF CELL COUNT
A	515.0 ±33.28	50.60 13.13	27.80 1 2.78	51.2013.39	80.415.38
B	400.8 ± 17.7	31.1311.43	24.9912.45	74.1915.36	75.46 1 2.46
C	360.8 ± 15.86	29.0611.20	30.7314.96	75.013.14	55.3915.58
D	493.6 ± 18.08	53.1612.99	34.8611.11	87.9914.22	116.8613.97

* MEAN ± STANDARD ERROR

STATISTICAL COMPARISON BETWEEN

A & B	P < 0.01	P < 0.001	P < 0.5	P < 0.01	P <0.5
A & C	P< 0.001	P < 0.001	P < 0.5	P < 0.001	P <0.02
A & D	P < 0.5	P < 0.5	P < 0.05	P < 0.001	P < 0.001
B & C	P <0.1	P <0.1	P <0.1	P <0.1	P < 0.05
B & D	P < 0.01	P < 0.001	P <0.01	P < 0.05	P < 0.001
C & D	P < 0.001	P < 0.001	P <0.1	P < 0.05	P < 0.001

TABLE 3

*** MEAN HEIGHT (um) OF SECRETORY CELLS**

GROUPS	SURFACE MUCOUS CELLCOUNT	MUCOUS NECK CELLCOUNT	PARIETAL CELL COUNT	CHIEF CELL COUNT
A	12.55 ±0.74	8.89 ±0.24	12.36 ±0.29	8.45 ± 0.27
B	12.32 ±0.38	9.78 ± 0.22	11.53 ±0.29	8.88 ±0.29
C	11.23 ±0.59	9.43 ±0.31	11.29 ±0.93	8.95 ±0.11
D	14.18 ± 0.12	10.27 ±0.25	11.67 ± 0.16	9.16 ± 0.14

* MEAN ± STANDARD ERROR

STATISTICAL COMPARISON BETWEEN

A & B	P < 0.5	P < 0.05	P < 0.5	P<0.1
A & C	P<0.1	P<0.1	P < 0.5	P<0.1
A & D	P < 0.05	P < 0.001	P < 0.05	P < 0.05
B & C	P<0.1	P < 0.5	P < 0.5	P < 0.5
B & D	P< 0.001	P<0.01	P < 0.5	P < 0.5
C & D	P< 0.001	P < 0.05	P < 0.5	P<0.1

FIGURE 1: Photograph of gastric mucosa showing areas of erosions (E), marked by brownish black pigment in an animal treated with fenopron. (Photograph x 10).

FIGURE 2: Section of gastric mucosa stained with H & E, showing entire thick ness of gastric mucosa in a normal control animal. (Photomicrograph x 160).

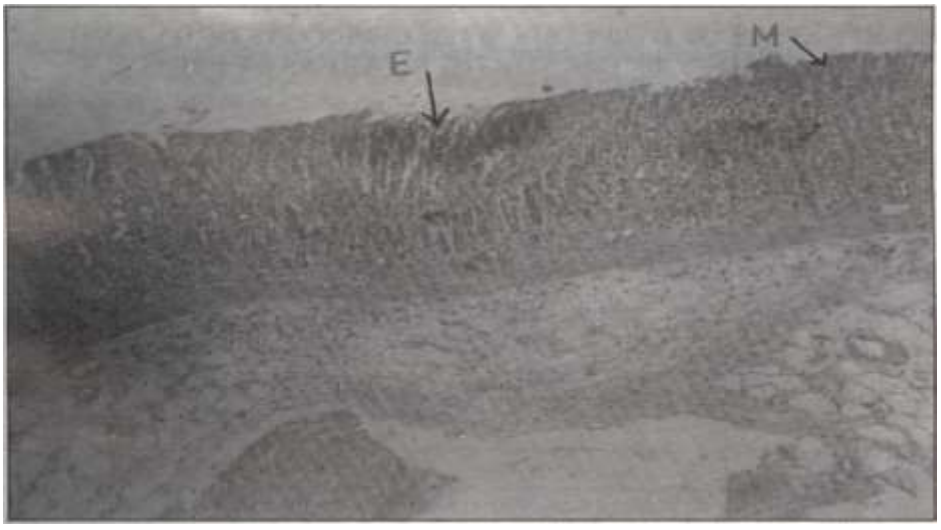

FIGURE 3: Showing section of the stomach stained PA.S., showing an acute erosion (E) with mucous contents (M) in an animal treated with fenopron. (Photomicrograph x 64)

FIGURE 4: Section of gastric mucosa stained with H & E showing flattening of surface epithelial lining with pyknotic nuclei (arrow) in an animal treated with paracetamol followed by fenopron. (Photomicrograph x 160).