Sulindac solid dispersions: development, characterization and *in vivo* evaluation of ulcerogenic activity in rats

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ABSTRACT

Sulindac is a poorly soluble nonsteroidal anti-inflammatory drug associated with gastrointestinal intolerance as its serious side effect. This work investigated the ability of Eudragit Ll00-55 (Eud L100-55), Cellulose acetate phthalate (CAP) and β-cyclodextrin (β-CD) to ameliorate its gastric ulcers induced in rats. Binary solid dispersions (SD) using solvent evaporation method were fabricated for the drug with different drug to polymer weight ratios of 1:1, 1:2 and 1:3. SD and physical mixture were characterized through *in vitro* dissolution, infrared spectroscopy, differential scanning calorimetry, X-ray diffraction and scanning electron microscopy. The best enteric SD and SD using β-CD was tested *in vivo* for their ulcerogenic activity. Sulindac was highly dispersed inside CAP system that efficiently limited its release inside the stomach while no occurrence of any physicochemical interactions with the drug. β-CD improved the drug aqueous solubility, however it couldn't protect against gastric ulcers induced by sulindac. SD using CAP as enteric polymer at a ratio of 1:2 significantly suppressed gastric ulceration. Direct exposure of sulindac to the stomach wall had the major contribution to its ulcerogenic activity rather than its poor gastric solubility. The gastrointestinal intolerance of sulindac could be addressed by avoiding its acute local contact with the ulcer-prone areas.

INTRODUCTION

Concepts about gastro duodenal mucosal injury induced by nonsteroidal anti-inflammatory drugs (NSAIDs) have been evolved from a simple theory of topical injury to other theories involving multiple mechanisms with both local and systemic effects (Wolfe *et al.,* 1999) The systemic effects were largely resulted from the inhibition of endogenous prostaglandin synthesis which in turn, led to sharp decrease in epithelial mucus and bicarbonate secretion, epithelial proliferation, mucosal blood flow and there for the mucosal resistance to injury (Schoen and Vender, 1989; Wolfe and Soll, 1988). Topical mucosal Injury of NSAIDs was initiated by their acidic properties represented by their lower dissociation constant. These weak acids remained as lipophilic non-ionized form in the highly acidic gastric environment, such conditions favor its migration through the gastric mucus across plasma membranes into surface epithelial cells, where NSAIDs are dissociated resulting in trapping of hydrogen ion that can directly kill epithelial cells (Allen *et al.,* 1993; Schoen and Vender, 1989; Somasundaram *et al.,* 1995).

NSAIDs can also induce topical mucosal damage by decreasing the hydrophobicity of gastric mucus, thereby allowing endogenous gastric acid and pepsin to injure and damage the surface epithelium (Darling *et al.,* 2004; Lichtenberger *et al.,* 2006; Wolfe and Soll, 1988). Although NSAIDs had a well-established place for the management of osteoarthritis and rheumatoid arthritis, its chronic use was accompanied with significant gastrointestinal (GI) toxicity (Lanas, 2010). Moreover, 1–4% of patients chronically taking these drugs clinically developed significant ulceration, bleeding, and obstruction (Silverstein *et al.,* 2000). Recent clinical trials done over six months revealed that 17.1% of patients showed clinically significant ulcers after treatment with conventional NSAIDs (Scheiman *et al.,* 2006). Sulindac, (*cis*-5-fluoro-2-methyl-1-[(*p*-methylsulfinyl)benzylidene] indene-3-acetic acid) is a NSAID, chemically related to indomethacin, with strong analgesic and antipyretic properties that was clinically advocated for therapeutic use in rheumatoid arthritis, osteoarthritis, degenerative joint disease, ankylosing spondylitis and acute gout (Plakogiannis and McCauley, 1984). Sulindac has been proved to decrease the incidence of colorectal adenomas and carcinomas thus it can be used as a cancer chemo preventive agent for those disorders (Thun *et al.,* 2002). The most frequently reported adverse effect of sulindac was that affecting the gastro-

intestinal tract (GIT). The highest rate of upper gastrointestinal bleeding was reported for sulindac users among different users of other NSAIDs (Carson *et al.,* 1987). In addition to, 15.4% of patients taking sulindac either alone, or in combination with aspirin developed different gastric, pyloric and duodenal ulcers (Larkai *et al.,* 1987). The significant differences in the toxicity of NSAIDs were closely related to the incidence of their gastrointestinal adhesions. Occurrence of gastrointestinal adhesions was more frequent in mice treated with sulindac than other NSAIDs at selected doses used for the treatment of rheumatoid arthritis and osteoarthritis. Gastrointestinal adhesion of sulindac was directly related to its cumulative retention inside the GIT (Jalbert and Castonguay, 1992). Moreover, according to the Biopharmaceutical Classification System, sulindac is regarded as a class II drug which characterized by its low water solubility and high permeability. Bioavailability for class II drugs is limited by their dissolution rate which would be increased by improving the drug dissolution rate. Low water solubility is another major problem related to sulindac's bioavailability besides its possible impact on its local adverse effects (Leuner and Dressman, 2000; Yazdanian *et al.,* 2004).

Sulindac is a potential candidate for the aforementioned reasons to investigate the possible relation between its local contact with the gastric mucosa and the occurrence of GI intolerance that will help to manage its serious side effects. Previous literatures reported different fabrication solutions for a similar NSAIDs as aceclofenac to overcome both of these problems by formulating a soft capsule containing drug and solubilizers (Yong *et al.,* 2005), solid dispersions using mixed surfactants (Joshi and Sawant, 2006), complexation with HP-β-cyclodextrin (Dahiya and Pathak, 2006), combination of immediate-release prostaglandins and extended-release NSAID (Franz, 2007), dual-release compositions of Cox-2 inhibitors (Desai *et al.,* 2007), spherical agglomerates using sodium alginate and PVP (Muatlik *et al.,* 2007), chitosan–drug cocrystals (Muatlik *et al.,* 2008), drug-loaded agarose beads (Yesmin *et al.,* 2008), fast-dissolving tablets (Margret Chandira *et al.,* 2008; Setty *et al.,* 2008) and enteric coated immediate release pellets (Kilor *et al.,* 2010). Unfortunately, all these approaches seemed to be more attractive for improving the dissolution of these drugs rather than avoiding their GI adverse effects.

Thereby, there is a critical need to prepare a new sulindac formulation not only to improve its solubility but also to minimize its GI side effects. It is also essential that such a formulation should involve simple and reproducible technique so that it can be easily applied at a commercial level.

Solid dispersion can be defined as a type of solid state material where molecular dispersion of one or more pharmaceutically active drugs in an inert carrier matrix occurred (Chiou and Riegelman, 1971; Singh *et al.,* 2011).The solid dispersion technique has been reported to be highly successful in improving the solubility and bioavailability of poorly soluble drugs because it is simple, economic, and easily applicable to various types of drugs (Shah *et al.,* 2007; Vasconcelos *et al.,* 2007).

Our hypothesis outlined the feasible formulation of different sulindac solid dispersion systems using two types of protective polymers that could address both of these problems while improving its bioavailability at the same time. The first attempt was to use enteric polymers like Eud L 100-55 and CAP which are resistant to acidic media (Kilor *et al.,* 2010; Lin and Kawashima, 1987) to encapsulate the drug and subsequently decrease the direct contact between the drug dispersed inside the polymer matrix and the gastric mucosa. On the other hand, protective polymers like cyclodextrins which commonly used tominimize the ulcerogenic effect of various NSAIDs on the stomach besides its key role as solubility enhancers for poorly soluble lipophilic drugs (Challa *et al.,* 2005; Loftsson and Duchene, 2007;Uekama *et al.,* 1998). The aim of this study is to compare the magnitude of gastric irritations and gastric ulcers induced in rats after oral treatment with free sulindac and its different solid dispersion systems using enteric polymers or β-CD.

MATERIALS AND METHODS

Materials

Sulindac, Eudragit L 100-55 and Cellulose acetate phthalate were obtained from Sigma-Aldrich Chemical Company (St. Louis, MO, USA). Beta-cyclodextrin was purchased from Fluka Chemical Company (Chemie GmbH, Buchs, Switzerland), Acetone (SDS, France), Isopropanol (Fisons, England), Ethanol (Fischer scientific, USA), Potassium dihydrogen ortho phosphate (Reidel de Haein, Germany) were purchased from El-Nasr Pharmaceutical Company, Cairo, Egypt. The water used all over the study was double distilled deionized water.

Preparation of solid dispersion systems
Preparation of solid dispersion systems using enteric polymers

Solid dispersions using solvent evaporation technique were employed to coat sulindac with enteric polymers Eud L 100-55 and CAP at different drug to polymer weight ratios of 1:1, 1:2 and 1:3. All formulation were prepared by dissolving the appropriate amount of the polymer in a mixture of isopropanol and acetone (1:1 v/v), with continuous stirring using magnetic stirrer (Stuart, Germany). An amount of sulindac equivalent to 150 mg was dissolved in a minimal amount of the solvent mixture at 40°C. The polymer solution was added gradually to the drug solution over a period of five minutes with continuous stirring. Organic solvents were allowed to evaporateover a period of 24 h under stirring conditions (150 rpm) at room temperature till a dry film was obtained. The resultant film was left in an oven for an additional 2 h at 40°C to ensure a full removal of the organic solvents from the samples. The dried film was then observed microscopically to observe any grittiness or drug precipitation. The dry film formed was granulated through a sieve (450 μm) (Fritsch Gmbh, Germany) in order to obtain drug granules with a homogenous particle size which eventually stored at room

temperature in dark tight containers in a desiccator over anhydrous calcium chloride (Serajuddin, 1999).

Preparation of solid dispersion system using β- cyclodextrin

The solid dispersion of sulindac using β-CD at drug to polymer ratio of 1:1 was prepared by co-evaporation of equimolar drug – β-CD in ethanol-water (1:1 v/v) solution on a water bath at 50°C (Rijendrakumar *et al.,* 2005). Powder mass was screened using the same sieve to get uniform particle size.

The physical mixture was prepared from the exactly weighed amounts of the drug and the polymers which were pulverized in a porcelain mortar, geometrically mixed and finally passed through the same sieve used for solid dispersions.

Characterization of solid dispersion systems
In vitro drug release

The dissolution studies were carried out using USP II dissolution apparatus (Erweka type DT. Germany) for transparent hard gelatin capsule containing either free drug or different sulindac solid dispersion systems containing equivalent to 150 mg of free drug at 0.1 N HCL (pH 1.0) and phosphate buffer of pH values of 3.0 and 7.4 for 2 h in each dissolution medium. The USP general delayed-release dosage form standard specifications were conducted with a paddle speed of 100 rpm, temperature of $37\pm0.5°C$ and 900ml of dissolution medium. Samples (5 ml) were withdrawn at predetermined time intervals along the period of 2.0 hours at l, 3, 5, 7, 10, 15, 20, 30, 45, 60, 90, and 120 min, filtered using Millipore filter (0.45 μm) and were assessed spectrophotometrically at a wavelength of 327 nm with a UV spectrophotometer (SHIMADZU, UV- 160A, Japan). Sample volume used for analysis was replaced by equal volumes of fresh dissolution medium preheated at 37°C to maintain the sink conditions.

Each batch was analyzed in triplicate and the calculated mean cumulative drug release values were used to plot the dissolution curve.

Fourier transform infrared spectroscopy (FTIR)

A qualitative IR analysis has been performed for plain sulindac, sulindac- CAP(1:2)physical mixture and sulindac- CAP (1:2) solid dispersion system. Infrared spectrums of these powders were carried out using FTIR analyzer (Perkin Elmer model, USA) according to the KBr disk method. All samples were grinded and mixed thoroughly with potassium bromide at a ratio of 1:100 (sample/KBr) followed by compressing the powders under pressure of 5 tons for 5 min using hydraulic press to form the KBr disk. Scans were obtained from 4000 to 450 cm^{-1} at a resolution of 2 cm^{-1}.

Differential scanning calorimetry (DSC)

Thermal analysis of sulindac, sulindac- CAP(1:2) physical mixture and sulindac- CAP (1:2)solid dispersion system were characterized by Du pontmodel Setaramlabsys TM (TG-DSC 16 analyzer, France). Approximately 2mg of powder sample was placed in a hermetically sealed aluminum pan (50 μL) with a pinhole at argon purge of 20 mL/min. The temperature difference between the sample and the reference is represented graphically in relation to the differential heat flow. The scanning rate of 20°C/min, from 40°C to 200°C was used in presence of argon.

Powder X- ray Diffraction Analysis (XRD)

Powder X-ray diffraction patterns were recorded using a powder X-ray diffractometer (Bruker AXS model D8 Advance, Germany) under the following conditions: target Cu; filter Ni; voltage 40kv; current 40mA; receiving slit 0.2 inches. The data were collected in the continuous scan mode using a step size of 0.01° at 20/s. the scanning range was 5-50° at a wave length of 1.54°A. Samples used for XRD analysis were exactly the same as those used for DSC analysis.

Scanning Electron Microscopy (SEM)

Visualization of surface morphology was carried out using electron microscope (Jeol JSM-S410 Scanning Microscope, USA). The same samples used for previous characterization were coated with a thin layer of colloidal gold applied in a cathodic vacuum evaporator before observation. The scanning electron microscope was operated at an acceleration beam voltage of 20-40kv with beam size (a few - 30°A). Resolution ranged from 10-1000°A with magnification power of 20-650000X.

In vivo Ulcerogenicity Studies

Adult male Wistar-strain rats weighing 190-210g were obtained from National researches center (Cairo, Egypt). *In vivo* ulcerogenicity studies were conducted according to the procedure reported by previous study (Alsarra *et al.,* 2010) with some modifications. Experimental design and animal groups was shown in (Table 1).

Table 1: Effect of different doses of sulindac and sulindac solid dispersion systems on ulcer incidence and ulcer index.

Group Number	Treatment[≠]	Ulcer Incidence	Ulcer Index[≠]
I	Control group	0% (0/6)	0.0 ± 0.0
II	Sulindac 5mg/kg	83.3% (5/6)	1.167 ± 0.307
III	Sulindac: β CD (1:1) 5mg/kg	100% (6/6)	1.33 ± 0.210
IV	Sulindac:CAP (1:2) 5mg/kg	33.3% (2/6)	0.33 ± 0.210
V	Sulindac 10mg/kg	100% (6/6)	1.33 ± 0.210
VI	Sulindac: β CD (1:1) 10mg/kg	100% (6/6)	1.5 ± 0.223
VII	Sulindac:CAP (1:2) 10mg/kg	66.6% (4/6)	1.67 ± 0.210
VIII	Sulindac 15mg/kg	100% (6/6)	4.167±0.167
IX	Sulindac: β CD (1:1)15mg/kg	100% (6/6)	4.33±0.210
X	Sulindac:CAP (1:2) 15mg/kg	83.3% (5/6)	1.33±0.210

Rats were maintained at 22 ± 1 °C on a 12 h light-dark cycle and allowed rat chow and water ad libitum. Ten groups of rats (n = 6 animals per group) were used. The allocation of the animals to each group was randomized. *In vivo* experimental protocols were approved by the Animal Care and Use Committee and were in accordance with all recommendations in the University Guide for the Care and Use of Experimental Animals. Before the start of the experiments, rats were housed individually

in wire mesh cages to avoid coprophagy under controlled environmental conditions. Food was withdrawn for 36 h but water was allowed ad libitum (El-Shitany, 2006) The absence of ulcers in some of the treated groups has revealed that the pre-fasting condition alone didn't induce any ulcers.

As described in the previous studies (Bhargava *et al.,* 1973;Brzozowski *et al.,* 2001; Schmassmann *et al.,* 1998), on the morning of the experiments each fasted rat was orally administered 1 ml suspension of the assigned drug by oral gavage in a dose equivalent to 5, 10 and 15 mg/kg of sulindac or different sulindac solid dispersion systems.

Magnetic stirring was utilized to obtain a well-dispersed suspension of each drug and solid dispersion treatment. Six hours later (Chandranath *et al.,* 2002), each animal was removed from its cage, anaesthetized with ether, and the abdomen was opened. Each stomach was excised, dissected along the greater curvature and contents were emptied by gently rinsing with isotonic saline solution. Each stomach was pinned out on a flat surface with the mucosal surface uppermost.

Macroscopic examination of gastric ulcers

The ulcer incidence represented by presence of hemorrhagic lesions and/or gastric ulcers were examined and assessed macroscopically with the help of a 10x binocular magnifier immediately after the animals were sacrificed. To quantify the induced ulcers in each stomach, the scoring system reported by (Alsarra *et al.,* 2010) was employed.

The induced ulcers were in the form of small spots punctiform lesions and each was given a score between 1 and 4. Ulcers of 0.5 mm diameter were given a score of 1 whereas ulcers of diameters 1 and 2 mm were given scores of 2 and 4, respectively.

Stomach with no pathology was assigned a score of zero. For each stomach, an ulcer index was calculated as the sum of the total score of ulcers. Six determinations were made for each drug suspension administrated. The average ulcer index is presented as the mean (n =6) ± standard error.

Histopathological Examination of stomach sections

For histological examination, all stomachs were removed and fixed overnight in 10 % w/v buffered formalin. Each specimen was sectioned, processed overnight and then embedded in paraffin. The paraffin blocks were sectioned and the slides were stained with a standard haematoxylin and eosin stain and photographed under 20 x magnifications using a Nikon Eclipse 80i light microscope (Nikon Corporation, Japan)**.**

Statistical Analysis

All data are presented as the mean ± the standard error (S.E.). Significant differences between different *in vitro* and *in vivo* values were determined by one- way analysis of variance (ANOVA) using the SPSS[®] (version 10, 1999, SPSS Inc.,

Chicago, IL). Statistical differences showing $P \leq 0.05$ were considered significant.

RESULTS AND DISCUSSION

In vitro drug release

Solid dispersion using solvent evaporation technique or film casting method can be used efficiently to yield molecularly dispersed form of the drug inside the carrier matrix besides its role in adjusting the right amount of drug, polymer and plasticizer combination that should be used (Shanbhag *et al.,* 2008; Wyttenbach *et al.,* 2013).

Mechanisms of drug release from solid dispersion systems are reliant on the dissolution behavior of both the drug and the polymer. The physicochemical properties of the polymer determine the drug release from the carrier in case of low drug concentration. On the other hand, high drug concentration loaded into polymer matrix made the physicochemical properties of the drug like its particle size to control the dissolution behavior (Craig, 2002; Srinarong *et al.,* 2011).

Eud L 100-55, is an anionic copolymer based on methacrylic acid and ethyl acrylate. The carboxylic groups start to ionize in aqueous media at pH 5.5 and above, rendering the polymer resistant to acidic media (Kilor *et al.,* 2010). Cellulose acetate phthalate was commonly used as an enteric film coating material, or as a matrix binder for tablets and capsules (Lin and Kawashima, 1987). Such coatings resisted prolonged contact with the strongly acidic gastric fluid, but readily dissolved in neutral intestinal environment releasing the drug immediately.

In vitro drug dissolution profiles for free drug showed that the percentage dissolved after a time point of 20 minutes of the free drug was 24.4, 26.46, and 86.37 % at pH values of 1.0, 3.0, and 7.4 respectively.

Solid dispersion of sulindac using both enteric polymers at a drug to polymer weight ratio of 1:1, showed that the polymers were unable to coat the drug efficiently to prevent its release at acidic pH values. Solid dispersion using enteric polymers adopting this ratio resulted in cumulative release of 26.67 ± 2.75%, 28.17 ± 3.3% at pH 1.0 and 22.65 ± 5.44%, 25.4 ± 4.36% after the same time at pH 3 from Eud L100-55 and CAP, respectively. High drug content of 91.23± 1.57% and 93.99± 3.24%, was released at pH 7.4 from Eud L100-55 and CAP, respectively.

Increasing the drug to polymer weight ratio to 1:2, CAP polymer showed significant decrease in drug released after 20 minutes of 12.84 ± 1.67% and 12.17 ± 0.89% compared to what released from Eud L100-55 polymer at both acidic pH values of the stomach 1.0 & 3.0, respectively (p value <0.05).This significant reduction in drug release is more clear at higher drug to polymer ratio of 1:3 from CAP rather than from Eud L100-55 polymer at both acidic pH values of the stomach (p value <0.05). However, there was no significant difference in drug release from either Eud L100-55 or CAP polymers at pH of the intestine of 7.4(p value >0.05).

Solid dispersions using water soluble carriers ascyclodextrins which commonly used as solubility enhancers in drug formulations led to formation of water-soluble non covalent inclusion complexes with poorly water-soluble drugs and consequently improved its poor aqueous solubility (Challa *et al.*, 2005; Loftsson and Duchene, 2007). Solid dispersion system using β-CD at drug to polymer ratio of 1:1, significantly increased the aqueous solubility of sulindac at all pH values (p value <0.05). This can be due to reduction of drug particle size to a nearly molecular level inside the solid dispersion. β-CD as a soluble carrier dissolved releasing sulindac to be exposed to different dissolution media as very fine particles resulted in quick dissolution and absorption (Dhirendra *et al.,* 2009; Vasconcelos *et al.,* 2007)
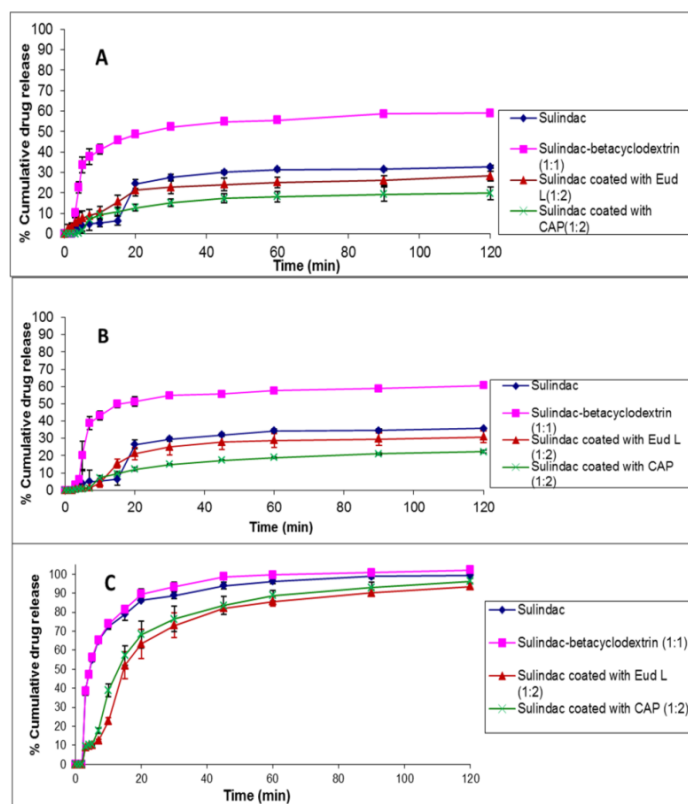
Figure 2 represented the release profile of sulindac (control), and sulindac solid dispersion systems (Eud L100-55 (1:3), CAP (1:3) and β-CD (1:1)) at pH 1.0, 3.0 and 7.4 (fig. 2-A, B and C, respectevely). The figure depicted that at acidic pH values of 1.0 and 3.0 there was a significant reduction in the release of sulindac from solid dispersion system using CAP at drug to polymer weight ratio of 1:3 where the total percentage released was 16.53 % and 14.68 % compared to 25.73% and 27.6 % release from Eud L 100-55. However, at pH 7.4, the release of sulindac from CAP was significantly decreased with a delayed release rate compared to the control.
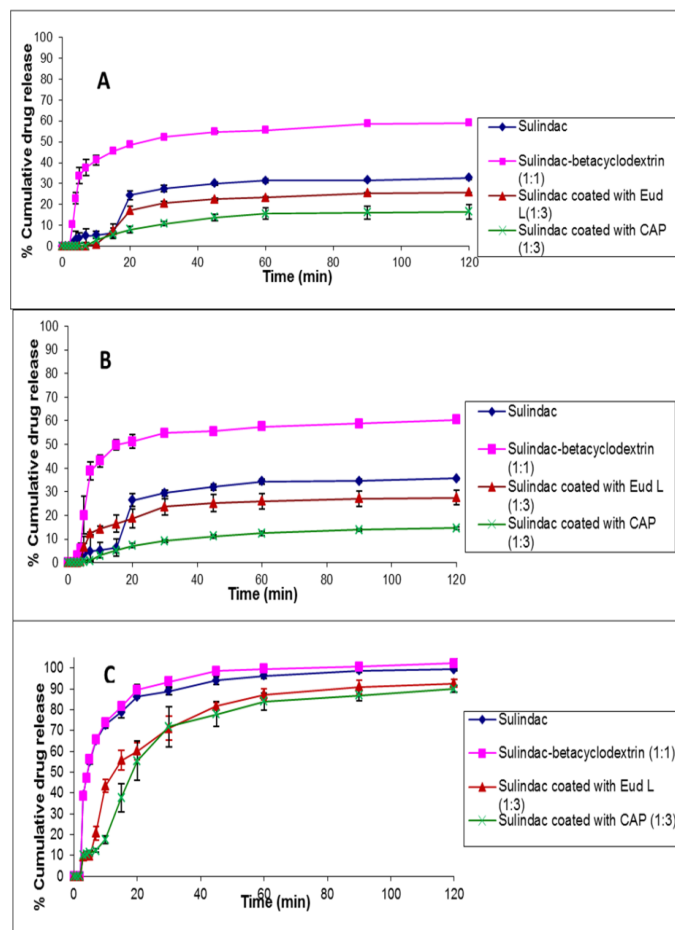

Fig. 1: Release profile of sulindac as a free drug (♦) and from solid dispersion systems using β-CD (1:1) (■), Eud L 100-55 (1:2) (▲) and CAP (1:2) (×) at pH 1(A), 3(B) and 7.4 (C) (±SE n=3).

Fig. 2: Release profile of sulindac as a free drug (♦) and from solid dispersion systems using β-CD (1:1) (■), Eud L 100-55 (1:3) (▲) and CAP (1:3) (×) at pH 1(A), 3(B) and 7.4 (C) (±SE n=3).

Figure 1 demonstrated the release profile of sulindac (control), and sulindac solid dispersion systems (Eud L100-55 (1:2), CAP (1:2) and β-CD (1:1)) at pH 1.0, 3.0 and 7.4 (fig. 1-A, B and C, respectively). The figure clearly showed that solid dispersion system using CAP at drug to polymer ratio of 1:2 achieved significant reduction in the total drug release of 19.84 % and 22.29 % compared to 28.21% and 30.84 % release from Eud L 100-55 at pH values of 1.0 and 3.0 respectively. At pH 7.4, there was no significant difference between total percentages released of sulindac from the three solid dispersion systems as well as the control due to the solubility of the three polymers at this pH value.

Screening the previous results, cellulose acetate phthalate was the best enteric polymer to be used for sulindac solid dispersion. This can be explained by sufficient thickness and uniformity achieved by increasing its amount which provided maximal protection against drug release at acidic pH values of 1.0 and 3.0 at both drug to polymer ratios of 1:2 and 1:3, however maximum dissolution at intestinal pH of 7.4 was only achieved at a weight ratio of 1:2. Cellulose acetate phthalate at a drug: polymer ratio of 1:2 was selected as the optimal solid dispersion using enteric polymer to conduct further *in vitro* characterization

and *in vivo* evaluation, since it was the lowest ratio which achieved significant reduction in drug release at both fasted and fed state of the stomach while performing higher dissolution at intestinal pH and consequently higher drug bioavailability. Moreover, enhancement of sulindac aqueous solubility using β-CD could be another pretty attractive approach to evaluate its possible role in minimizing the drug's GI intolerance *in vivo*.

Fourier transform infrared spectroscopy

FTIR studies were carried out to check for any physicochemical interaction between the drug and the polymer. The interaction between the drug and polymer often resulted in new identifiable changes or modifications in the IR profile of solid dispersion (Kilor *et al.,* 2010; Mahapatra *et al.,* 2011; Modi and Tayade, 2006). Free drug spectrum showed the principle peaks of sulindac at 1700 cm^{-1} (C=O), 1155 cm^{-1} (C – F), 1000-1020 cm^{-1} (S = O), 1580 & 1600 cm^{-1} (aromatic ring modes). The FTIR spectra of sulindac solid dispersion using CAP compared with those of pure sulindac and physical mixture powder showed all drug and polymer relative peaks at the same wave number but with a certain reduction in their intensity. This indicated that there was no interaction between the drug and the polymer in the solid dispersion system. Sulindac remained unaffected during solvent evaporation process and hence CAP can be properly used as an enteric polymer for sulindac.

Differential Scanning Calorimetry

The DSC thermograms of sulindac solid dispersion system in comparison to those of pure sulindac and the physical mixture confirmed no occurrence of any prominent interaction between the drug and the polymer. Free sulindac showed a sharp endothermal peak at188°C due to its crystalline form. In case of the solid dispersion, significant broadening and decrease in Tm of the drug endothermic peak was occurred in addition to a sharp decrease in the enthalpy compared to pure sulindac. This behavior suggested a significant reduction in drug crystallinity (Osawa *et al.,* 2014).The widening of the endothermic melting peak of the drug could be explained by the highly dispersed molecular form of the drug inside the matrix (Tros de llarduya *et al.,* 1998).

Powder X- ray Diffraction Analysis

Sulindac powder was crystalline as indicated from the appearance of the characteristic diffraction patterns which showed sulindac characteristic peaks. The X-ray diffraction pattern of solid dispersion system revealed no sharp peaks attributable to sulindac whilethe rest of the diffraction pattern was diffused as in the case of amorphous substances. This could be attributed to drug transformation from the crystalline form into non crystalline amorphous form during the solid dispersion process (Osawa *et al.,* 2014).

Scanning Electron Microscopy

Scanning electron microscopy (SEM) is the technique of choice to support visually the other qualitative and quantitative results by exploring the shape and surface morphology of the drug powder or granules (Kilor *et al.,* 2010). Surface morphology of sulindac, sulindac-CAP (1:2) physical mixture and sulindac- CAP (1:2) solid dispersion revealed sulindac crystals with similar crystal shapes and well-defined surfaces which almost disappeared in solid dispersion due to polymer matrix formation by where the drug was highly dispersed (Figure 3). These findings supported further results of X-ray diffraction which revealed transformation of the drug into amorphous form.
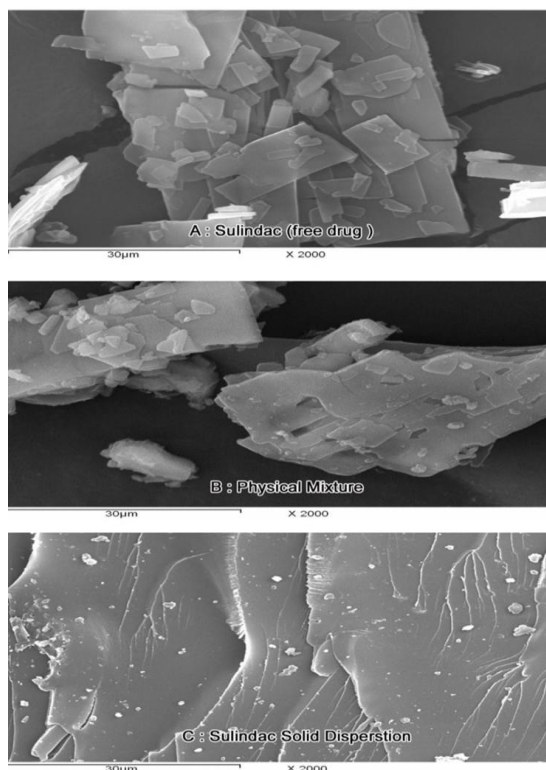

Fig. 3: Scanning Electron Microscopy of sulindac (A), sulindac-CAP(1:2) physical mixture(B) and sulindac coated with CAP (1:2) as solid dispersion (c) (20-650000X).

***In vivo* Ulcerogenicity Studies**
Macroscopic Analysis

Gastric mucosal injury was evaluated based on observation of rats' stomach treated with pure drug and different solid dispersion systems. Results in (Table 1) revealed that untreated rats developed no ulcers; however, group II rats showed ulcer incidence of 83.3% which confirmed that sulindac can induce gastric ulceration after a single dose administration of (5mg/kg) of free drug which was consistent with the dose used clinically (Glavin and Sitar, 1986; Jalbert and Castonguay, 1992). Increasing the dose of the free drug to higher level of 10 &15 mg/kg was to account for the cumulative behavior of sulindac which resulted from its higher ability of gastrointestinal adhesion as well as its chronic use by the patients with chronic rheumatologic disorders.

Gross study of gastric Lumina of control group showed completely apparent normal glistening mucosa regarding normal rauga and mucous covering layer. Single 5 and 10mg/kg doses of

sulindac evoked focal area of congestion, spots of hemorrhagic area (pin pointed hemorrhagic area covered with mucous) while wide spread hemorrhagic areas indicated by dark red spots which are blood clots appeared after treatment with 15 mg/kg dose. Solid dispersion using β-cyclodextrin revealed spots of hemorrhagic area beside lesions of deep perforating ulcers within gut sections for all doses. This could be due to dead mucosal cells which started the mucosal damage leading to ulceration (Karanachi *et al.,* 1997). Single doses of solid dispersion using CAP (1:2) showed apparently normal gastric mucosa at low dose of 5mg/kg and tiny hemorrhagic spots or just hyperemic areas covered with mucous at higher ones. In addition to, there were a significant reduction in both the number and the diameter of ulcer per rat treated with solid dispersion of sulindac with CAP rather than other treatment groups.

Figure 4 showed that formulation of sulindac with β-CD resulted in non-significant increase in the gastric ulcers induced by free sulindac for all doses. However, the solid dispersion system using CAP at drug to polymer ratio of 1:2, significantly suppressed the stomach ulcers for all doses. Based on the ulcer index values of all animal groups, the enteric coated formulation of sulindac with CAP resulted in a mean percentage reduction in the gastric ulcers by 71.7%, 44.5% and 68.1% compared to the control group and 75.5%, 50.3% and 69.3% compared to solid dispersion usingβ-CD following treatment with different doses of 5, 10 and 15mg/kg, respectively (Table 1).
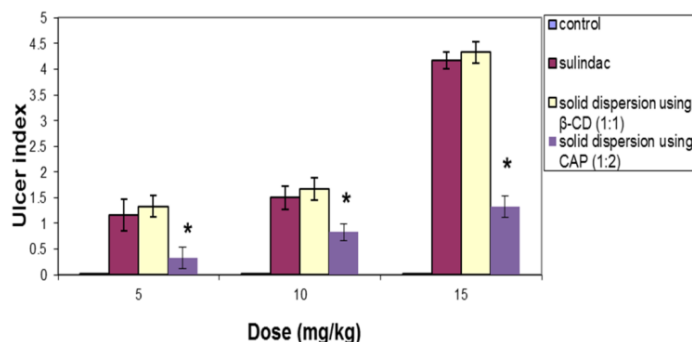

Fig. 4: Effect of different doses of sulindac & sulindac solid dispersion systems on the gastric ulcer measured as ulcer index. Rats were treated by three different doses of sulindac and sulindac solid dispersion systems (5, 10 and 15 mg/kg) and ulcer index was calculated. Each value is the mean ± S.E (n = 6). (*) ulcer index values of groups treated bysulindac solid dispersion using CAP (1:2) is significantly lower compared to corresponding drug group and solid dispersion using β-CD (1:1) of the same dose (P<0.05).

Histopathological Observations

The histological pattern of the mucosal specimens was studied for each treated and control samples. Histopathological examination of H&E stained stomach sections of control rats showed completely normal gastric mucosa with normal squamous epithelium and excess mucous layer (Figure 5.A). After treatment with 5mg/kg dose, histopathological examination of group II stomach sections revealed superficial focal ulceration with swallowed degenerated superficial mucosal covering layer infiltrated by inflammatory cells (Figure 5.B) while one stomach

showed mild congestion in the lamina properia. Mucosal specimens of Group III showed wide area of superficial degenerated swollen cells infiltrated by inflammatory cells (neutrophil infiltration) (Figure 5.C).However, the histological pattern of group IV experienced completely normal mucosal covering with a thin rim of mucous similar to control group (Figure 5D) while two rats showed mild mono nuclear cellular infiltration.
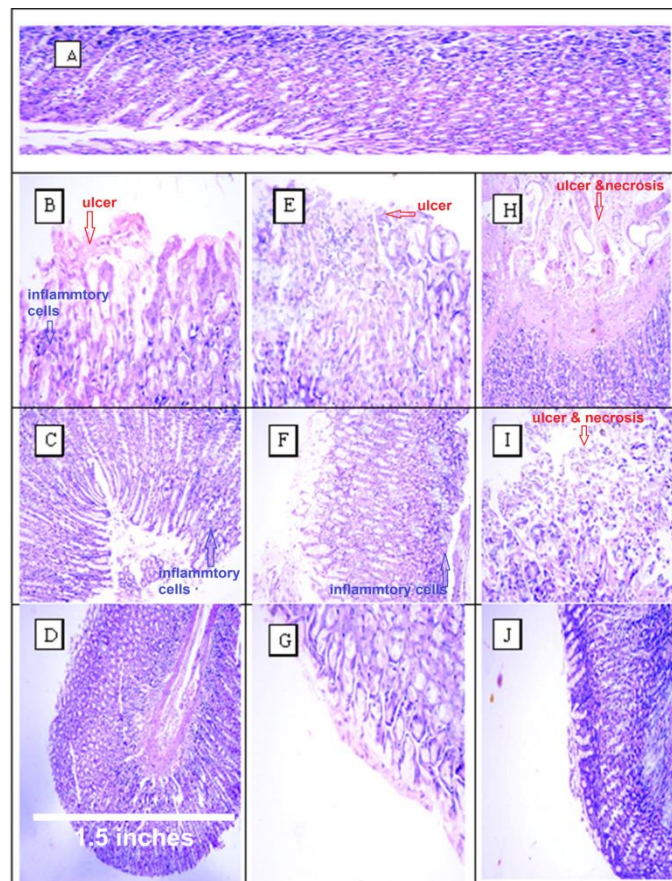

Fig. 5: Histopathological photographs of stomach specimens stained with hematoxylin and eosin (Hx&E x 250). A: from control rats; treatment with sulindac, solid dispersion using β-CD and solid dispersion using CAP with 5mg/kg represented as B, C &D , with 10mg/kg represented as E, F &G and with15 mg/kg represented as H, I &J, respectively.

Regarding rats treated with 10mg/kg dose, histopathological examination of group V, showed wide focal area of mucosal necrosis and excess cellular debris with mono nuclear infiltration (Figure 5.E) while all rat stomachs of group VI revealed wide areas of superficial necrosis with excess mucous covering infiltrated with inflammatory cells (Figure 5.F). However, group VII treated rats showed superficial swollen (edematous) mucosa covered with apparent thick mucus layer infiltrated with some inflammatory cells (Figure 5.G) while two mucosal specimens showed completely normal gastric mucosa.

The highest dose of (15 mg/kg) of group VIII, showed pronounced necrotic gastric mucosa with sever dilated congested blood vessels in the lamina properia with sever edema highly

infiltrated by inflammatory cells (neutrophil infiltration) (Figure 5.H) while all rats stomachs of group IX revealed marked congestion, necrosis, and edema in superficial mucosal layer heavily infiltrated with mononuclear inflammatory cells (lymphocyte and plasma cells) (Figure 5.I). In group VII treated rats experienced small focal necrotic gastric mucosa with excess covering mucosal layer studied with cellular debris and inflammatory infiltration (Figure 5.J) while one stomach showed apparently normal gastric mucous.

Histological examinations augmented the macroscopic results in the comparative evaluation of the role of β-CD as a protective polymer and CAP as enteric polymer in decreasing the gastric ulcer induced in rats by sulindac.

NSADs can produce GIT mucosal injury via local irritating and systemic effect (Engelhardt *et al.,* 1995). Local ulcerogenic activity of sulindac may be attributed to its local inhibitory effect on gastric prostaglandin E2 (PGE2) and prostaglandin I2 (PGI2) that are the main inhibitors of gastric acid secretion beside its poor gastric solubility and direct contact mechanism (Bhargava *et al.,* 1973; Ribeiro-Rama *et al.,* 2009). Various NSAIDs have been complexed with cyclodextrins, obtaining in this case many advantages such as dose lowering, taste masking and reduction of side effects particularly gastric irritation (Rijendrakumar *et al.,* 2005;Uekama *et al.,* 1998).β-CD are not absorbed in the gastrointestinal tract, it only enhance the absorption of drugs after oral administration. Inclusion complex of sulindac with β-CD acted to interfere with the local ulcerogenic action of sulindac by competitive inclusion complexation of local gastric prostaglandins or mucin. These proposed mechanisms are supported by the reported data for complexation of CDs in aqueous solutions with prostaglandins E1,E2, and F2, released as a result of stress especially in case of stress-induced ulcers (Silverstein *et al.,* 2000; Simon *et al.,* 1998; Simon *et al.,* 1999)

Sulindac is characterized by its greater ability for gastrointestinal adhesions which followed by high accumulation behavior that can induce gastric ulcer under normal conditions without stress by acute local contact with the gastrointestinal mucosa. In our study, two different approaches were adopted to overcome this problem. Decreasing the direct exposure between sulindac and the gastric mucosa played a major role in controlling its ulcerogenic activity. *In vitro* results showed that sulindac is widely dispersed inside the CAP as enteric carrier so that only sulindac allocated on the surface can contact with the stomach wall to induce gastric irritation while the rest is slowly released by low disintegration of the enteric matrix in the stomach. This key finding can be appreciated from the significant reduction of ulcer incidence and ulcer index following treatment with enteric dispersion formulation. On the other hand, β-CD not only could not counteract the drug-induced ulcer but also increased its ulcerognic activity by facilitating the chance of direct local contact of sulindac in the soluble form with the ulcer exposed areas.

CONCLUSIONS

It is clear that β-CD and CAP are safe for oral use. The major contribution of the local ulcerogenic effect of sulindac can be attributed to its acute local contact with stomach wall. Therefore, it was very likely that its GI toxicity was aggravated when more amount of soluble drug was available in the stomach at a given time and consequently it can be concluded that, β-CD was unable to protect against gastrointestinal disorders induced by sulindac in rats at normal conditions also at doses consistent with those used clinically. Encapsulating sulindac into CAP as an enteric carrier and its slow diffusion into the gastric lumen as confirmed by *in vitro* dissolution data could alleviate the problem of gastric ulceration by minimizing its direct exposure to the ulcer-prone area of the stomach. Solid dispersion characterization using FT-IR, DSC, XRD and SEM revealed that no significant changes occurred for sulindac. Solid dispersion of sulindac using CAP at drug to polymer weight ratio of 1:2 significantly reduced gastric irritations and gastric ulcers compared to the free drug and solid dispersion using β-CD meanwhile improving its bioavailability. It is worthy to mention that CAP can be utilized in the oral formulation of sulindac to avoid its typical ulceration side effects. Future work using the same approach can be applied for different NSAIDs.

DECLARTION OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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