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BIOAVAILABILITY AND EROSIVE ACTIVITY OF SOME NON-STEROIDAL ANTI-INFLAMMATORY DRUGS SOLID-DISPERSIONS.

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ABSTRACT

Solid dispersions of mefenamic acid, azapropazone, glafenine and floctafenine were prepared with PVP K_{25} and PEG 6000 in a ratio of 1:1 w/w. Bioavailability and erosive activity of these drugs were investigated using their solid dispersions. The obtained results revealed that the coprecipitate of such drugs with PVP enhances their bioavailability and significantly inhibits the ulceroqenic effect of the drugs under investigation. However, solid dispersions with PEG enhance bioavailability but slightly reduce their gastric ulceration.

INTRODUCTION

The anti-inflammatory analgesics are often used for long course treatment in patients with chronic and disabling conditions. Most of them cause gastrointestinal toxicity such as peptic ulceration and haemorrhage. A large number of new anti-inflammatory analgesics have been introduced and although their relative efficacy and safety remains to be established, there is evidence that some may produce toxic effects.

Mefenamic acid, azapropazone, glafenine and floctafenine are anti-inflammatory drugs of different chemical structures that have poor solubilities in water[1]. The gastrointestinal

complaints were the most symptoms encountered with medications of these drugs[1,2].

Polyvinyl Pyrrolidone (PVP) and Polyethylene Glycol (PEG) are widely used in the preparation of solid dispersions of insoluble drugs which are applicable in many pharmaceutical preparations. These facts together with the problems encountered with the poor bioavailability of the above mentioned drugs predomenate our investigation to formulate such drugs in solid dispersion with either PVP or PEG.

The surface and histological examination of the gastrointestinal tract of rats fed on these drugs either untreated or in a solid dispersion were also of interest to be investigated.

EXPERIMENTAL

1- Material and Equipment :

Mefenamic acid (El-Nile Co. for Pharmaceuticals, Cairo, Egypt); azapropazone (Siegfried, Zofinen, Switzerland); glafenine and floctafenine (Memphis Chem. Co. Cairo, Egypt). Formalin , sodium chloride, ethyl alcohol, eosin, methyl alcohol, chloroform, hematoxylin, xylol, hard paraffin, PEG 6000 and PVP K_{25} (analytical grades - Prolabo, France). Perkin-Elmer 505 Spectrophotometer and Aminco - Bowman Spectrophotofluorometer.

2- Preparation of Solid Dispersions:

Solid dispersions of each drug in a ratio of 1:1 w/w with PVP or PEG were prepared by solvent and fusion methods for PVP K_{25} and PEG 6000 respectively[3]. In the solvent method, drug-PVP physical mixture was dissolved in an organic solvent then evaporating off the later over a water bath. Methyl alcohol was used to prepare the coprecipitates of azapropazone and mefenamic acid while chloroform was chosen to prepare those of glafenine and floctafenine according to the solubility of drugs under investigation[1].

In the fusion method, each drug was mixed with PEG 6000 in a ratio of 1:1 w/w. The mixtures were carefully heated on electric hot plate till complete melting of PEG, then suddenly cooled in ice bath with continuous stirring. The coprecipitates and the frozen masses were scratched and stored in a desiccator overnight then pulverized, seived and the fractions of 45-63 µm were collected.

3- Bioavailability Study:

Adult male rabbits (2-2.25 Kg) were fasted for 24 hr, while water was allowed freely. The animals were divided into 4 groups each of 6 rabbits. Each group was separately fed with untreated drug and its solid dispersion or coprecipitate in a crossover design. All the administered medications had a particle diameter of 45-63 um and were filled in a hard gelatin capsule in a dose of 50 mg Kg^{-1}. Blood samples were collected at certain time intervals from the congested aural vein into glass tubes and drug concentrations were determined.

4- Methods of Assay of Blood Samples:

a) Mefenamic acid:

Blood samples were taken into heparinized tubes, then centrifuged at 9000 rpm for 10 minutes. The plasma was assayed spectrophotometrically for the total mefenamic acid (parent drug and metabolites, free and conjugated) by the method of Glazko[4].

b) Azapropazone:

Serum was separated from the collected blood samples. The concentration of azapropazone was determined spectrophotometrically as described[5].

c) Glafenine and Floctafenine:

Floctafenine and glafenine have nearly similar chemical structures[1]. Thus, the spectrophotometric method reported by Mallein et al[6].

for assessment of glafenine was adopted to determine both glafenine and floctafenine in heparinized blood samples. The method involves the treatment of serum with n-butanol saturated with concentrated ammonia solution and the butanolic extract was measured spectrophotometrically at 360 nm. The assay was developed for analysing blood samples for both drugs and it was checked for its accuracy for floctafenine[7].

5- Gross-surface and Histological Study:

Male albino rats of 200-250 g weight were randomly divided into 12 groups each of three rats. All animals were fasted 24 hr before experiments but had free access to water. Each three groups received the drug, drug-PVP coprecipitate and drug-PEG solid dispersion. The drugs and their solid dispersions were given in a dose of 20 mg for floctafenine and glafenine. The doses of mefenamic acid and azapropazone were 10 mg of each. All drugs doses were given as suspension in one ml water by means of stainless steel canula. Seven hours after dosing, the animals were killed, stomach was excised, opened out along the lesser curvature and the contents were washed out with 0.9% w/v aqueous sodium chloride solution. Each stomach was stretched out and examined for the presence of ulcerations, fixed in 10% formalin solution. The tissues were processed by the usual paraffin method, sectioned of 6 um, stained by hematoxylin and eosin stain[8], and examined microscopically.

RESULTS AND DISCUSSIONS

a) Bioavailability Study :

The blood plasma concentrations at different time intervals for mefenamic acid, glafenine and floctafenine and the serum concentrations of azapropazone are given in Figures 1 a,c,d and b respectively. Area under blood data curves (AUCS) was

calculated from blood concentrations up to 12 hrs by trapozoidal rule and the values were summarized in Table 1. The obtained data showed that PVP and PEG enhanced the bioavailability of the investigated drugs from their solid dispersions. The blood level profiles were almost parallel to the untreated drug.

The peak time of blood concentrations was not affected by the type of polymer and the technique of dispersion used, however, the peak heightwas increased (Table 1).

The maximum blood concentrations (Table 1 and Figure 1) were in the following order : PVP coprecipitate > PEG solid dispersion > untreated drug. Statistical analysis of the obtained data using Student 't' test[9] revealed that a highly significant difference existed between coprecipitates and untreated drugs. These data indicated that the mean blood drug concentrations over 0-12 hour interval were affected by the type of polymer and method of its incorporation with drug.

The increase in bioavailability of the tested drugs from their solid dispersions may be due to particle size effect and the increase in the wettability of drugs during dissolution. This results are in agreement with the previously reported data[3].

2- Gastric Ulcerogenic activity:

The rats which received untreated drugs exhibited a considerable mortality within 7 hours and gastrointestinal haemorhage was established to be the cause of death, but no mortality was identified for those animals given the solid dispersions (Table 2). The oral administration of the selected drugs either untreated or in solid dispersion to rats showed quitely different effects on the gastric mucosa. Focal erosions in the corpous and body with evidence of bleeding in or around the eroded

areas after administration of the untreated drugs occurred. Some lesions were seen from the serosal surface as small brown areas. No erosions were evident after dosing of solid dispersions but there was extensive sloughing of the mucous layer The erosions were clearly visible to the naked eye and were generally focal or extended lengthwise down the mucosa. No damage occurred in the middle of the greater curvature in the fore-stomach. Most of the damage occurred in the middle of the greater curvature in the corpus with occasional damage in the antrum and pylours.

The microscopic examination of stomachs of all groups showed stricking abnormalities (Figs. 2-13). Extensive damage occurred, and the damaged cells in the mucosa below erosions stained poorly in stomach of rats receiving untreated mefenamic acid, azapropazone, glafenine and floctafenine (Figs. 2, 5, 8 and 11).

The solid dispersions of the tested drugs with either PVP or PEG seemed to decrease the ulcerogenic effects of all drugs (Figs. 2-13). The figures indicate that PVP inhibits the ulcerogenicity of azapropazone, glafenine and floctafenine (Figs. 7, 10, and 13). A typical gastric mucosa with normal gastric pits and oxyntic cells were observed in the stomach of rats receiving PVP coprecipitates of azapropazone, glafenine and floctafenine. However, mefenamic acid-PVP showed damaged and erosion area w which are still less delterious than untreated drugs (Figs. 2 and 4).

The oral administration of the tested drugs in the form of solid dispersion with PEG inhibits their ulcerogenic activities to certain extent with different variances (Figs. 3, 6, 9, and 12). Enlargement of the area between damaged and undamaged

cells (Fig. 3) was found in animals receiving mefanamic acid-PEG solid dispersions. However, there is a sharp distinction between damaged and undamaged cells (Fig. 6) for azapropazone-PEG solid dispersion. Meanwhile, few cells have been sloughed away but the remainder had been clearly either damaged severely in the erodded area or remained intact as in case of glafenine-PEG (Fig. 9). The damage is confined to the superfacial mucosal cells occurred and the internal cytoplasm of the superfacial mucous cells distrupted as a consequence of discharging large number of mucous granules (Fig. 9). In contrast the gastric mucosa of the rats receiving floctafenine-PEG solid dispersion showed absence of any ulceration in the mucosal surface. Only inflammatory infiltrate, consisted of eosinophils, lymophocytes and plasma cells was found (Fig. 12).

It is noteworthy that the used anti-inflammatories induce peptic ulceration and bleeding when administered orally, which is in agreement with the reported findings[1,2].

Several mechanisma have been proposed to account for the development of gastric damage [10-15]. Among these explanations is the direct physical damage by the drug particles and loss of the protective mucous layer[10] and acidity influence of the drugs[11]. Many attempts were reported to inhibit these ulcerogenic activities utilizing different routes of administration, microencapsulation and different dosage forms[16-19]. In this study, it was found that coprecipitation of such drugs with PVP inhibits these peptic ulceration. In addition, the dispersion of such drugs with PEG decreased this effect. The drug may be in the molecular form (coprecipitate) or in very fine crystalline particles thet convyed with PEG (solid dispersion), and consequently enhancement in the dissolution and absorption of such drugs may occurs[3].

Accordingly, the time of contact of such drugs with the mucosal surface is decreased, and hence their local effects may be inhibited. In conclusion solid dispersions and coprecipitates of the tested drugs with PVP and PEG can be recommended in the oral therapy with NSAID.

Table 1: Blood level[x] data of rabbits administered different NSAID untreated and as solid dispersions.

Drug	Mefenamic acid			Azapropazone			Glafenine			Flocta fenine		
Form-	Untreated	PVP coppt	PEG S.d	Untreated	PVP coppt	PEG S.d.	Untreated	PVP coppt.	PEG S.d.	Untreated	PVP coppt.	PEC S.d
Peak hight ug/ml	52 ±3.20	89 ±3.17	64 ±3.09	1160 ±6.14	1220 ±8.12	1150 ±7.15	85.33 ±4.11	157.95 ±6.3	122.15 ±5.2	66.4 ±3.12	83.75 ±6.7	76.3 ±4.1
Peak time (hrs)	2±0	2±0	2±0	3±0	3±0	3±0	2±0	2±0	2±0	2±0	2±0	2±0
AUC_{o-12} µg/ml.hr	324.75 ±14.1	524** ±16.3	369* ±14.7	7497 ±12.7	8257** ±20.5	7767* ±16.2	769.69 ±15.3	999.84*** ±20:4	862.33* ±17.7	401.93 ±8.2	532.6** ±9.7	465.85* ±8.3

x Average of 6 rabbits for each treatment.
± Standard deviation (plasma or serum)
* Insignificant difference (P>0.05)
** Significant difference (P<0.1)
*** Very high significant difference (P<0.001) on comparing with untreated drug by student's t-test .
coppt. = coprecipitate
S.d. = solid dispersions.

Table 2: Mortality rate of rats administered different NSAID untreated and as solid dispersion.

Drug Form	Mefenamic acid			Azapropa-zone			Glafenine			Floctafenine		
Mortality time (hr)	A	B	C	A	B	C	A	B	C	A	B	C
2	1	-	-	1	-	-	-	-	-	1	-	-
4	1	-	-	1	-	-	2	-	-	1	-	-
7	1	-	-	-	-	-	1	-	-	1	-	-
Total*	3	-	-	2	-	-	3	-	-	3	-	-

* : Number of died animals.

A : Untreated drug.

B : PVP corecipitate.

C : PEG solid dispersion.

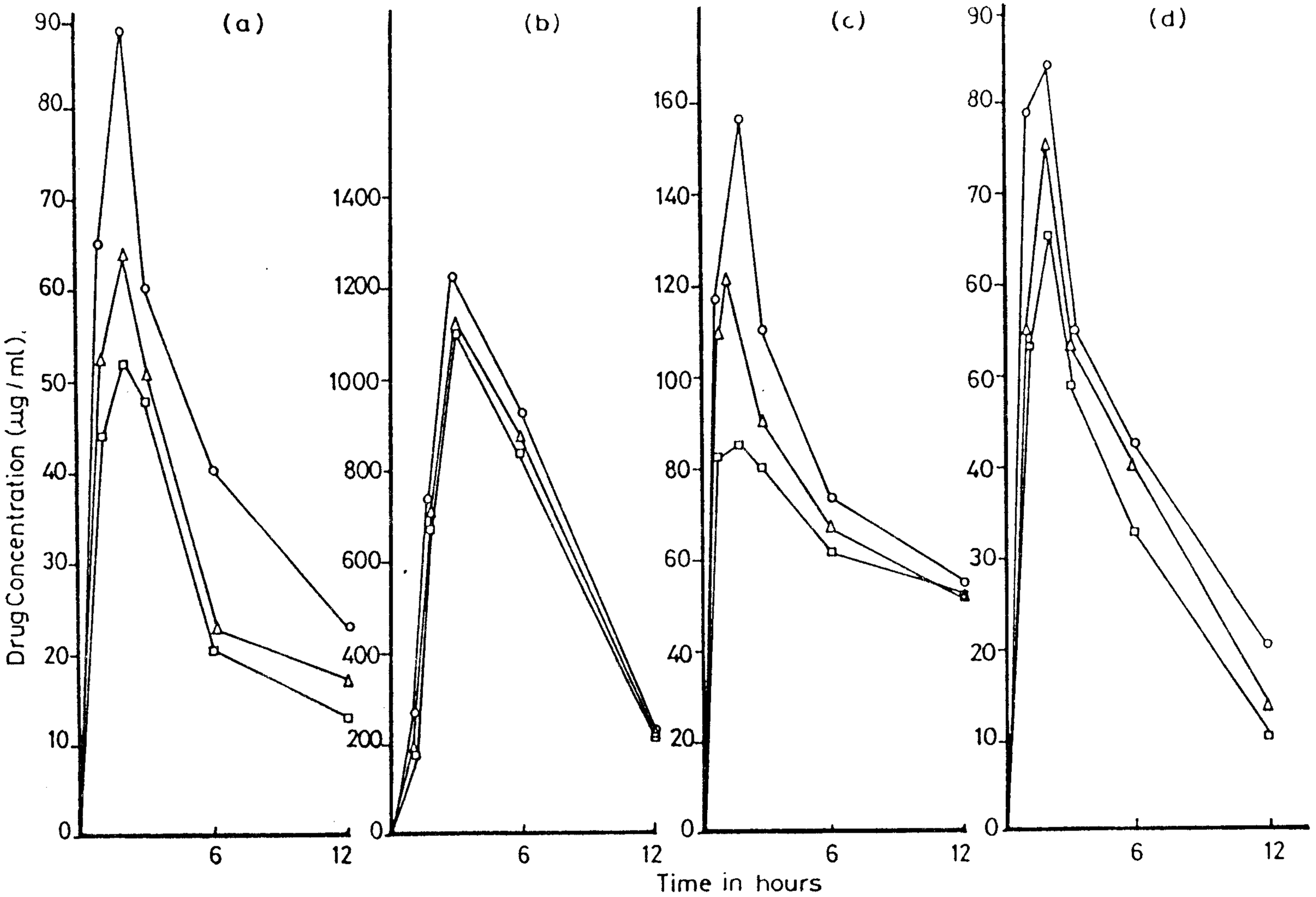

Figure 1. Mean blood concentration of mefenamic acid(a), azapropazone(b), glafenine (c), and floctafenine(d).

- ▫ Untreated drug
- ▵ Drug - PEG solid dispersion
- ○ Drug - PVP coprecipitate

Fig. 2: Gastric mucosa of a rat after oral administration of 10 mg untreated mefenamic acid (Hx. & E.X 100).

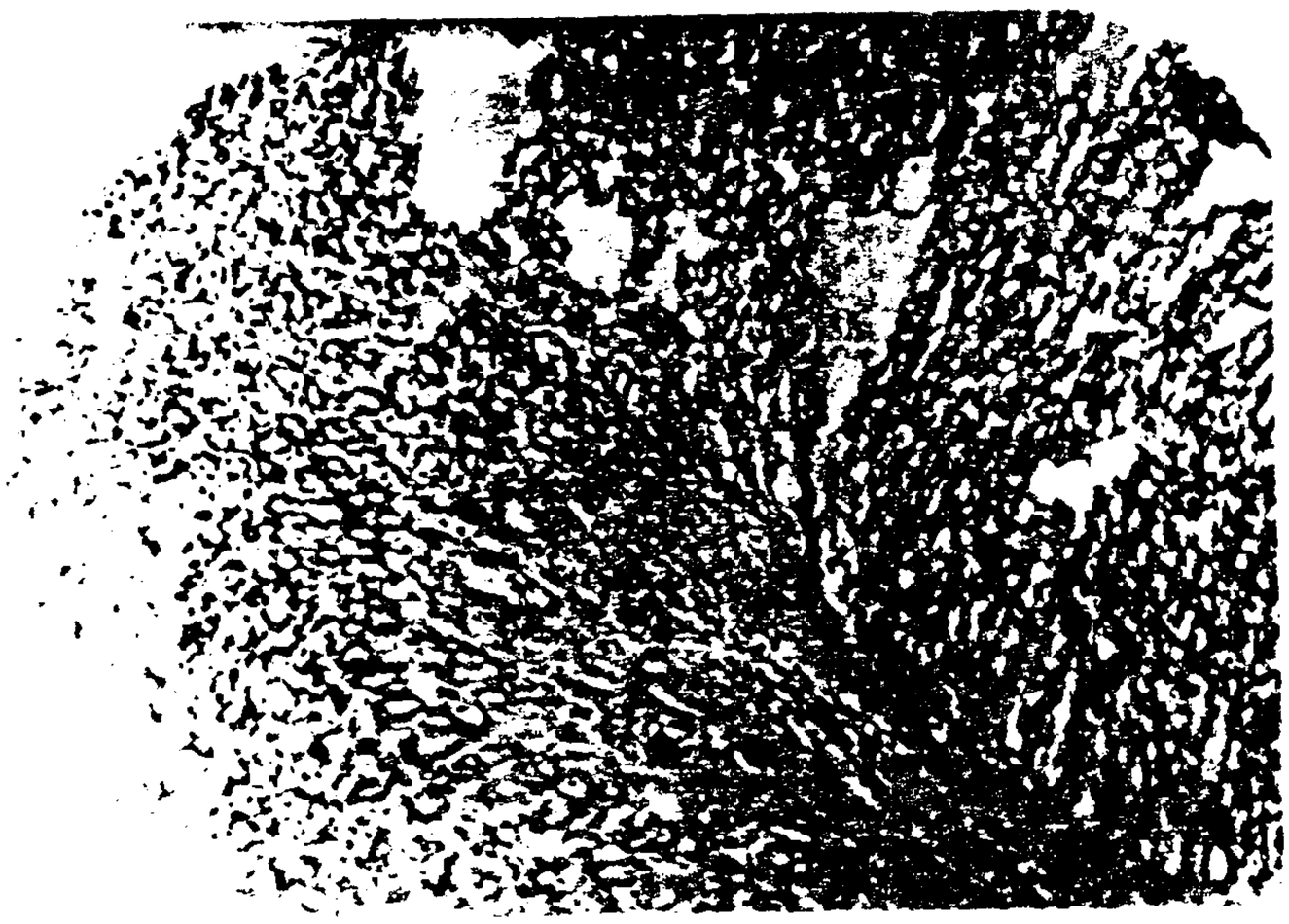

Fig. 3: Gastric mucosa of a rat after oral administration of 20 mg mefenamic acid-PEG solid dispersion (Hx & E.X 100).

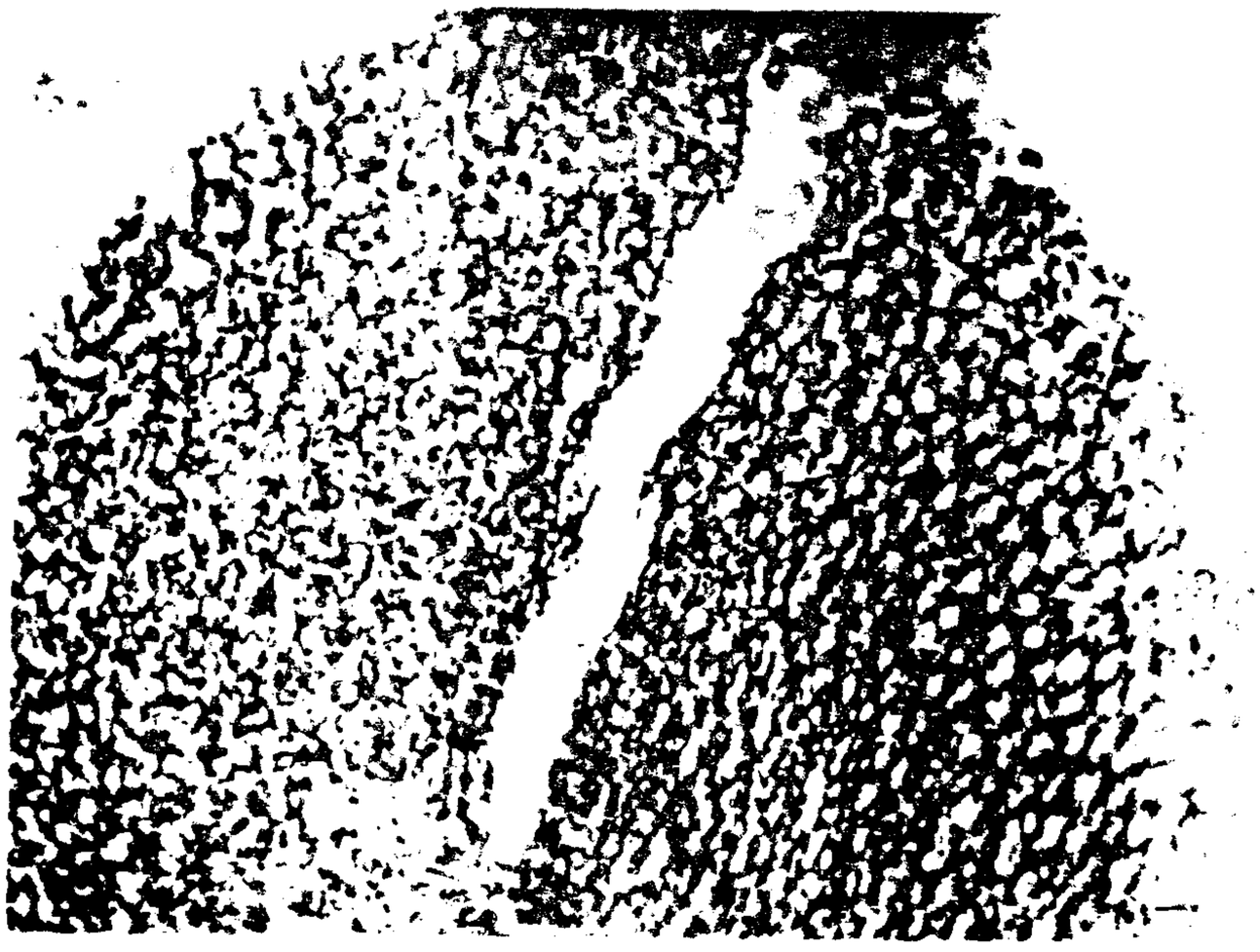

Fig. 4: Gastric mucosa of a rat after oral administration of 20 mg mefenamic acid-PVP coprecipitate (Hx & E.X 100).

Fig. 5: Gastric mucosa of a rat after oral administration of 10 mg untreated azapropazone (Hx. & E.X 100).

Fig. 6: Gastric mucosa of a rat after oral administration of 20 mg azapropazone-PEG solid dispersion (Hx & E.X 100).

Fig. 7: Gastric mucosa of a rat after oral administration of 20 mg azapropazone-PVP coprecipitate (Hx & E.X 100).

Bioavailability and Erosive Activity of some Non-Steroidal Anti-Inflammatory Drugs Solid-Dispersions.

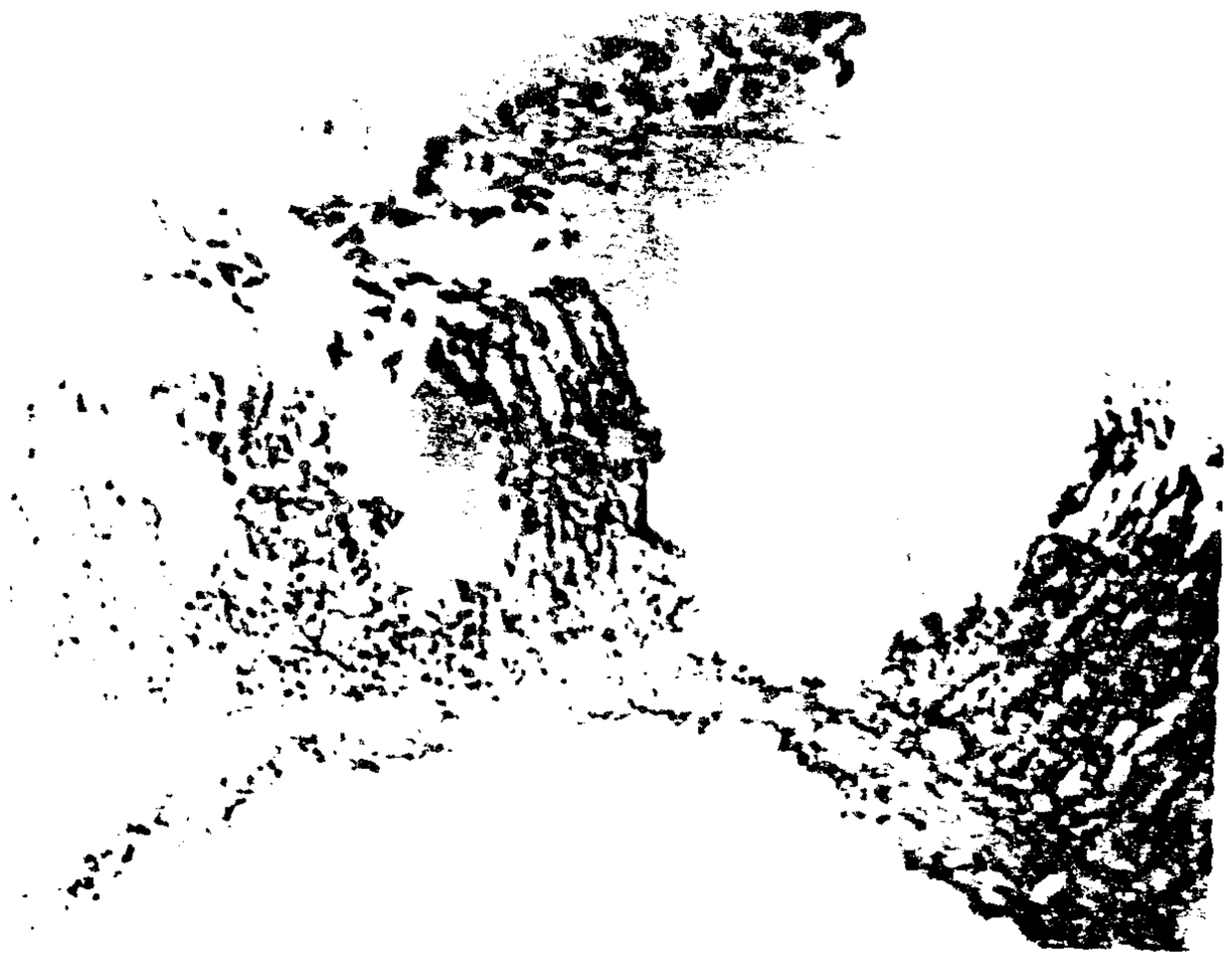

Fig. 8: Gastric mucosa of a rat after oral administration of 20 mg untreated glafenine (Hx. & E.X 100).

Fig. 9: Gastric mucosa of a rat after oral administration of 40 mg glafenine-PEG solid dispersion (Hx. & E.X 100).

Fig. 10: Gastric mucosa of a rat after oral administration of 40 mg glafenine-PVP coprecipitate (Hx & E.X 100).

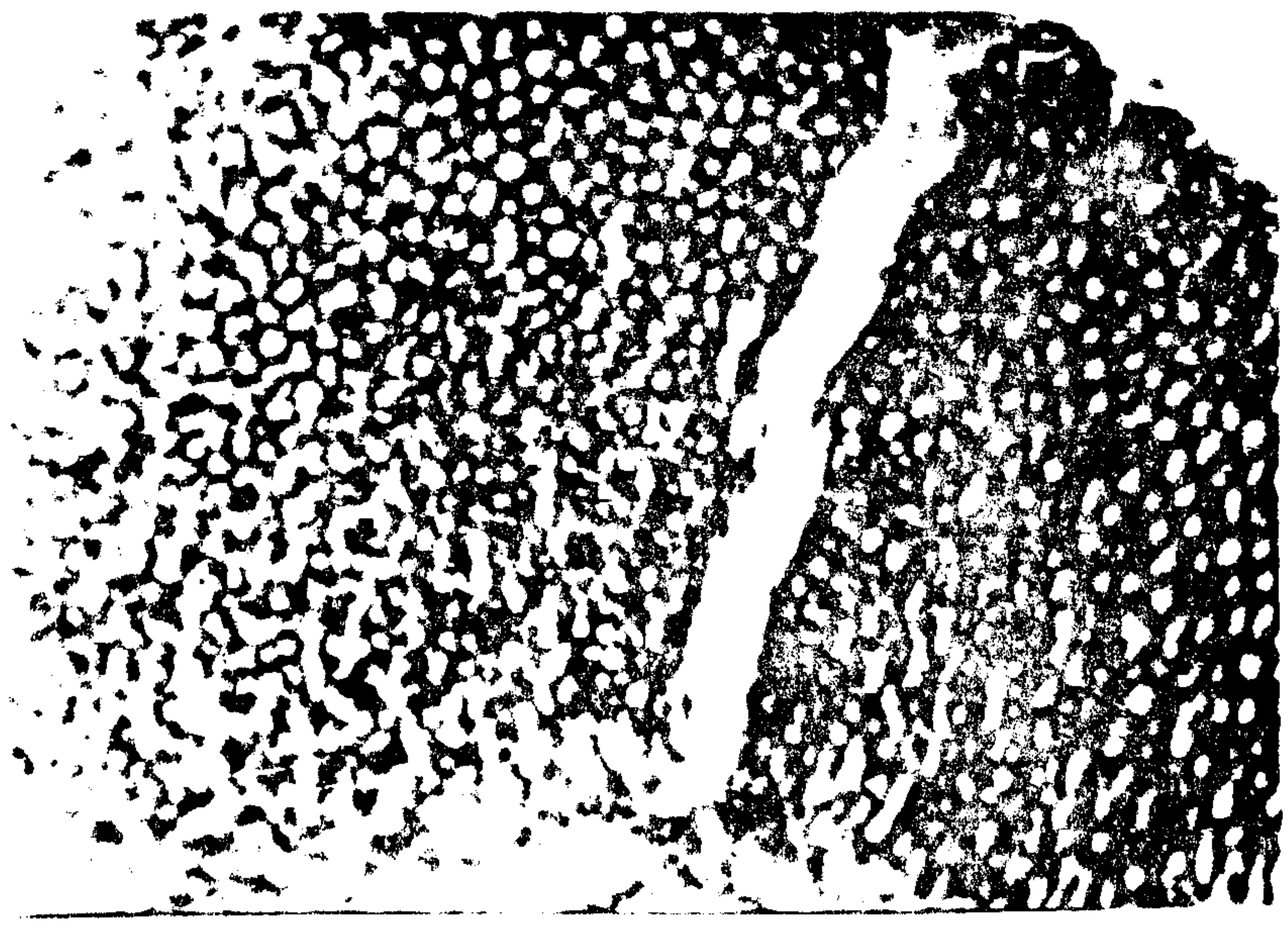

Fig. 11: Gastric mucosa of a rat after oral administration of 20 mg untreated flectafenine (Hx & E.X 100).

Fig. 12: Gastric mucosa of a rat after oral administration of 40 mg floctafenine-PEG solid dispersion (Hx & E.X 100).

Fig. 13: Gastric mucosa of a rat after oral administration of 40 mg floctafenine-PVP coprecipitate (Hx & E.X 100).

REFERENCES

1) W. Martindale,"The extrapharmacopeia",The Pharmaceutical press London 28 th Ed. 1982 p. 246-260.

2) L.F. Prescott in "Side Effects of Druas" Annual 2nd Ed., by M.N.G. Dukes, 1978 (2) Chapter 9, p. 91.

3) W.L. Chiou, and S. Riegelman J. pharm. Sci., 60, 1281 (1971).

4) A.J. Glazko in "Pharmacology of the Fenamates", Ann. phys. Med. Suppl., Ed. Bailliere, Tindall and Cassell, London,1967 p. 23.

5) Uc Johan J. Rollar and F. Schatz,Arzneimittel-Forsch, 23, 666 (1973).

6) K. Mallein, J. Rodelet, J. Pottier and M. Boucherat., Soc Biopharmacie, Paris 160, 122 (1966).

7) E.M. Ramadan, A. El-Helw and Y. El-Said., Pharmazeutische Industrie (In Press).

8) D.J. Hinqson and S. Ito, Gastroenterology, 61, 156 (1971).

9) J.H. Burn, D.J. Finney and L.C. Goodwine in "Biological Standardization" 2 nd Ed, Oxford University Press, 1952,p.32.

10) J.L.A. Roth., A. Valdes-Davena. In "Salicylates International Symposium" edited by A. St. J. Dixon, B.K. Martin, M.J.H. Smith and P.H.N. Wood, Churchill, London, 1963, p. 224.

11) H.W. Davenport, New Engl. J. Med., 276, 1307 (1967).

12) I.P.T. Hakkinen, R. Johensson and M. Pantio Gut, 9, 712 (1968).

13) A.A.M. Abdel-Galil and P.B. Marshall., Brit. J. Pharmac. Chemother., 33, 1, (1968).

14) A. Robert, Prostaglandins, 6, 523 (1974).

15) K.D. Rainsford, B.M. Peskar, and K. Brune, J. Pharm. Pharmac., 33, 127 (1981).

16) *E.P. Frenkel, M.S. McCall, C.C. Douglass and S. Eisenberg., J. Clin. Pharmac., 8, 347 (1968).*

17) *L.P.J. Holt, and C.F. Hawkins, Br. Med. J. 1, 1354 (1965).*

18) *Y. Wada, H.Y. Etoh, A. Ohira, H. Kimata, F.Koide, H. Ishihama and Y. Mizushima J. Pharm. Pharmac,, 34, 467 (1982).*

19) *R. Saziki and S. Okabe., Oyo Yakuri, 8, 617 (1974) .*

الاتاحة الحيوية والنشاط التآكلى لبعض المنتشرات الصلبة للادوية المضادة للالتهابات

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حضرت المنتشرات الصلبة لحامض الميفيناميك والاذابرباذون والجلافينين والفلوكتافينين مع عديد فينيل البيروليدون ك ٢٥ وعديد الايثيلين جليكول ٦٠٠٠ بنسبة ١ : ١ وقد تناول البحث دراسة الاتاحة الحيوية لهذه الادوية ومنتشراتها الصلبة بالاضافة الى دراسة النشاط التآكلى لهذه الادوية ومنتشراتها الصلبة على معدة الفئران .

وقد اشارت النتائج الى وجود زيادة واضحة فى الاتاحة الحيوية لهذه الادوية من منتشراتها الصلبة بينما قلت خطورتها التآكلية على معدة الحيوانات .

وقد كان هذا التأثير واضحا من المنتشرات الصلبة للادوية مع عديد فينيل البيروليدون عنه فى حالة عديد ايثيلين جليكول .

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