Acute Gastrointestinal Toxic Effects of Suspensions of Unencapsulated and Encapsulated Ibuprofen in Rats

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Purpose. The study examined the gastrointestinal (GIT) toxicity effects of suspensions of encapsulated and unencapsulated ibuprofen in male Wistar rats.

Methods. Rats were randomly divided into four experimental groups and four control groups, and dosed with suspensions of encapsulated and unencapsulated ibuprofen (17 mg/kg and 44 mg/kg). Bethanechol chloride, a cholinomimetic agent (5 mg/kg), was administered 30 minutes after the dosing, to induce gastric irritation. Blood plasma concentrations were monitored in another set of rats for 12 hours using the encapsulated and unencapsulated systems, to establish drug release and exposure to the mucosa.

Results. Evaluation of the upper GI segments after 7 hours revealed that the 44 mg/kg dose of the encapsulated drug significantly reduced the number of lesions present compared to the unencapsulated drug ($p < 0.05$). At 17 mg/kg, the encapsulated drug reduced toxicity, but not significantly compared to the unencapsulated ibuprofen. Necrosis of the mucosa was observed histopathologically in the unencapsulated drug at both doses, whereas the encapsulated drug treatment revealed preserved mucosa. The encapsulated system had a maximum plasma concentration, Cmax, and time taken to reach Cmax, (Tmax) of 26.7 μg/ml ± 1.5 and 3.6 ± 0.2 hr, respectively. The area under the plasma concentration-time curve, (AUC_{0-12}), was 158.8 ± 23.5 μg·h/ml, confirming drug release and absorption.

Conclusions. Encapsulation of ibuprofen significantly reduced gastrointestinal toxicity especially at the higher dose level and drug was released enough to subject the GI mucosa to irritation, but without the usual toxic effects.

KEY WORDS: ibuprofen; microspheres; suspension; drug release; gastric lesions; rats.

INTRODUCTION

Ibuprofen, a non steroidal anti-inflammatory drug (NSAID), is available as either a suspension, syrup, or immediate release tablets. It is commonly prescribed as an anti-inflammatory drug for the treatment of such diseases as arthritis, rheumatism, and as an analgesic or antipyretic (1). However, ibuprofen, in the conventional dosage forms, induces gastrointestinal (GIT) ulcers and bleeding, especially in the elderly and pediatric populations for which the drug is most often prescribed (2). As an aspect of preformulation in drug development and dosage formulation, pharmaceutical technology processes, such as encapsulation, coating, or other modification of the drug release into a controlled or sustained release product, have been employed with the intent of decreasing NSAID-induced gastrointestinal toxicity (3, 4, 5). These processes make it possible for less drug to be in contact with the gastric mucosa per unit time than the conventional or immediate release forms (6).

Evaluations of drug-induced gastrointestinal toxicity for various NSAIDs have been reported by several investigators (7, 8, 9, 10). Rainsford (7), for example, studied the ulcerogenic activity of various NSAIDs in stressed rats (exposure to cold temperature) and reported that six drugs (azapropazone, benoxaprofen, meclofenamic acid, oxaprozin, proquazone and sulindac) did not produce gastric ulcerogenic activity. However, ibuprofen was shown to induce gastric damage and this was thought to be due to the inhibition of prostaglandin biosynthesis. In a later study, Rainsford used a cholinomimetic agent (bethanechol) to induce gastric irritation in mice instead of exposure to cold temperatures. Numerous lesions and hemorraghic areas were observed, as measured by visual image analysis (8). In another study, Fujii *et al.* used gastroscopy to determine the temporal relationship between gastric damage and selected NSAIDs (aspirin, indomethacin, mefenamic acid and fenoprofen calcium) (9). They reported that mefenamic acid showed the least gastric mucosal damage, whereas indomethacin produced greater gastric damage than aspirin. Leyck, *et al.* (10) coadministered polyene phosphatidylcholine (Phospholipon® 100) along with various NSAIDs in assessing gastric toxicity. The aim of their study was to protect the mucosa with phospholipid since it has been reported that the phospholipid content, especially that of phosphatidylethanolamine, is markedly reduced in the gastric mucosa of rats with stress-induced ulcers (11) and that lipid intake ameliorated gastric ulceration (12). Leyck and coworkers (10) found improved gastric tolerance to the NSAIDS used, however, in all these reports, only the unencapsulated drug was used.

Most reports on the use of novel drug delivery systems in reducing NSAID-related gastrointestinal toxicity have been extrapolations from *in vitro* drug release studies. Studies to correlate the *in vitro* release with potential for reduced toxicity using controlled-release delivery systems *in vivo* have not been widely documented. Since toxicity is due to a great extent on the rate of drug release, release mechanism, and amount of drug that is exposed to the GIT mucosa, it is important to establish that the drug has been released and subsequently absorbed into the blood, and that the results obtained are not due to insufficient or lack of drug release from the drug delivery matrix. The present study was designed to compare the gastrointestinal toxicity effects of encapsulated (microspheres) and unencapsulated ibuprofen suspensions, and to establish that the drug was released from the wax matrix into the GIT by determining relevant pharmacokinetic parameters.

MATERIALS AND METHODS

Materials

Ibuprofen was obtained as a gift from Boots Company (USA), Shreveport, Louisiana, while bethanechol hydrochloride and methylcellulose were purchased from Sigma Chemical Company. Ceresine wax was donated by Frank Ross Waxes of New Jersey and buffered formaldehyde fixing solution was

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purchased from Fisher Scientific Company of Pittsburgh, Pennsylvania. All other reagents used were of analytical grade.

Male Wistar rats, weighing between 250 and 300 grams, were purchased from Hilltop Laboratories, Scottdale, PA and were acclimated to the animal care facility for several days. Animals were maintained on a 12-hour light-dark cycle. They were fed a standard rat diet and given water *ad libitum.* The animal protocols were approved by the Duquesne University Animal Care and Use Committee.

Methods

Preparation of Microspheres and Suspensions

The microspheres used were prepared by the congealable disperse microencapsulation process in which the drug particles were coated with paraffin wax (3). Respective amount of microspheres corresponding to the drug used in the toxicity or absorption studies is equivalent to 172 mg/g of microspheres. There was batch to batch reproducibility of this formulation as reported by Adeyeye and Price (4). The microspheres (165 μm) were suspended in methylcellulose (MC) solution containing 1% Tween 80. The unencapsulated drug (48 μm size) suspension was similarly prepared using MC solution.

In Vitro Drug Release

Drug release was first determined *in vitro,* using simulated intestinal fluid and a modified USP apparatus type II in which the microspheres were placed in mini stainless steel baskets as reported by Adeyeye and Price (4).

In Vivo Evaluation

A plasma concentration-time profile was determined to confirm the release of drug from the wax matrix. Four precannulated male Wistar rats were fasted overnight and administered a suspension of encapsulated microspheres, equivalent to 20 mg/kg of ibuprofen, via gastric gavage. Serial blood samples were obtained using the cannulated jugular vein at 0, 0.25, 0.5, 0.75, 1, 2, 4, 6, 8, 12 hours after drug administration. Blood samples were centrifuged and the plasma was frozen at −20°C until the ibuprofen assay was performed. The assay used to quantitate ibuprofen blood concentrations was a HPLC method previously reported by Mehvar, *et al* (13). The drug concentration was recorded as total plasma concentration, although the assay method could distinguish individual enantiomers. To determine the extent of drug absorption, such parameters as maximum plasma concentration (Cmax), time to reach Cmax (Tmax), and area under the plasma concentration-time curves (AUC_{0-12}) which was estimated using the trapezoidal rule, were calculated. Unencapsulated ibuprofen suspension was used in a cross-over study and the resultant pharmacokinetic parameters were used for comparison with the data obtained from the encapsulated drug suspension.

Determination of Acute Gastrointestinal Toxicity

To determine the effect of encapsulated and unencapsulated suspensions of ibuprofen on gastrointestinal toxicity, a 2 × 2 factorial experimental design consisting of two treatments (dose and dosage form) was used. Male Wistar rats, weighing between 250 and 300 grams, were fasted overnight and randomly divided into eight groups (A through H).

Groups A through D were used as control groups (N = 4 rats) and consisted of animals treated with saline only, bethanechol (BCL) only, suspension of blank microspheres alone, and suspension of blank microspheres and bethanechol, respectively (Table IV). Groups E and F (N = 6 rats) were given suspensions of unencapsulated ibuprofen at 17 mg/kg and 44 mg/kg, respectively. Groups G and H (N = 6 rats) were given suspensions of encapsulated ibuprofen at the same two doses, respectively. Additionally, Groups E through H were given bethanechol chloride (5 mg/kg; i.p.) 30 minutes after the dosing, to induce gastric irritation according to the method of Rainsford (8). Animals were euthanized, using ethyl ether, 7 hours post dosing followed by the excision of the stomach and intestinal tract.

Tissue Preparation and Quantitation of Lesions

The stomach and the first 12 cm of the duodenum were opened and carefully washed with saline to remove any residual debris and fixed in 10% buffered formaldehyde. Tissues were examined macroscopically (× 75 magnification) for lesions and hemorrhages using top and bottom illumination. The evaluation of lesions or hemorrhages was based on the Ulcer Index (UI) reported by Leyck *et al.* (10) and expressed as the sum total of lesions per treatment. The scoring units used ranged from 0 to 5, where 0 represents no macroscopically visible lesions; 1 represents one to three small hemorrhages less than 4 mm in diameter; 2 represents more than three small hemorrhages or one large hemorrhage greater than 4 mm in diameter; 3 represents one large (> 4 mm) and several small lesions; 4 represents several large (>4 mm) hemorrhages; and 5 indicates tissue perforation.

Representative stomach and duodenal tissue samples were embedded in paraffin wax, sectioned with a microtome, stained with hematoxylin-eosin, and observed under a 100 X magnification. An Erosion Index (EI) was developed for scoring the histological changes in these tissue samples. The EI units ranged from 0 to 3, where 0 represents normal gastric fundus and normal villous duodenum with preserved surface epithelium; 1 represents acute inflammatory infiltrate on the mucosal surface with small lesions present, but with some preservation of the mucosal cells; 2 represents acute inflammatory infiltrate on the mucosal surface; and 3 represents the presence of coagulative necrosis of the entire mucosal surface with acute inflammatory infiltrate.

RESULTS AND DISCUSSION

In Vitro Drug Release and Plasma-Concentration Time Profiles

The encapsulated ibuprofen drug suspensions were shown to be released slower compared to the unencapsulated drug. Figure 1A shows that the encapsulated drug was released in a controlled manner with an *in vitro* T_{50} (time taken for release of 50% of drug from within the ceresine wax matrix) of 4 hours. The drug release kinetics of the encapsulated formulation were found to follow the Higuchi square root model (i.e., linear relationship between drug release and square root of time), indicating that the drug was released from the matrix via diffu-

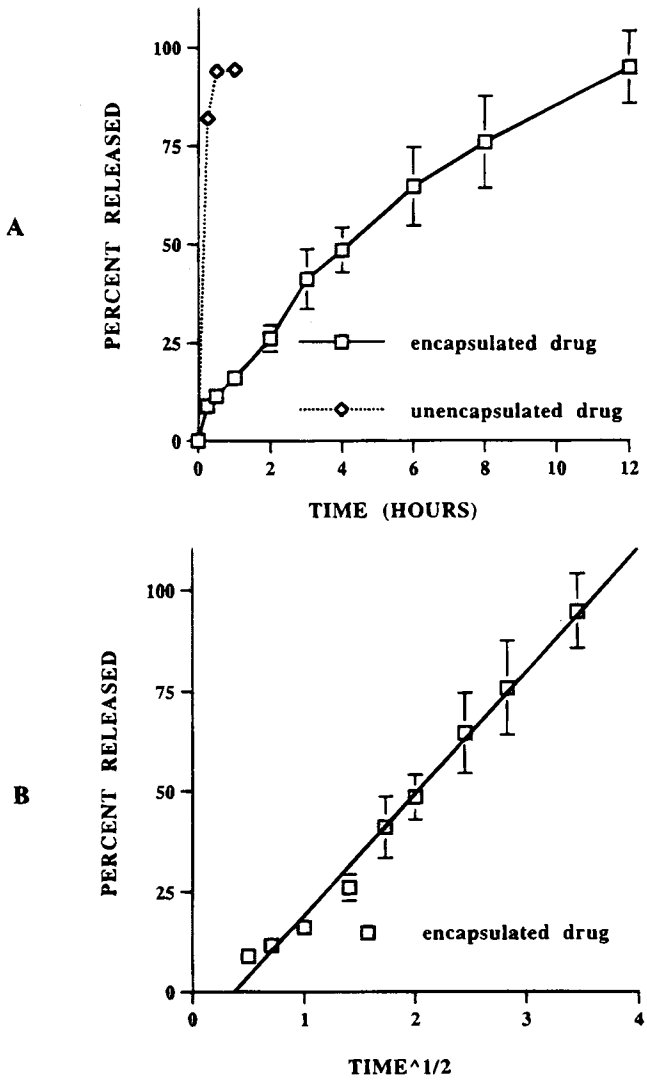


Fig. 1. In vitro drug release profiles of ibuprofen from encapsulated (wax coated) drug and unencapsulated drug (A); Higuchi square root of time model for drug release from encapsulated drug (B).

sion (Figure 1B). The results were as expected and correlated with results previously reported by Adeyeye and Price (4). *n vitro* dissolution of the unencapsulated ibuprofen was very rapid, reaching 87% in 15 minutes and 95% within 1 hour (Figure 1A).

Since one of the aims of this study was to establish that sufficient drug was released into the gastrointestinal tract to cause either direct or indirect toxicity, several *in vivo* pharmacokinetic parameters for encapsulated and unencapsulated ibuprofen suspensions were determined. Plasma concentration-time profiles and the respective mean pharmacokinetic parameters of the encapsulated and unencapsulated ibuprofen suspensions are reported in Table I and Figure 2. Unencapsulated drug suspensions was shown to absorb into the bloodstream very quickly with a Tmax of 0.32 ± 0.23 hr compared to the encapsulated drug suspension (Tmax = 3.6 ± 0.2 hr), even though the AUC_{0-12} values were similar for both suspensions within the same sampling period (Table I and Figure 2). The Cmax of the unencapsulated drug was also much greater (60.6 ± 2.33 μg/ml) than the encapsulated drug suspension (26.71 ± 1.5 μg/ml). These data clearly indicate that the gastrointestinal mucosa was exposed to less drug per unit time with the encapsulated drug suspensions. Nevertheless, the concentration in the plasma was still within the therapeutic range.

Table I. In Vivo Disposition Parameters of Rats Dosed with Oral Ibuprofen Suspensions (20 mg/kg)

	Suspensions of Encapsulated Drug	Suspensions of Unencapsulated Drug
	Mean ± S.D.	Mean ± S.D.
Cmax (μg/ml)	26.71 ± 1.5	60.6 ± 2.33
Tmax (h)	3.6 ± 0.2	0.32 ± 0.23
AUC_{0-12} (μg.h/ml)	158.83 ± 23.5	141.7 ± 12.5

Acute Gastrointestinal Toxicity

Macroscopic Observations

The number of lesions observed in each rat tested and the relative extent of tissue damage as expressed by the Ulcer Index scale (0 to 5) is presented in Table II for unencapsulated preparations and Table III for encapsulated preparations. The average number of lesions observed at each dose tested is presented in Figure 3. There are significant differences in the number of lesions present when comparing gastrointestinal tissues from rats treated with encapsulated drug suspensions to unencapsulated suspensions. Also, the number of lesions observed in animals treated at the low dose, 17 mg/kg (Groups

Table II. Macroscopic Lesions Observed 7 Hours Post Dosing in Individual Rats Dosed with Suspensions of Unencapsulated Ibuprofen, 17 mg/kg (Group E) and 44 mg/kg (Group F)

	Number of Lesions (Group E)					
	Rats					
Lesion Index	1	2	3	4	5	6
0	—	—	—	—	—	—
1	8	1	2	1	1	2
2	—	—	—	—	—	—
3	—	—	—	—	—	—
4	—	—	—	—	—	—
5	—	—	—	—	—	—
Total	8	1	2	1	1	2
	Number of Lesions (Group F)					
	Rats					
Lesion Index	1	2	3	4	5	6
0	—	—	—	—	—	—
1	29	22	18	12	23	21
2	—	—	1	—	7	2
3	—	—	—	—	2	—
4	—	—	—	—	—	—
5	—	—	—	—	—	—
Total	29	22	19	12	32	23

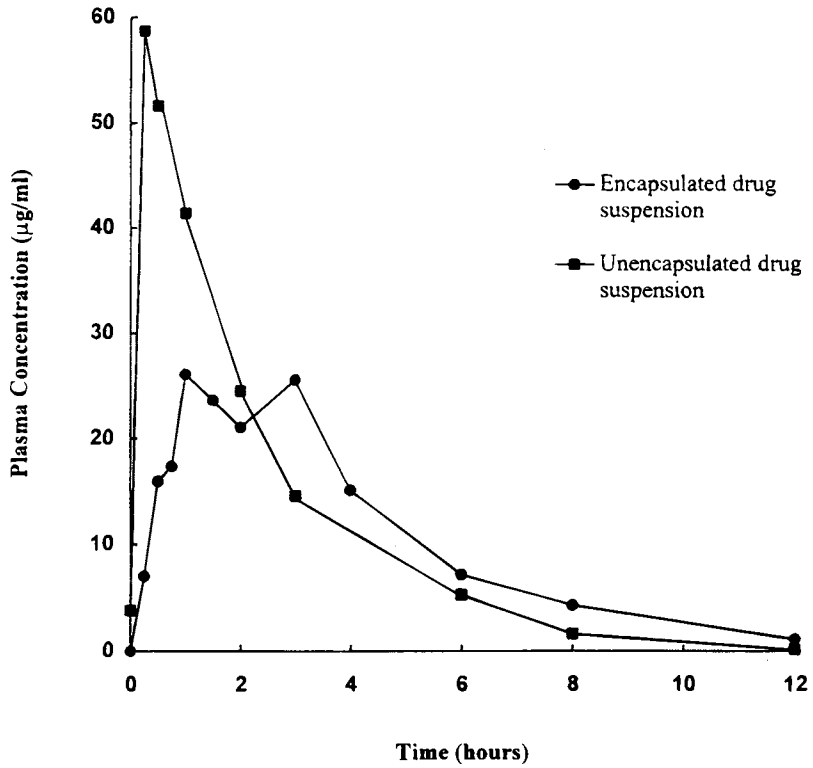


Fig. 2. Mean plasma concentration-time profiles of encapsulated and unencapsulated ibuprofen suspensions in rats.

Table III. Macroscopic Lesions Observed 7 Hours Post Dosing in Individual Rats Dosed with Suspensions of Encapsulated Ibuprofen, 17 mg/kg (Group G) and 44 mg/kg (Group H)

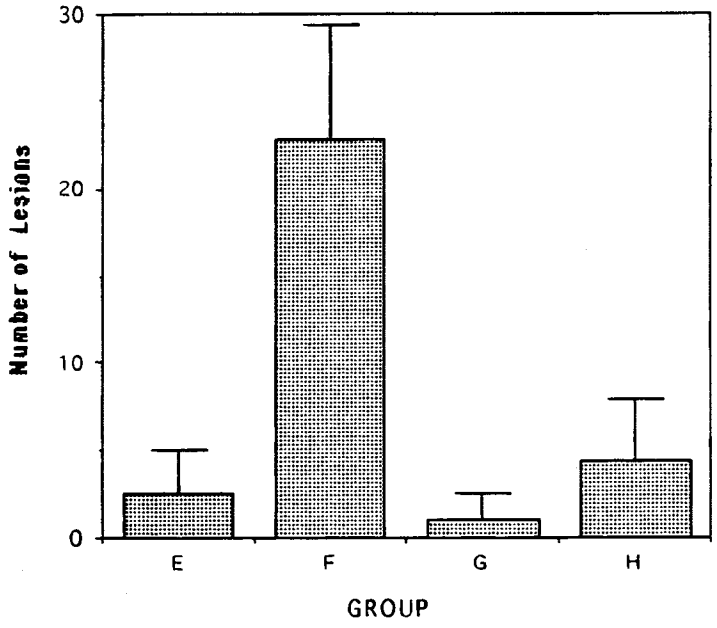
	Number of Lesions (Group G)					
	Rats					
Lesion Index	1	2	3	4	5	6
0	—	—	—	—	—	—
1	4	2	—	—	—	—
2	—	—	—	—	—	—
3	—	—	—	—	—	—
4	—	—	—	—	—	—
5	—	—	—	—	—	—
Total	4	2	—	—	—	—
	Number of Lesions (Group H)					
	Rats					
Lesion Index	1	2	3	4	5	6
0	—	—	—	—	—	—
1	1	1	2	10	7	6
2	—	—	—	—	—	—
3	—	—	—	—	—	—
4	—	—	—	—	—	—
5	—	—	—	—	—	—
Total	1	1	2	10	7	6

E and G), was significantly less than the number observed at the high dose, 44 mg/kg (Groups F and H), for both encapsulated and unencapsulated preparations. Statistical analysis of the stomach lesions (repeated measures design, ($p < 0.05$), based on the total number of lesions, revealed that the encapsulated ibuprofen suspension (44 mg/kg) significantly reduced gastric irritation and lesions when compared to the unencapsulated drug suspension at the same dose ($p = 0.014$). In rats that were given 17 mg/kg, the encapsulated form also reduced toxicity but to a lesser extent when tested at the same dose, compared to the unencapsulated drug suspension, ($p = 0.102$).

Table IV summarizes the treatment groups and presents the number of rats treated which produced any lesions, the total number of lesions per treatment group, and the Ulcer Index (UI) or total number of lesions divided by number of rats for each treatment group. The Ulcer Index for unencapsulated drug suspensions at the two dose levels (Groups E and F) were higher, 2.5 and 22.83, compared to 1.0 and 4.5 for the encapsulated drug suspensions (Groups G and H), respectively. Photographs of the macroscopic examination of stomach tissue of rats dosed with both preparations and controls are shown in Figures 4A to 4D; and 5A and 5B, respectively.

Microscopic Observations (Histopathology)

From the histopathology evaluation, an erosion index (EI) was developed to score the extent of the microscopic damage produced on the surface epithelium of the gastric and duodenal mucosa. The unencapsulated drug suspensions had the highest EI values of 3 compared to an EI of 0 for the encapsulated drug suspensions and controls. The stomach tissue sections of



E = 17 mg/kg unencapsulated ibuprofen suspension + Bethanechol 5 mg/kg
F = 44 mg/kg unencapsulated ibuprofen suspension + Bethanechol 5 mg/kg
G = 17 mg/kg encapsulated ibuprofen suspension + Bethanechol 5 mg/kg
H = 44 mg/kg encapsulated ibuprofen suspension + Bethanechol 5 mg/kg

Fig. 3. Mean number of lesions for all treatment groups.

Table IV. Summary of Treatment Groups and the Incidence of Macroscopic Lesions 7 Hours After the Drug Administration

Groups	Number of Rats with Lesions	Total Number of Lesions per Treatment Group	Ulcer Index
A (Saline)	0/4	0/4	0.00
B (Bethanechol-BCL)	0/4	0/4	0.00
C (Blank MC suspension)	0/4	0/4	00.00
D (Blank MC suspension + BCL)	0/4	0/4	0.00
E (17 mg/kg Susp. of unencapsulated drug + BCL)	6/6	15/6	2.5
F (44 mg/kg Susp. of unencapsulated drug + BCL)	6/6	137/6	22.83
G (17 mg/kg Susp. of encapsulated drug + BCL)	2/6	6/6	1.00
H (44 mg/kg Susp. of encapsulated drug + BCL)	6/6	27/6	4.50

the rats dosed with unencapsulated ibuprofen suspensions at 17 mg/kg and 44 mg/kg (Groups E and F) displayed necrosis of the mucosal surface even though macroscopy examination indicated numerous small lesions (Figures 6A and 6B). In contrast, the surface of the epithelial layers of the tissue sections of rats dosed with encapsulated drug suspensions (Groups G and H) were well preserved with the parietal and chief cells (secretory cells) and the villous architecture of the duodenum was intact (Figures 6C and 6D). The tissues from the control groups (Groups A, B, C, and D) showed normal gastric fundus and preserved surface epithelium and no mucosal erosion (Figure 7A and 7B).

CONCLUSIONS

Gastrointestinal toxicity associated with the use of ibuprofen or similar NSAIDs can be reduced by pharmaceutical technology processes such as microencapsulation, in which the drug is developed into coated microparticulates using innocuous excipients such as paraffin wax. Encapsulation of ibuprofen was shown to reduce toxicity as demonstrated by significantly fewer lesions present at either a low dose of 17 mg/kg or a high dose of 44 mg/kg. In contrast, the unencapsulated ibuprofen suspensions resulted in significant increase in gastrointestinal toxicity both doses. Since the doses used in the study were within therapeutic range for humans, the observations gathered in this study may be applicable in a clinical setting.

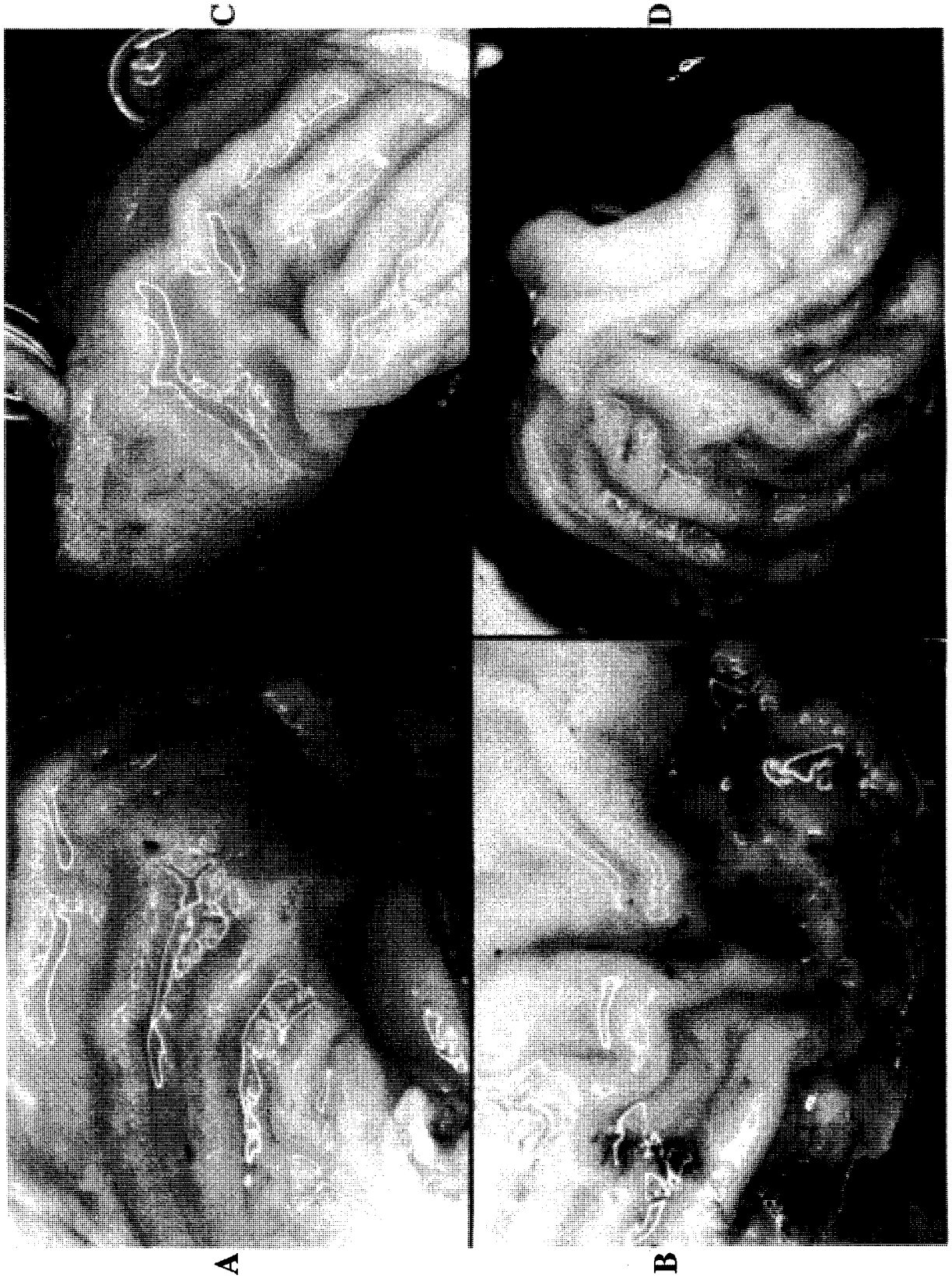


Fig. 4. Photomacrographs of stomach tissues of rats dosed with 17 mg/kg (A), 44 mg/kg (B) of suspensions of unencapsulated ibuprofen; and with 17 mg/kg (C), 44 mg/kg (D) of suspensions of encapsulated ibuprofen.

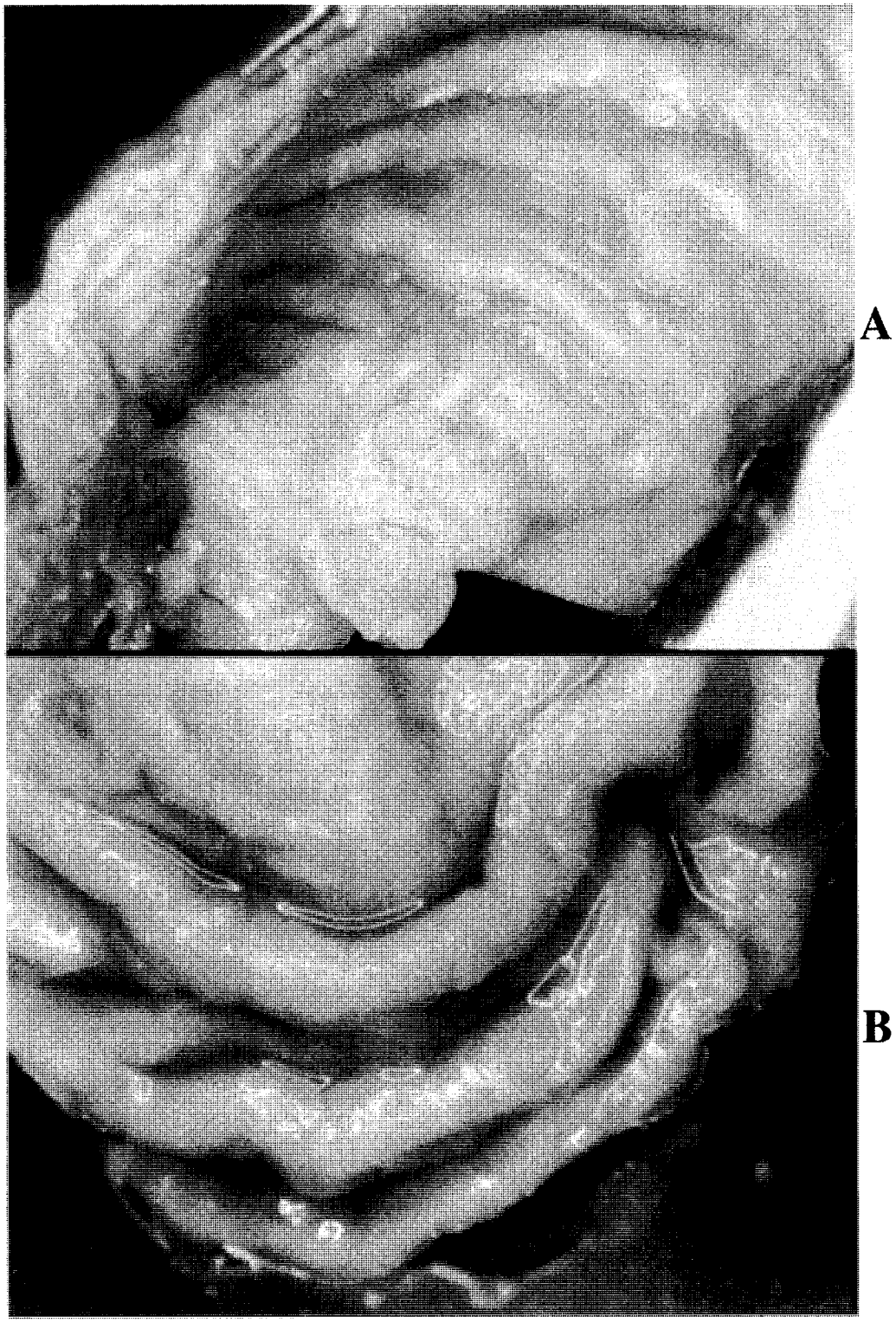


Fig. 5. Typical photomacrographs of stomach tissues of rats dosed with bethanechol (A); suspensions of blank microspheres and BT; or with only 1% suspending agent—MC, (B).

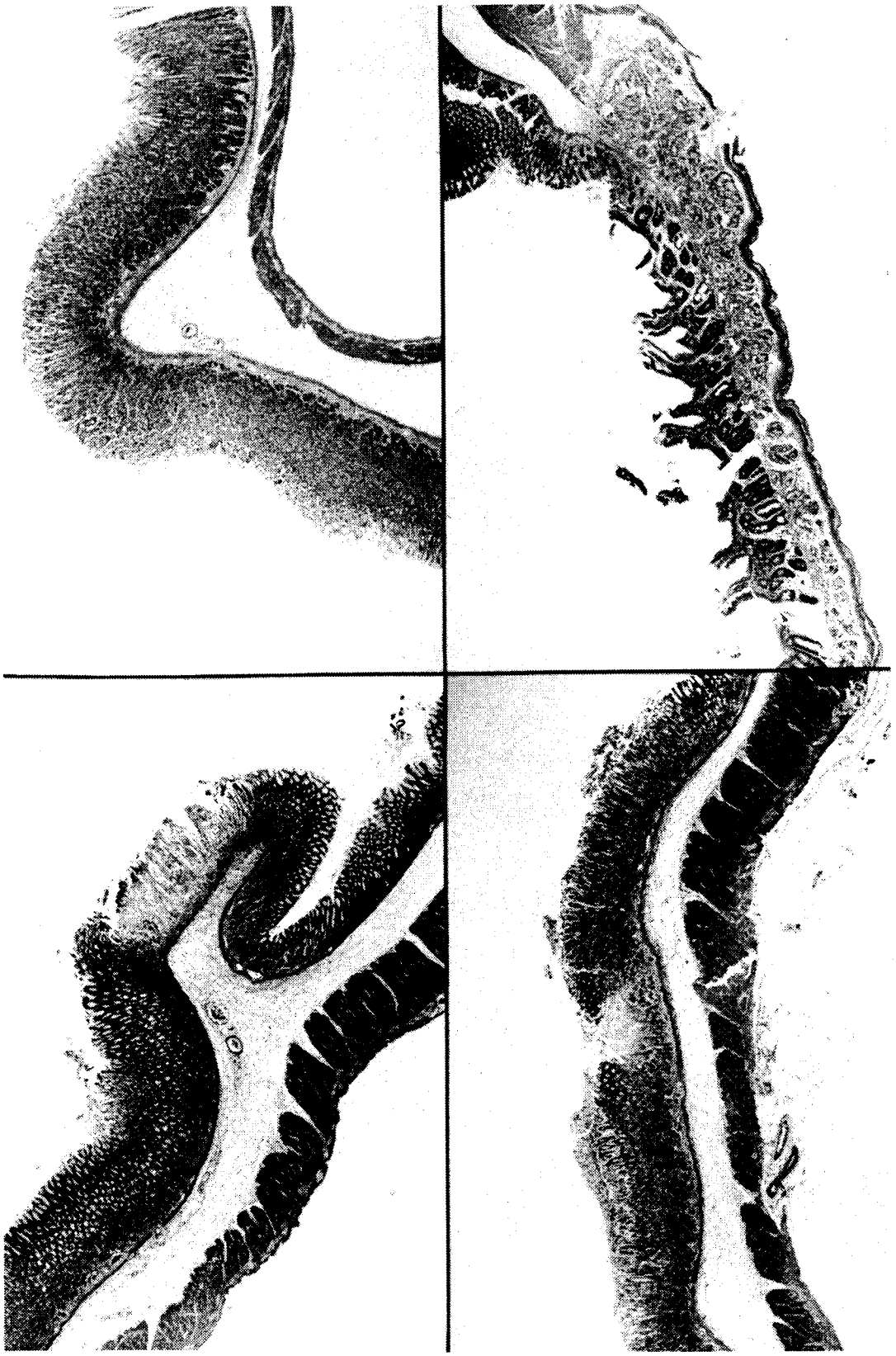


Fig. 6. Histological appearance of gastric fundus showing erosion and coagulation necrosis with acute inflammatory infiltrate in rats dosed with suspensions of ibuprofen, A=17 mg/kg, B=44 mg/kg of unencapsulated drug; C=17 mg/kg, D=44 mg/kg of encapsulated drug. The histology of C and D shows well preserved parietal cells and chief cells, or the architecture of the duodenum.

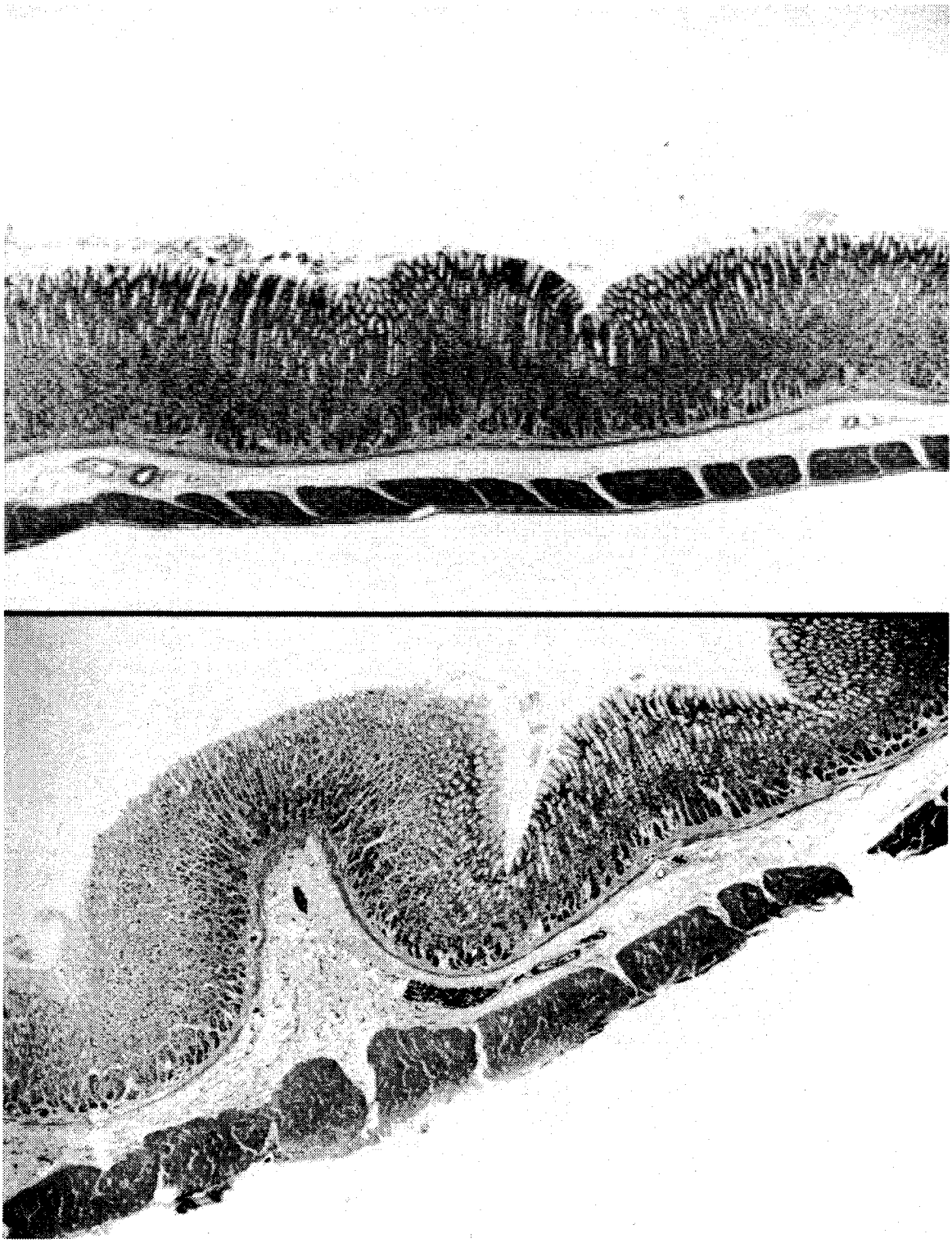


Fig. 7. Typical histological appearance of surface epithelium and normal gastric fundus of rats dosed with BCL alone, BCL and suspension of blank microspheres (A), and with neither drug nor any agents (B). Note the well preserved secretory gland cells.

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