

ROLE OF PARACETAMOL AGAINST THE EROSIIVE ACTION OF RECTIFIED SPRIT ON STOMACH IN ALBINO RATS

Umar Draz and M.Zahoor Janjua

ABSTRACT

The protective role of paracetamol against rectified spirit (non specific injuring agent) on the gastric mucosa of albino rat was observed under dissecting as well as laboratory micro-scope. A dose of 250 mg/kg body weight provided complete protection under disseting micro-scope. However, gum acacia with rectified spirit treatment provided significant decrease in lthe erosion score. Under laboratory microscope a significant increase in the thickness of the mucosa was observed in combined treatment of paracetamol with rectified spirit as compared to rectified spirit alone or gum acacia with rectified spirit. A slight exfoliation and flatterning of the surface epithelium may probably be due to increase in the secretory activity of chief cells. A significant increase in the secretory activity of the mucous neck cells in the group treated with rectified spirit plus paracetamol may be attributed to the increased bio-synthesis of prostaglandin from the mucous neck cell, which provides partial protective role against ulcerogenic agent.

INTRODUCTION

Most of the non-steroidal anti-inflammatory drugs (NSAID) can cause erosions and ulcer in the gasteroin-testinal tract⁵. Acetaminophen has been a suitable substitute for aspirin for its analgesic and antipyretic use in patients in whom aspirin is contraindicated e.g. those with peptic ulcer⁴. In human a single dose of acetaminophen significantly reduced gastric mucosal damage (measured by net ion reflex, potential difference and endo-scopeic examination) produced by acidified aspirin and ethanol. The protective effect by acetaminophen pretreatment may be either due to stimulation of endogenous gastric mucosal prostaglandin production or prevention of mucosal prostaglandin degradation⁸. So it was presumed that paracetamol may also protect the gastric nucosa against the non-specific injuring influence. In some of the Asian countries including Pakistan rectified spirit is commonly used as a substitute for alcohol. Therefore, the present study is designed to test theeffect of paracetamol on the erosive activity of the rectified spirit in gastric mucosa.

Adult male albino rats weighing between 200 and 350 grams, bred in animal house of Jinnah Post Graduate Medical Centre, Karachi were used in the present study. Paracetamol was administered as suspension in one per cent. gum acacia in distilled

From: Ayub Medical College, Abbottabad- Pakistan.

UMAR DRAZ, MBBS, Assistant Professor, Department of Anatomy.

M.ZAHOOR JANJUA Prof. Basic Medical Sciences Institute JPMC Karachi.

MATERIALS & METHODS:

water by oral intubation in a volume of one ml per 100 gm. body weight. The protected dose of paracetamol was assessed by administering different doses, (Ranging between 30-250 mg/kg body weight against rectified spirit). The dose of 250 mg/kg body weight was found optimum to protect mucosal lining of the stomach and was used for further detailed study. Rectified spirit was administered in a dose of 0.5 ml per 100 gm body weight¹⁰.

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

GROUP 'A': Served as normal control, were given gum acacia in the volume as used in the treated group.

GROUP 'B': These animals received rectified spirit only.

GROUP 'C': These animal received gum acacia in same volume as used for paracetamol followed by rectified spirit.

GROUP 'D': These animals received Paracetamol followed by rectified spirit.

The erosions produced by rectified spirit was observed under dissecting microscope and assessed according to the procedure adopted by van kalfschoten et al., 1983 and score was given to erosions according to arbitrary scale designed by Bonta¹.

ARBITRARY SCALE OF BONTA

Approximate	Diameter of Erosion/ Ulcer in mm.	Score
Less than	1	0.5
	1 - 2	1
	2 - 3	2
	3 - 4	4
More than	4	8
Perforation		12

The cumulative score of one group was divided by num of animal and expressed as median erosion score of the group. The erosions were subjected to the detailed histological study with the help of laboratory microscope to observe the change in the thickness of mucosa the distribution and height of the surface mucous epithelium, mucous neck, chief and parietal cells of the gastric gland to assess their secretory activity. The general morphology of the erosions was studied under 6 micron thick paraffin embedded H & E stained sections and the count of secretory cells in PAS stained section. The cell count was done under 40X objective and 8X ocular in a strip

covering the whole field measuring 150m in width extending from the surface to the base of the gastric gland.

STATISTICAL ANALYSIS OF THE DATA

The statistical significance of difference of various quantitative changes between experimental groups and control was evaluated by student "t" test using casio FX-350 scientific calculator. The difference was regarded statistically significant if the P' value was equal to or less than 0.05. th P' value was found by means of t' distribution table.2

OBSERVATIONS & RESULTS

(A) NORMAL CONTROL GROUP: Opening along lthe greater curvature, the stomach in seven animals revealed food contents mixed with pale yellow fluid. However, in the remaining three animals little muddy coloured food contents were observed. Under dissecting microscope the internal surface of each stomach was clearly identified into two parts by a raised ridge. The greyish white parts continuous with the oesophagus was squamous part (rumen) while the pink area raised into fold (rugae) continous with duodenum was identified as secretory or glandular part mucosal thickness was 548.0 um 29.39 (table 3). The surface mucous cells were tall columnar lying on the basement membrane, eosinophilic granular cytoplasm. The nuclei were round to oval in shape and basaly located. Their mean cell count was 52.60 3.82 with the mean height of 12.35 um 0.57 (table 2 & 3). The round to tubular gastric glands were lined by three type of cells; the mucous neck cells parietal cells and chief cells. Mucous neck cells were deformed by the neighbouring cells and therefore tend to be quite irregular in shape with wide base and narrow apex or broad apex and narrow base with long axes of the nuelei perpendicular to the long axis of the cells. Their mean cell count was 28.20 2.96 while their mean height was 9.05 un 0.25 (table 2 & 3). The large parietal cells with ight pink eosinophilic cytoplasm and central nuclei, lie at adluminal side between chief cells and basement membrane at the base of the gland. Their mean cells count observed was 55.80 2.80 while mean size was 12.20 um 0.33 (table 2&3). The pyramidal shaped chief cells with round or oval nuclei showing basophilic granular cytoplasm and apical vacuolation were located at the base of the gland. The mean values of the chief cells count was 77 6.13 unit area with the mean height of 8.13 um 0.27 (table 2&3).

(B) RECTIFIED SPIRIT TREATED GROUP

Opening the stomach along the greater curvature, red elongated spots were visible with naked eyes on the fundic part of the stomach. Under dissecting microscope 4-6 elongated red coloured haemorrhagic spots were seen on the convexities of the various rugae of the fundic area. Moreover, few spots were also seen on the antrum. The neighbouring rugae showed numerous spider web like dilated tortuous blood vessels. No such spots were observed on rumen. The median erosion score was claculated as 4.10 0.42 (Table 1).

TABLE 1 MEDIAN EROSION SCORE OF RATIFIED SPIRIT

GROUPS	MEDIAN EROSION SCORE
A	0
B	4.10+0.42
C	2.8+0.25
D	0

P value for B and C < 0.05

TABLE 2 MEAN HEIGHT(um) OF SECRETORY CELLS

Group	Surface mucous cells	Mucous neck cells	Partial cells	Chief cells
A	12.35±0.57	9.05±0.25	12.20±0.33	8.13±0.27
B	10.68±0.67	8.90±0.42	12.26±0.26	9.07±0.25
C	10.84±0.49	9.74±0.37	12.45±0.21	9.88±0.18
D	12.17±0.25	10.1±0.31	12.43±0.12	9.14±0.17

TABLE 3 MEAN VALUE OF MUCOSAL THICKNESS AND COUNT OF SECRETORY CELLS

Groups	Mean mucosal thickness (um)	Surface mucosal cell count	Mucous neck cell count	Partial cell count	Chief cell count
A	548.0±29.4	52.60±3.8	28.20±2.9	55.8±2.8	77.0±6.1
B	373.6±11.4	28.59±1.6	24.46±2.8	70.2±3.5	76.1±4.5
C	363.2±22.7	27.59±1.0	27.19±4.4	59.7±4.5	69.1±5.6
D	496.0±13.7	49.19±1.1	33.73±3.2	64.1±4.4	101±5.6

The mean mucosal thickness (373.6 um) 11.14) was significantly decreased when compared with group A and D (Table 3). Under laboratory microscope well marked erosive areas were observed in all animals. These areas contained congested and dilated blood vessels with numerous R.B.Cs in them. The epithelial lining was completely lost, but the outlines of the connective tissue elements of the lamina propria was left intact. the erosions infiltrated about 1/2 to 1/3 of the thickness of the mucosa. Marked degree of dilatation of blood vessels was observed in the mucosa which extended to muscularis mucosae as well. Inflammatory exudate including numerous lymphocytes, plasma cells a few neutrophils were observed in this area (Fig 2).

A significant decrease in the surface mucous cells count (28.59 1.56) was observed when compared with group A and D (Table 3). The mean height of these

cells (10.68um 0.67) was relatively decreased when compared with group A, C and D but it was not statistically significant (Table 2). the mean value of mucous neck cell count (24.46 2.89) was significantly decrease when compared with group D. The mean height of these cells (8.90 m 0.42) was also significantly decreased as compared with group D (Table 2 & 3). The mean value of parietal cells count (70.19 3.47) was significantly increased when compared with group A. The mean size of these cells (12.26 um 0.26) did not whown statistically significant difference when compared with the other groups (Table 2 & 3). The mean value of the chief cells count (76.06 4.54) was significantly decreased when compared with group D (Table 3) where as the mean height of these cells (9.07 m 0.25) was increased significantly when compared with group A and decreased when compared with group C.

The sub-mucosa showed numerous lymphocytes, eosinophils, neutrophils, plasma cells, few macrophages and numerous dilated blood vessels.

(C) GUM ACACIA FOLLOWED BY RECTIFIED SPIRIT GROUP:

The stomach was found empty on naked eye examination, and was Similar to that found in group B. Under dissecting microscope the median erosion score (2.80 0.25) was significantly decreased when compared with group B (Table 1). Under laboratory microscope mucosa showed similar type of findings as observed in group B. The mean mucosal thickness (363.3 um 22.8) was significantly decreased when compared with groups A and D (Table 3). The mean value of surface mucous cell count (27.59 0.99) Was significantly decreased when compared with group A and D. While the mean height of these cells (10.84 um 0.49) was not significant statistically when compared with other groups (Table 2 and 3). The mean value of the parietal cells count (59.66 4.54) and mean size of these cells (12.45 um 0.21) showed no statistically significant difference when compared with other groups (Table 2 and 3). The mean value of chief cells count (69.3 5.60) was significantly decreased when compared with group A, B and D (Table 2 and 3).

The submucosa showed inflammatory exudate similar to group B, the muscle coat a serosa appeared normal.

(D) PARACETAMOL FOLLOWED BY RECTIFIED, SPIRIT GROUP.

On examination of the stomach, it contained normal gastric secretions without food contents. The mucosa was normal looking on naked eye examination. Under dissecting microscope numerous spider like dilated blood vessels were observed on the anterior and posterior surfaces of the fundac area. These veessels were also ovserved on antral area in few cases. Under laboratory microscope the mucosal lining epithelium showed slight exfoliation at places. The nuclei became pyknotic at places and marked grade of dilatation of blood vessels was observed (Fig-3).

The mean mucosal thinkness (496 um \pm 13.7) was significantly increased when compared with group B and C (Table 30). The mean value of surface mucous cells count (49.19 \pm 1.06) was significantly increased when compared with group B and C while the mean height of these cells (12.17 UM \pm 0.25) showed no statistically

significant difference when compared with other groups (Table 2 and 3). The mean value of mucous neck cells count (33.73 ± 3.20) was significantly increased as compared with group B. However, the mean height of these cells ($10.12 \mu\text{m} \pm 0.31$) showed significant increase when compared with group A and B (Table 2 and 3).

The mean value of parietal cells count (64.06 ± 4.41) and height ($12.43 \mu\text{m} \pm 0.12$) showed no statistically significant difference when compared with other groups (Table 2 and 3).

The mean value of the chief cells count (100.93 ± 5.96) was significantly increased when compared with groups A, B and C respectively. While the mean height ($9.14 \mu\text{m} \pm 0.17$) of these cells was significantly increased when compared with group A and C (Table 2 and 3). The submucosa, muscle coat and serosa appeared normal.

DISCUSSION

Previous work 3,7,8,10 on the hypothesis of protection of gastric mucosa by paracetamol against ulcerogenic agents was just superficial i.e. by measuring various gastric secretions biochemically or by observing the changes under dissecting microscope. In the view of these pitfalls the present study was designed to observe the protective effect of paracetamol against rectified spirit, by localizing the site of lesion under dissecting microscope and thereafter subjecting these sites to the detail morphological study with the help of laboratory microscope for changes, in the thickness of the mucosa, in the epithelium of gastric gland with reference to various secretory cells.

As regard the protective dose of paracetamol the data available showed wide range of variations by different investigators. One group of workers³ observed that the dose of 80 mg per kg body weight significantly decreases the ulcerogenic effect of aspirin and absolute ethanol while another group⁷ observed that the dose of 150 mg per kg body weight completely protect the stomach against the effect of aspirin. However a third group¹⁰ used 500 mg per kilogram body weight to study the protective effect and observe partial protection against indomethacin and complete protection against ethanol (66% v/v). However in the present study there was complete protection against rectified spirit by dose of 250 mg/kg body weight in albino rat under dissecting microscope but this proved to be partial under laboratory microscope. This observation is supported by the experimental results through the inclusion of an extra group - C in our study which did not catch the attention of previous workers.

The mucosal thickness increased significantly in group treated with Paracetamol plus rectified spirit as compared to the group B and C but was relatively decreased when compared with normal control group. This also shows partial protection of gastric mucosa by paracetamol. The height and number of surface mucous cells showed significant increase in group D as compared with group B, which reveals that there has been recovery in the secretory activity of the cells under paracetamol treatment.

The decrease in the height and number of mucous neck cells in group B reveal the ulcerogenic effect of rectified spirit on the gastric mucosa which has decreased the activity of these cells. While the increase in the number and height of these cells in

group D as compared to group B and even to normal control (group A) showed elevated secretory activity of mucosa by mucous neck cells which might have provided a protective shield of barrier between damaging agent and the surface mucosa. This is in partial agreement with a study⁹ based on the culture of fundic epithelial cells which showed increased mucous by prostaglandin E_2 in the gastric mucosa of the rat. This prostaglandin may be responsible for the protective effect of paracetamol because few workers⁸ reported that no protection was observed when they administered indomethacin (a prostaglandin inhibitor) before the administration of paracetamol against ulcerogenic agents. Similar findings were reported by another group of workers⁶ and they observed protection of gastric mucosa by prostaglandin against ethanol, 0.6 N hydrochloric acid, 0.2 N sodium hydroxide, 25% sodium chloride and even boiling water.

The increase in the number and height of the chief cells in group D as compared to group B suggests increased pepsin secretion from the gastric mucosa. This might be responsible for the flattening of the surface mucous cells and slight exfoliation at certain places in group D, a sign of partial protection by paracetamol. The decrease in the size and significant increase in number per unit area of parietal cells in group B reflect the suppressive activity of these cells, whereas relative increase in number without affecting the size of the cells in group D was observed when compared with group B which reveals no significant effect on the production of hydrochloric acid of stomach. This finding could be correlated with some workers⁶ who stated that the protection of gastric mucosa is neither by decreasing the gastric secretion nor by the amount of hydrochloric acid.

On the basis of discussion above we can say that paracetamol provides partial protection to gastric mucosa in albino rat in the dose of 250 mg per kg body weight. This dose appears very high but it is specific for the species used because it is said that smaller the animal higher the dose needed to produce desired effects. This may act as base-line information for the extension of the project to the humans which may bring fruitful results in minimizing the danger of erosion when ulcerogenic agents are used in combination with paracetamol in patients.

REFERENCES

1. Bonta, L.I. A study of the effect of some glucocorticoids and ACTH on the artificially induced gastric ulcers of the rat, *Arch. Int. Pharmacodyn.*, 132: 147-163.
2. Burn, J.H. Lecture notes on pharmacology 6th edition, Oxford, Blackwell Publication, 1961.
3. Konturek, S.J., Brzozowski, T., Plastuch, I. and Tradecki, Prevention of ethanol and aspirin induced gastric mucosal lesions by paracetamol and salicylate in rats: Rate of endogenous prostaglandin *Gut.*, 1982, 83, 536-540.
4. Peter, B.H. Frain, C. J and Morel, B.E. (1983) comparison of 650 mg aspirin and 1000 mg acetaminophen with each other and with placebo in moderately severe headache. *Am J. Med.*, 74 (6A) 36-42.
5. Prescott, L.F. (1976) Anti-inflammatory and analgesics and drugs used in the treatment of Rheumatoid arthritis and Gout. In *Meylet's side effect of drugs* (M.N.G. Duker, ed) *Excerpta Medica Amsterdam* pp 207-240.
6. Robert, A., James, E.N., Cleo, L., Alexandor, J.H. Cytoprotection by prostaglandin in rats. *Gastroenterology*, 1979. 77: 433-443.
7. Seegers, A.J.M., Jager, L.P., Noord Wijk, T.V. Effect of phenacetin, paracetamol and acetylsalicylic acid in the rat stomach. Dose response relationship time course of erosion development

- and effect on acid secretion. *J. Pharm, Pharmacol*, 1979. 31:840-848.
8. Stern, A-I, Hogan, D.L., Khon, L.H. and Isenbergh, J.I., Protective effect of acetaminophen against aspirin and ethanol induced damage to human gastric mucosa, *Gastroenterology*, 1984; 86:728-733.
 9. Terano, A. Ivey, K.J., Stachura, J., Mckenzie, W.N. Krause, W.J. Herojina, H. Wyche, J.H. and Sekhon, S. Cell culture of rate gastric mucosa. *Gastroenterology*. 1982; 82:1195(abstract).
 10. Van Kolschoten, A.A., Zandberg, V.P., Jaggar, I.P., Noordwijk, J.V. Protection by Paracetamol against various gastric irritants in the rat. *Toxicol. Appl. Pharmacol*, 1983, 69:73-42.