Original Article

Stability, bioavailability, and ulcerative activity of diclofenac sodium-mastic controlled release tablets

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ABSTRACT: Controlled release tablets containing 50 mg diclofenac sodium (DS) and 40% mastic with other natural additives were prepared. Drug release was examined and stability was studied using non-isothermal and isothermal thermogravimetric analysis (TGA). The bioavailability of two controlled release tablet formulations was studied and compared to that of commercial tablets, and rabbit stomachs were also histologically examined 24 h after administration of the various tablets.

Additives of pectin and sodium alginate indicated the controlled release profile of the drug. Non-isothermal TG revealed two stages of thermal decomposition for all formulations. Isothermal TG revealed that degradation of the drug in the tablet formulations follows first-order kinetics. The obtained degradation rate constants at various temperatures were plotted according to the Arrhenius equation. The degradation rate constant at 25°C was determined and used in estimation of shelf life. The obtained shelf lives of all formulations ranged from 3.38-4.92 years. In comparative studies with commercial tablets, the bioavailability of the drug from the two formulated tablets had no statistically significant difference in terms of the AUC and produced prolonged blood levels of DS with a delayed peak. The two controlled release tablet formulations resulted in no histological alterations in the stomach in terms of mucous surface cells and glands; in comparison, commercial tablets resulted in a disrupted mucous layer, necrotic ulcerations, hemorrhaging, and inflammatory cell infiltration along the base of the gastric glands.

Keywords: Diclofenac sodium, natural additives, tablets, dissolution rate, ulcerative activity, *in vivo* studies

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1. Introduction

Stability studies should be conducted to evaluate the physical and chemical degradation of pharmaceutical products. Such products should maintain their identity, strength, quality, and purity within specified limits through their period of storage and use (*1*).

The use of thermal methods is of great importance in the determination of drug purity, the quantitative and qualitative analysis of drug formulations, tests of stability, and determination of kinetic parameters. Thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) have been used simultaneously to determine the thermal stability of drugs, excipients, and pharmaceutical products (*2-6*).

Thermogravimetrical methods have been used to determine drug stability either as non-isothermal TG (the temperature is increased at a constant rate) or as isothermal TG (the temperature is set at a predetermined temperature). Non-isothermal TGA with its derivative (thermogravimetry, DTG) were used to study the thermal degradation kinetics of vinyl polyperoxide copolymers (*7*) and non-isothermal TGA at different heating rates in flowing nitrogen was used to estimate the thermal stability and thermal degradation kinetics of polyimides (*8*). Isothermal TG was used to study the thermal kinetics and determine the shelf-life of different ampicillin products (*9*). Both non-isothermal and isothermal TGA have been used to determine the kinetic parameters and shelf-lives of many drugs and drug products, including anti-hypertensive drugs (*10*), generic hydrochlorothiazide formulations (*11*), prednisone powder and tablets (*2*), and metronidazole powder and tablets (*12*).

The bioavailability of diclofenac sodium (DS) from various drug delivery systems was previously studied in humans (*13-15*). Different kinds of animals have also been used for bioavailability studies of DS from various drug delivery systems. Yong *et al.* (*16*) studied the pharmacokinetics of DS delivered by a poloxamer-based suppository in rats. Sarvavanan *et al.* (*17*) attempted to target DS to its site of action using gelatin magnetic microspheres in rabbits.

The aim of the current study was to determine the

order of decomposition and shelf-lives of DS controlled release tablet formulations using non-isothermal and isothermal TGA. The bioavailability of DS from controlled release tablet formulations was also assessed and compared with that of commercial tablets. The histology of stomach tissues of untreated animals and animals 24 h after administration of a single dose of both formulations of controlled release tablets and commercial tablets was studied.

2. Materials and Methods

2.1. *Materials*

Dilofenac sodium was obtained from Novartes Pharmaceutical Industry, Cairo, Egypt. Pectin was from Winlab, a division of Wilfrid Smith, UK. Sodium alginate and lactose monohydrate were from El Nasr, Pharmaceutical Chemical Co., Cairo, Egypt. Commercial grade mastic was from Chios' Gum Mastic Growers Association. Avicel PH 101 was from Fluka AG, VH9470, Buchs, Switzerland. Commercial DS tablets (Olfen tablets containing 50 mg drug, Lot No. 060034) were produced by Medical Union Pharmaceuticals Co. Abu Sultan, Ismailia, Egypt, under a license from MEPHA, Basel, Switzerland. Acetonitrile and methanol (CHROMASOLV®) were from Sigma-Aldrich Chemie GmbH D-39555, Steinheim, Germany. Mefenamic acid was kindly supplied by the Nile Co. for Pharmaceutical and Chemical Industries (El-Nile), Cairo, Egypt.

2.2. *Preparation of DS granules and tablets*

Pectin, sodium alginate, avicel PH 101, or lactose monohydrate was individually mixed in a porcelain mortar with DS and mastic using the least amount of chloroform to produce a doughy mass. The mass obtained was converted into granules by forcing it through a 2 mm sieve. The granules obtained were dried at room temperature for 24 h in open air and further dried at 40°C for 48 h in a hot air oven.

Tablet formulations were prepared from the equivalent weight of granules to contain 50 mg drug, 60 mg mastic (40%), and 187 mg of one of the tested additives (pectin, sodium alginate, avicel, or lactose). Magnesium stearate at a concentration of 1% was used as a lubricant. Tablets containing pectin, sodium alginate, avicel, or lactose were designated formulations A, B, C, and D, respectively. All batches were prepared using a single-punch Eraweka tablet press (G.M.B.H., Germany) at a constant pressure.

2.3. *Release study*

Drug release from different tablets was carried out using a USP dissolution apparatus with six jars (DA-6D, India). Phosphate buffer adjusted to a pH of 7.4 and temperature of 37 ± 0.2°C was used as a dissolution medium with a volume of 500 mL. A single tablet was placed in each basket, which was rotated at 50 rpm. Samples were withdrawn at different time intervals and replaced with the same volume of fresh medium. The drug released was measured spectrophotometrically at 276 nm (Spectrophotometer, UV-Visible; JASCO, V-530, Japan). All experiments were conducted in triplicate and the mean values were calculated.

2.4. *Non-isothermal and isothermal TG studies*

The different tablet formulations were tested with non-isothermal TGA. The data and curves of non-isothermal TGA were obtained with a Shimadzu model TGA-50H thermo-balance (Japan) in an atmosphere of nitrogen with a flow rate of 50 mL/min and a heating rate of 10°C/min up to 600°C. The sample mass used was 4-6 mg. The isothermal TG data and curves for DS tablet formulations were obtained in the region before the initial temperatures of thermal decomposition of the tablets in non-isothermal curves. The sample mass was in a range of 5-9 mg. The samples were heated at a heating rate of 10°C/min up to the isothermal temperature, where the temperature was maintained for 120 min (*11*,*12*). The isothermal TG curves were measured at 150, 160, 170, and 180°C for 120 min. Isothermal TG data were used to determine the stability of the different tablet formulations to reveal differences (*12*).

2.5. *Analysis of the isothermal data*

The degradation order of DS tablet formulations was determined by analysis of the isothermal data using zero, first, and second-order kinetics (*18*). The kinetic order of thermal decomposition of DS tablet formulations was chosen based on the highest correlation coefficient (r^2) obtained from the statistical data for each reaction order. The rate constants (K) were calculated for the chosen order at each temperature. Then, the natural logarithm (Ln) of the obtained rate constants (K) at different temperatures was plotted with respect to the reciprocal of the corresponding temperature (1/T) using the Arrhenius relationship. The degradation rate constant at room temperature (25°C or 298 K) was obtained by extrapolation to that temperature. The degradation rate constant at room temperature was used to calculate the shelf life (t_{90}) by substitution in the first-order equation.

2.6. *Bioavailability study*

2.6.1. *Tested formulations*

Formulations A and B were selected for *in vivo* study as

they have acceptable physical characteristics, the best controlled drug release behavior, and good stability. These formulations were compared with commercial tablets (Olfen tablet 50 mg).

2.6.2. *Study design*

Male Albino rabbits weighing 2.0-2.5 kg were randomly selected for the bioavailability study. The animals were divided into three groups, and each group of six rabbits received one of the tested formulations, namely formulation A, formulation B, and commercial tablets. The animals fasted overnight before tablet administration and during the experiment but were given free access to water. The tablets were given orally to the rabbits using a balling gun to deliver the tablets to the animal's stomach. The ear vein was cannulated with a 19-gauge butterfly (scalp) needle and blood samples (2 mL) were withdrawn from the vein before dosing (zero time) and at different time intervals after dosing, namely 1, 2, 3, 5, 8, 12, and 24 h, using heparinized tubes. The collected samples were immediately centrifuged and plasma was separated and stored at -20°C until analysis.

2.6.3. *Analysis of plasma level of diclofenac sodium*

High-performance liquid chromatography as reported by Liu *et al.* (*13*) was performed using mefenamic acid as the internal standard. An HPLC model L-7100 equipped with a Rheodyne injector valve with 20 µL loop and L-7400 UV detector, Merck Hitachi limited, Tokyo, Japan, was used. The plasma protein was precipitated with acetonitrile. Separation was performed on a Nucleosil 100-5 (C_{18}) column (150 × 4.6 mm). The mobile phase was acetonitrile-0.01 M potassium dihydrogen phosphate (pH 6.3) in a ratio of 35:65 (v/v), and the flow rate was 1 mL/min. A wavelength of 278 nm was used to monitor the drug and the mefenamic acid. The peak area ratio served as the basis for quantification.

2.6.4. *Pharmacokinetics calculation*

Pharmacokinetics parameters were calculated according to a non-compartmental model. The plasma concentration versus time curves was used to determine the maximum plasma concentration (C_{max}), time to achieve maximum plasma concentration (T_{max}), and the area under the concentration-time curve with respect to the respective sampling AUC_{0-t}.

The maximum concentration (C_{max}) and the time of its occurrence (T_{max}) for each individual animal were determined from the plasma concentration time data. The area under the curve (AUC) for each rabbit was calculated by the trapezoidal role method using GraphPad Prism Project software.

2.6.5. *Statistical analysis*

One-way analysis of variance (ANOVA) was used to estimate the difference between the different formulations, namely formulation A, formulation B, and commercial tablets, with regard to the AUC_{0-24}, C_{max}, and T_{max}. A *t*-test was also performed on pairs of formulations at a confidence interval of 5% (*19*).

2.7. *Histological study*

Rabbits were sacrificed 24 h after administration of a single dose of A or B formulated tablets or commercial tablets. Control rabbits were also sacrificed and examined. The stomach was isolated from other parts of the gastrointestinal tract, which was opened longitudinally and washed thoroughly with saline. The stomach was sliced into two parts, namely the fundus and the pylorus. The fundus and pylorus were then sliced into small pieces and treated separately by immersion in 10% neutral formalin for two days. They were then dehydrated, cleared, and embedded in paraffin wax. Paraffin sections (6 µm thick) were prepared and stained with hematoxylin and eosin stain (*20*).

3. Results and Discussion

3.1. *Preparation of DS Tablets*

The physical characteristics of DS tablets containing mastic and each of the selected additives (formulations A, B, C, and D) were within pharmacopeial limits. With regard to disintegration time, none of the tablet formulations displayed disintegration for 3 h except for formulation C containing avicel, which disintegrated after 37 min.

3.2. *Release study*

The release rate of DS from tablet formulations (Figure 1) decreased in the following order: formulation C > D > A and B. The highest release rate from formulation C may be due to the disintegrating effect of avicel. Formulation D had an intermediate release rate due to tablet erosion of the water-soluble lactose. That said, formulations A and B provided the best control of drug release. This may be attributed to the hydrophilic gel nature of both pectin and sodium alginate (*21*).

3.3. *Stability study*

The non-isothermal TG curve and Dr TGA of formulation D of DS tablets (Figure 2) are shown as a representative example. The results indicate an initial minor % weight loss as the temperature increased up to 200°C. This may be attributed to desorption of moisture as hydrogen bound water to the polysaccharide

structure of the additives (*6*).

The non-isothermal TG curves and Dr TGA indicate that the onset of thermal decomposition started at 200°C or above for all formulations and the Dr TG peaks were at 241.9, 236.61, 291.52, and 210.44°C for formulations A, B, C, and D, respectively. Li *et al*. (*7*) stated that the lower peak temperature of TG, the higher the thermal decomposition of the material(s). The current results also indicate that after the onset temperature, all formulations exhibited a two-stage decomposition pattern. These intervals are based on the change in the slope of the % weight loss as a function of temperature. The stages of thermal decomposition of different formulations and their onset temperature differ from one formulation to another depending on the melting point of the compound(s) and their stability when exposed to high temperatures (*4,10,12*).

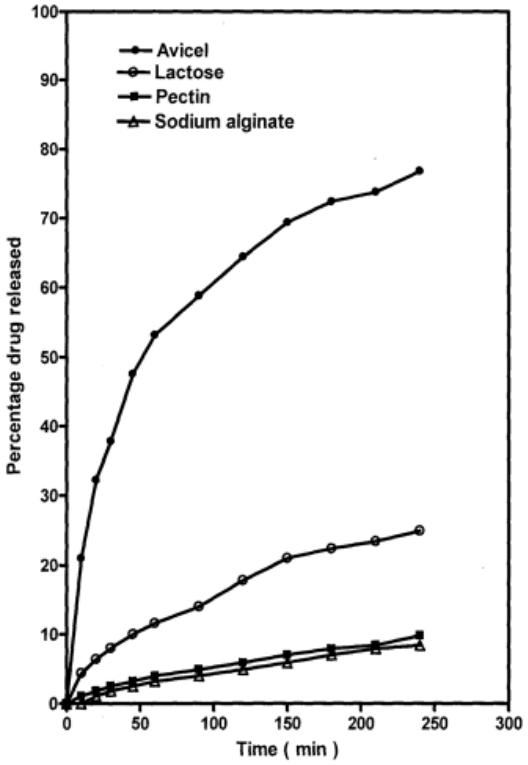

Figure 1. Release profile of DS from tablets containing 50 mg drug, 40% mastic, and various additives.

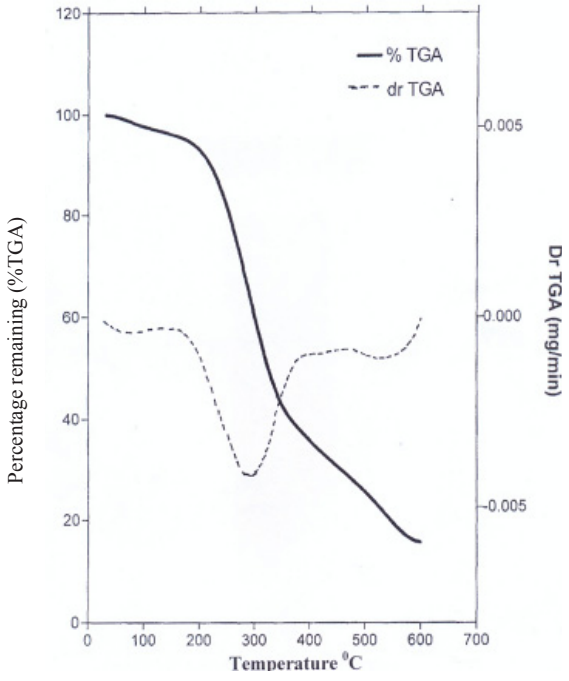

Figure 2. Percentage TGA and the differential TGA for DS tablets containing mastic and avicel.

The isothermal TG data at 150, 160, 170, and 180°C for DS formulations were used to determine the reaction order and the rate constants for thermal decomposition of DS tablet formulations. The decomposition of all formulations followed first-order kinetics. Table 1 shows the first-order rate constant of thermal decomposition for DS tablet formulations containing various additives under isothermal conditions.

The natural logarithm of the rate constants (Ln *K*) of all tablet formulations at different temperatures was plotted as a function of 1/T (Kelvin) in Figure 3. A straight line was obtained according to the Arrhenius relationship. The rate constants at 25°C (298 K) for DS formulations were calculated by extrapolation of the line to that temperature (Table 2). The values of the rate constants at 298 K were used to estimate the shelf-lives of DS tablet formulations. The estimated shelf-lives (Table 2) of DS tablet formulations A, B, C, and D were 4.512, 4.046, 4.915, and 3.376 years, respectively. Based on these rate constants, the different formulations had decreasing stability in the following order: formulation C > A > B > D.

Tablets of formulation A containing pectin and formulation B containing sodium alginate achieved the best control of drug release and also had good stability.

Table 1. First-order rate constant and its correlation coefficient for DS tablets containing 40% mastic with different additives

Type of additive	Temperature		r^2 *	Rate constant K (min^{-1})
	°C	K		
Pectin	150	423	0.997	5.210×10^{-4}
	160	433	0.995	8.350×10^{-4}
	170	443	0.989	1.430×10^{-3}
	180	453	0.991	2.232×10^{-3}
Sodium alginate	150	423	0.993	5.460×10^{-4}
	160	433	0.994	9.120×10^{-4}
	170	443	0.991	1.489×10^{-3}
	180	453	0.993	2.377×10^{-3}
Avicel	150	423	0.994	4.830×10^{-4}
	160	433	0.992	7.870×10^{-4}
	170	443	0.988	1.231×10^{-3}
	180	453	0.994	2.155×10^{-3}
Lactose	150	423	0.995	6.050×10^{-4}
	160	433	0.992	1.027×10^{-3}
	170	443	0.995	1.595×10^{-3}
	180	453	0.993	2.640×10^{-3}

*r^2 is the correlation coefficient for the first order.

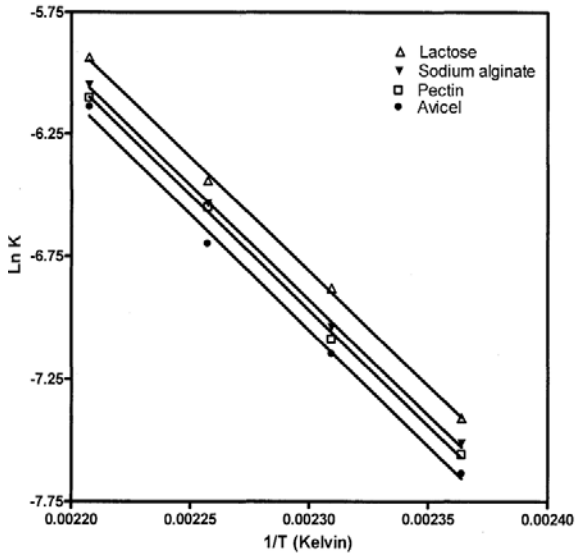

Figure 3. Plot of Ln *K versus* 1/T of the isothermal data of DS tablets containing mastic with pectin, sodium alginate, avicel, or lactose.

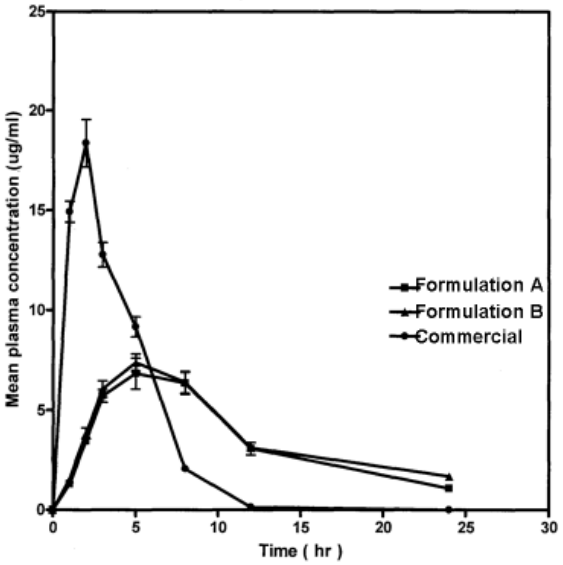

Figure 4. Plasma concentration time curve of DS obtained from formulation A, formulation B, or commercial tablets.

Table 2. First-order rate constant at 25°C and shelf life of DS tablets containing mastic and various additives

Type of additives	r^2 values of Arrhenius plot	Rate constant at 25°C (min^{-1})	Shelf-life (year)
Pectin	0.988	4.4276×10^{-8}	4.512
Sodium alginate	0.973	4.9369×10^{-8}	4.046
Avicel	0.990	4.0644×10^{-8}	4.915
Lactose	0.989	5.9166×10^{-8}	3.376

Table 3. The mean pharmacokinetic parameters of DS tablets after administration to rabbits (n = 6) of a single dose (50 mg) of either mastic-containing tablets or commercial tablets

Formulation	C_{max} (µg/mL) Mean ± S.D.	T_{max} (hr) Mean ± S.D.	AUC_{0-24} (µg/mL•h) Mean ± S.D.
Formulation A	8.04 ± 0.43	6 ± 1.54	85.60 ± 7.39
Formulation B	7.93 ± 0.35	6 ± 1.54	90.42 ± 8.36
Commercial tablets	18.75 ± 0.218	1.8 ± 30.4	83.60 ± 5.56

Therefore, they were chosen for the bioavailability study.

3.4. *Bioavailability study*

Diclofenac sodium-mastic tablets containing pectin and sodium alginate as selected additives (formulations A and B) as well as commercial tablets were administered to rabbits. The plasma concentration of DS in individual rabbits after oral administration of both A and B formulated tablets and the commercial tablets was determined. The mean plasma DS concentration-time profiles following administration of a single oral dose of either the mastic-containing tablets (A and B) or the commercial DS tablets are shown in Figure 4. As is apparent from Figure 4, the two profiles of the formulated tablets are quite comparable and at the same time differ vastly from that of the commercial tablets. Pharmacokinetic parameters (C_{max}, T_{max}, and AUC_{0-24}) were calculated individually on the basis of concentration-time data. Mean values ± S.D. were obtained from individual pharmacokinetic parameters and are shown in Table 3 for both the formulated tablets and commercial DS tablets.

The commercial DS tablets achieved a high C_{max} value of 18.75 µg/mL in 1.83 h. In contrast, tablets (A and B) containing mastic had lower C_{max} values of 8.04 and 7.93 µg/mL that occurred at a longer T_{max} of 6.00 for both. The mean AUC_{0-24} of both formulations A and B and commercial tablets was 85.48, 90.42, and 83.60 µg/mL•h, respectively.

Statistical analysis of the different bioavailability parameters using an ANOVA test (*19*) revealed no significant difference in the mean AUC_{0-24} values for all of the tested tablets ($p > 0.05$). That said, there was a significant difference in the mean values of C_{max} and T_{max} for the tested tablets ($p < 0.05$). Further statistical analysis using a student's t test revealed no significant difference in the C_{max} and T_{max} for formulations A and B but it did reveal a significant difference between the commercial tablets and both formulations A and B.

The results obtained indicate that all three formulations are equivalent with regard to the AUC. The A and B formulated tablets have a longer T_{max} and smaller C_{max} compared to the commercial tablets. This indicates that a prolonged plasma concentration of DS was obtained from the formulated tablets containing 40% mastic and pectin (formulation A) or sodium alginate (formulation B) in comparison to the commercial tablets. A similar finding was reported by Rani and Mishra (*22*) working on fabricated controlled release DS tablets. They stated that formulations containing HPMC, ethyl cellulose, or microcrystalline cellulose that they fabricated resulted in prolonged blood levels with a delayed peak in comparison to commercial tablets.

3.5. *Histological examination of the rabbit stomach*

Figure 5 shows the normal structural features of the stomach fundus of an untreated rabbit; the mucosa is covered by a continuous mucous layer and its surface is lined by healthy surface columnar epithelium. The ducts of fundic glands dip down into the lamina propria and their secretory parts (intact fundic glands) lie perpendicular to the surface. Histological examination of the stomach fundus of rabbits 24 h after administration of commercial tablets (Figure 5) indicated that the mucosa is covered by a disrupted mucous layer and that the surface epithelium displays necrotic ulceration. The lamina propria also displays hemorrhaging and cellular infiltration and the fundic glands are irregularly arranged with wide spaces in between. These results indicated damaged cells and tissues in the stomach fundus of the gastrointestinal tract after administration of the conventional commercial DS tablets. Photomicrographs of rabbit stomachs after administration of formulations A and B show that the mucosa is covered by a mucous layer and that both the surface epithelium and the fundic glands are intact but display mild hyperemia. Comparison to previous results clearly revealed that the administration of DS tablets in the form of formulations A and B reduced the erosion of the mucous membrane of the fundus, hemorrhaging, and cellular infiltration of the lamina propria. In addition, the fundic glands remained intact in comparison to when commercial tablets were administered.

Figure 6 shows the normal pylorus of an untreated rabbit stomach. The mucosa is covered by an intact, continuous epithelium layer and an underlying healthy lamina propria. Pyloric ducts extend into the lamina propria and the secretary portions of pyloric glands are transversely cut. Histopathological examination of the rabbit stomach pylorus (Figure 6) after administration of commercial tablets revealed that the surface columnar epithelium had necrotic ulceration and both hyperemia and cellular infiltration in the lamina propria. In addition, the underlying pyloric glands were disrupted. Once again, these results indicate damaged cells and tissues in the pyloric portion of the stomach after administration of the conventional commercial DS tablets. Photomicrographs of the rabbit stomach pylorus after administration of formulations A and B (Figure 6) show that the mucosa is covered by healthy, intact epithelium, pyloric ducts, and pyloric glands. That said, the lamina propria displays mild hyperemia with a trace of cellular infiltration. The preceding results demonstrated that the administration of DS in formulations A and B reduces progressive necrotic ulceration and cellular infiltration in the lamina propria of the stomach. Furthermore, disruption of the underlying pyloric glands is absent. However, mild hyperemia is present.

Histological examination of both the fundus and pylorus of rabbit stomachs revealed no histological alterations in mucous surface cells and glands following administration of formulations A and B. In contrast, when the commercial formulation was administered the stomach wall had a disrupted mucous layer, necrotic ulceration, hemorrhaging, and inflammatory cell infiltration along the base of the gastric glands.

This study clearly indicated that formulations A and B did not produce any ulcerative effect where the structural integrity of the stomach was completely intact. These results prove that the presence of pectin and mastic in formulation A or sodium alginate and

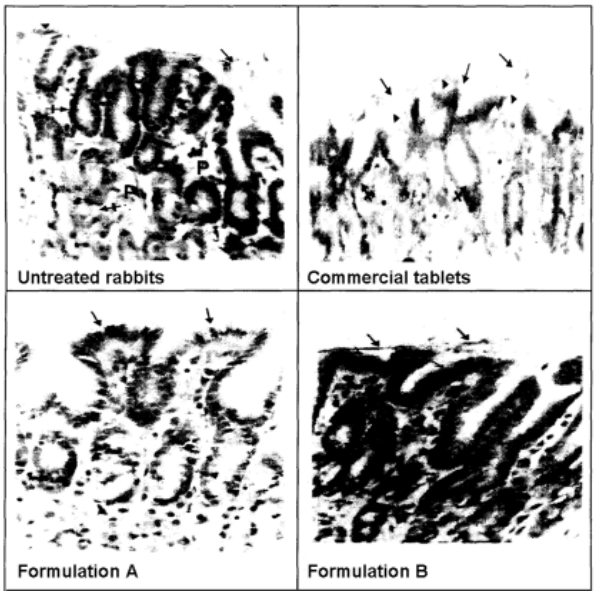

Figure 5. Photomicrographs of rabbit stomach fundus after administration of a signal dose of different formulations.

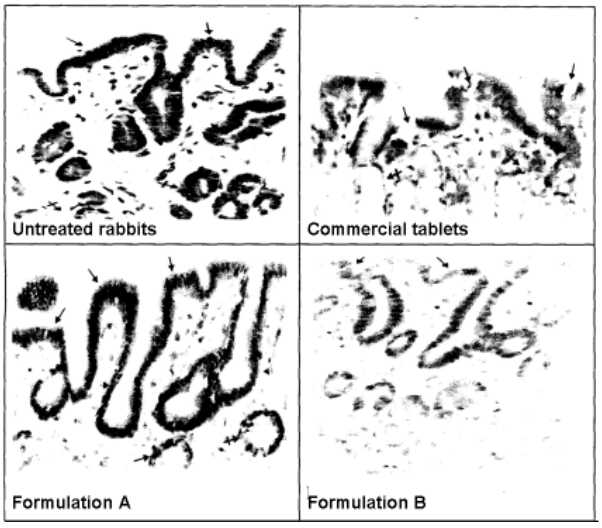

Figure 6. Photomicrographs of rabbit stomach pylorus after administration of a signal dose of different formulations.

mastic in formulation B act to protect the mucous layer of the stomach. This gastroprotective action may be due to the lining of the stomach surface by the gel-forming materials pectin and sodium alginate and/or the oily gum resin of mastic. In addition, mastic has cytoprotective and mild antisecretory properties as evidenced in experimentally induced gastric and duodenal ulcers in rats (*23*) and both pectin and sodium alginate have gastroprotective action (*24-27*).

4. Conclusion

In conclusion, formulation A (containing pectin and mastic) and formulation B (containing sodium alginate and mastic) proved satisfactory as evident in their controlled release, acceptable stability, good bioavailability, and prevention of gastric ulcers. These formulations may thus lead to improved patient compliance.

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