Evaluation of Newly Formulated Antiulcer Drug on Experimental Ulcer Model

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ABSTRACT: The present study was conducted to evaluate the potential gastric protective and therapeutic effects of ranitidine mucosal adhesive hydrogel formulae against ethanol induced gastric ulceration in rats. Adult female albino rats weighing between 200-220 g were randomly divided into two comparisons, protective and therapeutic effects of newly developed ranitidine mucosal adhesive hydrogel formulae containing polymer mixture of Chitosan and Hydroxypropyl methyl cellulose (HPMC) at ratio 9:1 (F1) or mixture of Sodium carboxymethyl cellulose (NaCMC) and HPMC at ratio 9:1 (F2). Each experiment has five groups, seven rats each; group 1 serves as control and group 2 serves as ulcer control since received a single oral dose of absolute ethanol (5ml/kg body weight). Group 3 ulcer group received an oral dose of ranitidine (27mg/kg), while groups 4 and 5 ulcer group received newly formulae of ranitidine F1 & F2 respectively. In the present study some gastric parameters as ulcer index, total acidity, gastric volume and pH besides some biochemical parameters as alanine aminotransferase (ALT), aspartate aminotransferase (AST) and total antioxidant capacity (TAC), total protein (TP) level and alkaline phosphatase (ALP) activity were carried out at the end of the experimental period. The present study showed that F1 revealed more protective and therapeutic potency than F2 as it was significantly reduced ulcer index, total acidity, gastric volume and pH in comparison to ulcerated group (p<0.05). Also, the biochemical markers ALT, AST and TAC were decreased significantly compared to ulcerated group in both experiments. TP level and ALP activity were altered among different treatments. Moderate improvement in mucus secretion was recorded for F1 & F2 treatments than the reference drug. The present results were confirmed by the histopathology findings. Collectively, the current study confirmed the better therapeutic action of formulae 1 & 2 over the pure drug and that F1 was the most potent formula. Also, it encouraged the use of F2 as a curative agent of ulceration rather than a protective one.

Key words: Ranitidine, mucosal adhesive hydrogel formulation, liver function markers, physiological ulcer indexes, mucin secretion

INTRODUCTION:
Gastric ulcer is a major health hazard in terms of both morbidity and mortality (Chaturvedi et al., 2007). Ulcer therapy has progressed from vagotomy to anticholinergic drugs, histamine H2 receptor antagonists, antacids and to proton pump inhibitors (Wallace & Granger, 1996). Even though the wide range of available drugs, many of them do not fulfill all the requirements and have many side effects. Although histamine H2 receptor antagonist drugs as famotidine and ranitidine are considered to be the safest one (Bourd et al., 2005), these drugs have been reported to have low bioavailability and short biological half-life (Hawkins & Hanks, 2000). Ranitidine is a competitive, reversible inhibitor of the action of histamine at histamine H2-receptors, including receptors on gastric cells, with a minimal effect on H1-receptors (Novak, 2002 and Chavda & Patel, 2010). It is one of the drugs of choice for the treatment of active duodenal ulcers, gastric ulcers, Zollinger-Ellison syndrome, gastroesophageal reflux disease, and erosive esophagitis (McCarty-Dawson et al., 1996). The indicated oral dosage of Ranitidine is 150 mg, twice daily, or 300 mg once daily. It has been found that the dose of 300 mg leads to fluctuations in its plasma levels (Chavda & Patel, 2010). Also, the drug has a short biological half-life of approximately two to three hours, an absolute bioavailability of only 50%, and it is absorbed only in the initial part of the small intestine (Grant et al., 1989; Lauritsen et al., 1990 and Gramatt et al., 1994). Colonic metabolism of Ranitidine is also partly responsible for the poor bioavailability of Ranitidine from the colon (Basit & Lacey, 2001). Hence, these drugs have promising future if controlled release formulations are developed. Several trails have been used Ranitidine to develop many gastroretentive drug delivery systems, but it failed in many cases (Gnanaprakash, 2013). Theoretical and applied studies stated that gastroretentive mucosal adhesive dosage form can remain in the gastric region for several hours and hence significantly prolong the gastric residence time of drugs (Hagerstorm, 2003 and Dua & Trivedi, 2013). Prolonged gastric retention improves bioavailability,
reduces drug waste. It is also suitable for local drug delivery to the stomach and proximal small intestines (Ben et al., 1994 &1996; Hagerstorm, 2003 and Dua, & Trivedi, 2013). Mucoadhesive dosage forms have been widely used for site-specific targeting for both local and systemic drug delivery (Chen et al., 1997; Nagahara et al., 1998; Harding et al., 1999; Wang et al., 2000; Cuna et al., 2001 and Bardonnet et al., 2006). Hydrogels found applications in formulating controlled and mucoadhesive drug delivery systems (Bansal et al., 2011). Hydrogels are cross-linked hydrophilic polymers with a network structure. Chitosan and Sodium Carboxymethyl Cellulose (Sod. CMC), have attracted attention. However, these hydrogels can swell slowly and exhibit low loading capacities, (Henriksen et al., 1996; Burmania et al., 2003 and Leonard et al., 2004) which restrict their use in effective drug delivery. Chitosan, is a linear random copolymer of D-glucosamine and N-acetyl D-glucosamine and is obtained by N-deacetylation of chitin (Domard & Cartier, 1992 and Roberts, 1992). Chitosan hydrogel can be formed by covalent cross-linking or ionic cross-linking. By definition, mucoadhesive hydrogels are a class of polymeric biomaterials that exhibit the basic characteristic of a hydrogel to swell by absorbing water and to interact by means of adhesion with the mucus that covers epithelia (Nikalje et al., 2012). The goal of mucoadhesive drug delivery system is to increase the residence time of therapeutic molecules at the specific sites within the gastrointestinal (GI) tract for absorption of the drug into the circulation (Hassan & Gallo, 1990; Roberts, 1992; Takeuchj et al., 1994 and Needleman & Smales, 1995). The approach involves the use of bioadhesive polymers such as Chitosan - hydroxyethyl propyl methyl cellulose (HPMC) that can adhere to the epithelial surface of the GIT (Sadgir et al., 2014). The adhesion of the polymers with the mucous membrane may be mediated by hydration, bonding or receptor mediation (Park & Robinson, 1984). The current study aimed to assessment the potential gastric protective and therapeutic effects of ranitidine mucoadhesive hydrogel formulations on ethanol induced gastric ulcer in rats compared to ranitidine powder.

MATERIALS AND METHODS:

Drugs and chemicals

Ranitidine HCl (R-HCl) was kindly donated by Amoun pharmaceutical company. Chitosan was purchased from Sigma, Chitosan (≥ 85% deacetylated, Mol. Wt ~ 600,000 Mol. Wt.; ~400 m Pa,s, 1 % in acetic acid at 20 °C, Sigma Aldrich, Germany), Hydroxypropyl methyl cellulose (HPMC), and sodium carboxymethyl cellulose (NaCMC) were purchased from Alfa, Germany. Double distilled water (DDW) was prepared in the laboratory. All other chemicals used were of analytical grade.

Preparation of hydrogel formulations

Two formulae were prepared using weighed amount of polymer mixture of Chitosan and HPMC at ratio 9:1 dissolved in 1% acetic acid to prepare F1; and weighed amount of polymer mixture of NaCMC and HPMC at ratio 9:1 dissolved in distilled water to prepare F2.

336 mg of ranitidine HCl was added to each formula. The formed solutions were casted into Petri dishes and left to dry at room temperature. Then the dried hydrogel films were cut into 2 x 2 mm square pieces and stored in tightly closed container at room temperature (Paloma et al., 2003).

Experimental animals

Induction of ulcer

All groups under investigation received single oral dose of absolute ethanol (5ml/kg body weight) according to the method described by Abdulla et al. (2010) except the control one.

Evaluation of Mucoadhesiveness of hydrogel

Six week aged female Swiss albino rats weighing 200-220 g were obtained from the animal house of National Organization for Drug control and Research (NODCAR, Giza, Egypt). The animals were acclimatized in Kaf El Gabal animal house for 2 weeks before the experiment. The animals were fed a standard diet (El-Nasr Company, Abou-Zaabal, Cairo, Egypt) and tap water ad libitum, and kept under controlled conditions of room temperature (21 ± 1 °C), relative humidity (55±5%) and a 12-h light/12-h dark cycle. Animals were fasted overnight prior to the experiment, but were allowed free access to water. Animals were divided into three groups, each group contains 25 albino rats, as follows: Group I: (positive control): this group contained a total of 25 albino rats and were administrated 5 ml of ranitidine HCl solution at concentration of 10 mg/ml, Group II: This group contained a total of 25 rats and administrated a hard gelatin capsule containing 2 x 2 square particles of formula I (F1) equivalent to 50 mg ranitidine. Group III: This group contained a total of 25 rats and administrated a hard gelatin capsule containing 2 x 2 square particles of formula II (F2) equivalent to 50 mg ranitidine. At 0.5, 1, 2, 3 and 4 hours following administration, 5 rats from each group (I, II and III) were euthanized by cervical dislocation, the stomachs were excised, cut along the greater curvature, and gently rinsed by 25 ml of 0.1N HCl and put in sonicator for dissolving of existed amount of ranitidine in rinsed solution then filtered through 0.45 µm Millipore filter. The amount of ranitidine remaining was evaluated by high-performance liquid chromatography using UV detector at wave length (λmax) 314 nm and C4 column, (250 x 4.6 mm; 5µ). The mobile phase consisted of 0.05 M potassium dihydrogen phosphate (pH 3): acetonitrile (25:75 v/v) and was delivered to the system at a flow rate of 1.2 ml/min. All assays were performed at ambient conditions. The remaining percentage of ranitidine as an index of residence in the stomach, i.e., Mucoadhesiveness, was calculated by the following equation:

Remaining percentage = (R/T) x100

Where R represents the amount of ranitidine remaining in the stomach and T represents the amount of ranitidine administrated. The method was validated for selectivity, linearity, accuracy and precision (Radi & Mansoor, 2004).
Evaluation of protective and therapeutic effect of ranitidine hydrogel

To achieve study goals, seventy healthy adult female Swiss albino rats, weighing between 200-220 g, were obtained from the animal house at the National Organization for Drug Control and Research (NODCAR). They were housed in wire cages with natural ventilation and illumination and allowed free water and standard diet for 10 days before beginning of the experiment. Throughout the experiment, all procedures, animals and the experimental protocols were approved by the Institutional Ethics Committee at NODCAR; and were carried out according to the criteria outlined in the “Guide for the Care and Use of Laboratory Animal”. Rats were fasted for 24 h prior to the experiment but allowed free access to water except for the last hour before the experiment. All experiments were performed during the same time of the day to avoid diurnal variations of putative regulators of gastric functions.

The animals were randomly divided into two experiments, the first to study the protective effects (pre-treated) of newly developed formulae while the second deals with their therapeutic effects (post-treated). Each classified into five groups (seven rats each); Group 1 serves as ulcer control (U), group 3 received ranitidine (R) as a reference antiulcer drug (27 mg/kg body weight) equivalent to human recommended dose (Chavda & Patel, 2010), while groups 4 and 5 received newly formulated ranitidine with the same concentration of ranitidine F1 and F2 respectively. In protective experiment (pre-treated) the animals in each group were receiving the dose of the treatment as mentioned above for 45 minutes before administration of 5ml of 95% ethanol. After further one hour from alcohol administration, blood samples were collected from retro-orbital plexus, then the animals were scarified by cervical dislocation; the stomach content was collected, then it was incised along the greater curvature and examined for gross and histological evaluation. In therapeutic experiment (post-treated) the animals were divided as mentioned above. The treatment was given daily for four successive days on empty stomach-starting one hour after induction of ulcer. On the fifth day blood samples were collected, then the animals were scarified by cervical dislocation, the stomach content was collected and then it was cut along the greater curvature, washed with saline and examined for gross and histological evaluation.

Blood sampling

Blood samples were allowed to clot for thirty minutes at room temperature and then centrifuged at 1000 x g and 4°C for ten minutes. The collected serum samples were stored at -80 °C for further estimation of total protein (TP) content, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) activity and total antioxidant capacity (TAC). Spectrophotometric procedures were used for their determination using commercial kit according to Gornall et al. (1949); Reitman & Frankel (1957); Moss (1982) and Koracevic et al. (2001) respectively.

Gastric volume and Total acidity

The animals were sacrificed and their stomachs were cut along the greater curvature, the content of each stomach was collected into small tubes and centrifuged at 3000 rpm for 5 minutes. The supernatant was separated and its volume was measured and expressed as ml/100 g body weight. The acid content was determined by titration method with 0.05N NaOH. Acidity was expressed as mEq/L/100 g of body weight (Maity et al., 2003).

Acidity= volume of NaOH x normality / 0.1 x100

Macroscopic evaluation of stomach

The macroscopic assessment of ethanol-induced gastric lesions was performed by an independent examiner who was blinded to the supplementation that the rats had received. The assessment of lesions was made according to quantitative scale described by Ismail et al. (1999). The stomachs were opened along the greater curvature, rinsed with saline to remove gastric contents and blood clot then photographed to assess the formation of ulcers. The numbers of ulcers were counted. Mean ulcer score for each animal will be expressed as ulcer index. The scale used was as follows: 5 = continuous lesions that occupied almost the entire length of the gastric fold, 4 = lesions which occupied almost 80% of the entire fold, 3 = presence of multiple lesions that measure 3mm in length on 80% of the folds, 2 = presence of at least two lesions approximately 2mm in length, 1 = presence of a single lesion with or without generalized erythema, 0.5 = presence of dot haemorrhage and 0 = no visible damage.

Curative ratio

The curative ratio from the ulcer was calculated according to Begum et al. (2014) for the treated groups by using the following equation.

Percentage (%) = [(CUI-TUI)/CUI] x 100

Where, CUI = ulcer index of control groups, TUI = ulcer index of treated groups.

Histological and Histochemical studies

Gastric tissue specimens from each group were fixed in 10% buffered formalin for twenty four hours. After that the tissue specimens were washed with tap water then serial dilutions of alcohol (methyl, ethyl and absolute ethyl) for dehydration. Specimens were cleared in xylene and embedded in paraffin at 56 degree in hot air oven for twenty four hours. Paraffin blocks were prepared for sectioning at 4 microns thickness by slide microtome. The obtained tissue sections were collected on glass slides, deparaffinized, stained by hematoxylin & eosin stain for routine examination as well as by alcian blue stain for detection of mucous through the light electric microscope (Bancroft & Stevens, 1996).

Statistical analysis

The results are presented as the mean ± standard error ( SE) of the six animals used in each group. Statistical significance was determined by one-way ANOVA at p< 0.05 followed by Dunnett t (2-sided) for comparisons between control and other groups while Duncan posthoc test was used for comparisons among all treated groups at p<0.05 using the Statistical Package for the Social Sciences software (SPSS 17).
RESULTS:
Evaluation of gastric mucoadhesion
Figure (1) showed the remaining percentage of ranitidine after 0.5, 1, 2, 3 and 4 hours in the different groups (I, II and III). It was observed that the remained amount of ranitidine 4 hours after administration of mucoadhesive hydrogel formulae F1 and F2 was 55.8±6.5%, 45.4±3.5%, respectively compared with 5.6±2.1% after administration of ranitidine powder. Thus, the mucoadhesive hydrogel formulae seem to be a potential as oral gastro retentive controlled drug delivery and targeting its site of action, the stomach, thus improving its oral bioavailability.

Evaluation of protective and therapeutic effect of ranitidine hydrogel
Antiulcer parameters
Table (1) showed the effect of pre-treated of R, F1 and F2 on gastric juice parameters and ulcer parameters. It was found that ethanol administration caused a significant increase in pH value, gastric volume and total acidity; it also caused a remarkably high ulcer index (2.80± 0.20) as compared with control group. While, pretreatment with F1 produced a significant decrease in pH value, gastric volume, and total acidity as compared to ulcerated group. In the protective study there was no significant difference between R & F2 in pH value, gastric volume and total acidity, while F2 showed a significant decrease in total acidity when compared to ulcerated group. Pre-treatment with R, F1 and F2 offered a significant protection against ethanol induced gastric ulcer in the experimental rats. Since Ranitidine reduced the ulcer index to (0.50±0.16) showing 82.14 % prevention whereas F1 reduced the ulcer index to (0.10±0.10) showing 96.43% prevention. While F2 reduces the ulcer index to (1.1±0.24) showing 60.71% prevention.

Table (2) showed the effect of post-treated of R, F1 and F2 on gastric juice parameters and ulcer parameters. It was observed that ethanol administration caused non-significant changes in pH value and gastric volume while total acidity was increased significantly as compared with control group, also caused a ulcer index (2.20±0.20). Post-treatment with R, F1 & F2 resulted in a significant reduction in pH value. Gastric volume showed a non-significant increase in all treated groups. Post-treatment with F1 showed a significant reduction in total acidity as compared to ulcerated group. While R & F2 revealed a non-significant change in total acidity. Post-treatment with R, F1 & F2 produced a significant healing reached to 63.64%, 81.82% and 77.27% respectively.

Biochemical parameters
It was found that oral administration of ethanol caused a significant decrease in serum total protein (TP) in both pre- and post-treated experiments as compared with control group (tables 3 and 4). Pre-treatment with F1 and F2 showed a non-significant change in total protein as compared to ulcerated group. A significant increase in serum total protein levels were observed in F1 & F2 post-treated rats while non-significantly increased in R post-treated as compared to ulcerated group.

Ethanol administration showed a significant increase in the activities of the hepatic enzymes ALT& AST as compared to control group (tables 3 and 4). While the administration of F1& F2 in both pre- and post-treatment experiments resulted in a significant decrease in serum activity of these hepatic enzymes when compared to their activities in ethanol treated rats. Ranitidine administration caused a significant decrease in hepatic enzymes activity in the pre- treated rats and a non-significant increase in post-treated rats as compared to ulcerated group.

It was observed that ethanol caused a significant decrease in ALP levels and a significant increase in TAC level when compared with control group. Oral administration of R, F1 & F2 in pre- and post-treated rats significantly increased ALP level as compared with ulcerated group. Pre-treatment with F1 revealed a significant decrease in TAC as compared with ulcerated group while R and F2 showed a non-significant decrease. Whereas post-treatment with R, F1 & F2 revealed a significant reduction in TAC when compared with ethanol treated group tables (3 & 4).

Histopathological studies
The severity of histopathological changes in stomach of different treatments as pre- and post-treatments was summarized in tables (5) & (6) respectively. Table (5) showed that pretreatment with F1 before ethanol had alleviated the effect of ethanol. Thereby F1 was more effective than R & F2 groups. Table (6) revealed that post-treatment with R, F1 & F2 decrease the severity ulcer induced by ethanol. Formula 2 was more effective than R & F1 treatments.

Histochemistry studies
Histochemical studies Plate (1) showed that; in the control group; the mucous secretion was localized in the superficial surface and in the lining epithelium of the glandular structure. Rat's stomach under the protective (pre-treated) experiment revealed marked alteration compared to control group where, very few mucous was noticed in the mucosal layer among ulcerated rats. While rats administered ranitidine, the intact mucosa and glandular structure showed mucous secretion. F1 treated rats revealed minor alterations where, the blue color of the mucous was noticed in the desquamated mucosal epithelium as well as in the underlying intact glandular structure. In the other hand, the mucous was localized in the deep intact glandular structure of the mucosa among rat treated with F2.

In rats experimentally induced ulcer and sacrificed after four days (post-treated); the mucous was absent from the mucosa. There was very few mucous in the ulcerated surface of Ranitidine (post-treated) group. Rats under F1 treatment showed that mucous was localized in the deep layer of the mucosa in the glands. While in F2 rats group, little mucous secretion was detected in the deep area of the mucosal epithelium.

DISCUSSION:
The main target of this research was to evaluate the healing efficacy of the two newly developed gastroretentive drug delivery system with ranitidine F1& F2 to meet the therapeutic needs relating to
experimental pathological conditions in rats as gastric ulcer. Also, assessed their role in augmenting the mucosal defense against ethanol induce gastric ulcer. Disturbances in gastric indices parameters include ulceration; necrosis in mucosa; hemorrhage; edema in mucosa and submucosa beside inflammatory reaction in submucosa. As well as depletion of gastric mucus were recorded in the present study to alcohol administration. Ethanol–induced gastrointestinal lesion implicates a variety of mechanisms. It has been reported to cause disturbances in gastric secretion, damage to the mucosa, alterations in the permeability, gastric mucus depletion and free radical production (Al-Howiriny et al., 2003; Vinothapooshan & Sundar, 2010; Choudhary et al., 2014 and Olaibi et al., 2014). These may be attributed to the release of superoxide anion and hydroperoxy free radicals during metabolism of ethanol. In accordance, oxygen derived free radicals has been found to be involved in the mechanism of acute and chronic ulceration in the gastric mucosa (Jude & Paul, 2009). Alcohol rapidly penetrates the gastric mucosa apparently causing cell and plasma membrane damage leading to increase intracellular membrane permeability to sodium and water which evident by the current histopathology finding since edema present in all alcohol treatments. The massive intracellular accumulation of calcium represents a major step in the pathogenesis of gastric mucosal injury. This leads to cell death and exfoliation in the surface epithelium (Raju et al. 2009). In the current study, it has been shown that pre-treatment or post-treatment with F1 & F2 ameliorated the deleterious effects of ethanol on ulcer index parameters with advantage of F1. Bioadhesive dosage form (Nagahara et al., 1998 and Wang et al., 2001) extend the residence time of the drug in the stomach, the extended release of the drug can maintain a higher drug concentration in the gastric region thereby, improve the absorption and systemic bioavailability of the drugs that were normally poorly absorbed (Nagai & Machida, 1985) and improve the therapeutic efficacy. This may be due to local cytoprotective enhancement by the two formulae via their bioadhesion properties (adhering to epithelium) and mucoadhesion properties (adhering to mucus), since these formulations allow more opportunity to contact the epithelium or mucus and topically coating the injured tissue for facilitating healing. Also, they offer significant potential for increasing gastrointestinal tract residence time leading to improved ranitidine bioavailability (Hemant et al., 2010). Gastric mucus (mucin) is an important protective factor for the gastric mucosa and consists of a viscous, elastic, adherent and transparent gel formed by 95% water and 5% glycoproteins that cover the entire gastrointestinal mucosa. Moreover, mucus is capable of acting as an antioxidant, and thus can reduce mucosal damage mediated by oxygen free radicals (Repetto & Llesuy, 2002). The protective properties of the mucus barrier depend not only on the gel structure but also on the amount or thickness of the layer covering the mucosal surface (Penissi & Piezzi, 1999). In the present study, decreased mucin secretion after ethanol treatment indicating reduced ability of the mucosal membrane to protect the mucosa from physical damage and back diffusion of hydrogen ions. Mucosal damage can be easily produced by the generation of exogenous and endogenous free radicals (Naito et al., 1995). An increase in mucus production usually assists the healing process by protecting the ulcer crater against irritant stomach secretions (HCl and pepsin) thereby enhancing the rate of the local healing process. F1 & F2 protected the gastric mucosa from damage by increasing the mucin content significantly as evident by the current histochemical micrographs. The advantage of F1 may be attributed to gastric mucus layer enhancement probably by increasing the generation of mucosal prostaglandin E2 (Dashputre & Naikwade, 2011). Prostaglandin E2 was known to be a strong stimulant for gastric mucus secretion due to the presence of chitosan (Ishihara et al., 2001), and its likely reflect an increase in the newly secreted adherent mucus which consider to be more important as a protective physical barrier of gastric epithelium as supported by the current histochemical finding. In the present study ethanol administration revealed a significant decrease in serum total protein levels in both experiments concurrent with significant increase in ALT & AST enzymes activities indicating hepatic injury (Myagmar et al., 2004). These results are in accordance with Ahmed et al. (2002); Hornyak et al. (2003); Nwoye, (2013) and Choudhary et al., (2014). Improved total protein levels estimated in the current study among F1 and F2 treatments in pre and post-treated rats respectively than R treatment to the fact that mucoadhesive dosage formule increase the absorption and systemic bioavailability of the drugs that normally poorly absorbed as ranitidine (Hemant et al., 2010). Also, it may be attributed to the immunomodulatory and inflammatory properties of ranitidine which improved by the current mucoadhesive formulae F1 and F2 and evidenced by the current histologic findings since the presence of mild to moderate inflammatory cells infiltration could motivate the immune responses. In addition, F1 & F2 had a greater protective effect than ranitidine on hepatocytes against ethanol-induced damage and subsequent leakage of enzymes into the circulation. These results are in accordance with Choudhary et al., 2014. On the other hand, a significant decrease in ALP levels observed in ethanol treated rats was in agreement with the study observed by Ahmed et al. (2012). Oral administration of F1 & F2 was significantly increased ALP level among pre- and post-treatments. Therefore, it is possible that the differences in the effectiveness between the two formulae may be due to the strength and duration of adhesiveness of the developed formulae to the mucosa or the ulcerated area with the advantage of the local effects over the systemic one. **Conclusion:** The current study throw some lights on the role of mucoadhesive hydrogel formulations against ethanol induced gastric ulcer in rats and confirmed the better therapeutic action of these formulae over the pure drug which might be a promising drug delivery system for the ulcer treatment. The mucoadhesive hydrogel formulations of chitosan and Sod. CMC not only provide an excellent
preventive effect in gastric ulcer models, but also possesses a significant hepatoprotective effect. Also, it encouraged the use of F1 formulae as protective and therapeutic agent of ulcer since F1 was the most potent one and formula with Sod. CMC as a therapeutic agent. Further physiological studies needed to explore the role of chitosan in gastric inflammation induced by different factors.

![Figure (1): Remaining percentage of ranitidine in the stomachs of albino rats at different time intervals after oral administration of F1 (group I), F2 (group II) and ranitidine solution (group III).](image)

Table (1): Effect of different formulae on some gastric mucosal parameters as protective agents (pre-treated) against standard ranitidine treatment.

<table>
<thead>
<tr>
<th>Treatment Parameters</th>
<th>PH</th>
<th>GV ml</th>
<th>Total acidity mEq/Lit</th>
<th>Ulcer index</th>
<th>Curative ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>5.56±0.218</td>
<td>1.238±0.018</td>
<td>25.30±1.393</td>
<td>0.00±0.00</td>
<td>-</td>
</tr>
<tr>
<td>U</td>
<td>7.48±0.218</td>
<td>3.28±0.102</td>
<td>58.00±2.55</td>
<td>2.80±0.20</td>
<td>-</td>
</tr>
<tr>
<td>R</td>
<td>7.60±0.114</td>
<td>2.98±0.391</td>
<td>54.00±2.898</td>
<td>0.50±0.16</td>
<td>82.14%</td>
</tr>
<tr>
<td>F1</td>
<td>5.53±0.189</td>
<td>2.44±0.178</td>
<td>13.00±1.140</td>
<td>0.10±0.10</td>
<td>96.43%</td>
</tr>
<tr>
<td>F2</td>
<td>7.34±0.196</td>
<td>3.60±0.187</td>
<td>36.00±1.871</td>
<td>1.1±0.24</td>
<td>60.71%</td>
</tr>
</tbody>
</table>

- Results were expressed as mean±SE for each 6 rats.
- * Significance difference versus control at P < 0.05.
- Groups have the same letter mean non-significant while groups have different letters means significant at P < 0.05.

Table (2): Effect of different formulae on some gastric mucosal parameters as therapeutic agents (post-treatment) against standard ranitidine treatment.

<table>
<thead>
<tr>
<th>Treatment Parameters</th>
<th>PH</th>
<th>GV ml</th>
<th>Total acidity mEq/Lit</th>
<th>Ulcer index</th>
<th>Curative ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>5.54±0.220</td>
<td>1.244±0.012</td>
<td>25.10±1.756</td>
<td>0.00±0.00</td>
<td>-</td>
</tr>
<tr>
<td>U</td>
<td>5.65±0.022</td>
<td>1.320±0.046</td>
<td>44.00±1.696</td>
<td>2.20±0.20</td>
<td>-</td>
</tr>
<tr>
<td>R</td>
<td>4.82±0.107</td>
<td>1.320±0.026</td>
<td>40.20±2.818</td>
<td>0.80±0.12</td>
<td>63.64%</td>
</tr>
<tr>
<td>F1</td>
<td>4.06±0.284</td>
<td>1.284±0.035</td>
<td>37.20±2.518</td>
<td>0.40±0.19</td>
<td>81.82%</td>
</tr>
<tr>
<td>F2</td>
<td>3.76±0.083</td>
<td>1.268±0.036</td>
<td>46.20±1.855</td>
<td>0.50±0.00</td>
<td>77.27%</td>
</tr>
</tbody>
</table>

- Results were expressed as mean±SE for each 6 rats.
- * Significance difference versus control at P < 0.05.
- Groups have the same letter mean non-significant while groups have different letters means significant at P < 0.05.
Table (3): Effect of different formulae on some serum biochemical parameters as protective agents (pre-treated) against standard ranitidine treatment.

<table>
<thead>
<tr>
<th>Treatment Parameters</th>
<th>TP mg/dl</th>
<th>ALT U/L</th>
<th>AST U/L</th>
<th>ALK U/L (37°C)</th>
<th>TAC mM/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>7.46±0.21</td>
<td>20.743±0.66</td>
<td>30.029±0.43</td>
<td>44.382±1.621</td>
<td>2.059±0.002</td>
</tr>
<tr>
<td>U</td>
<td>6.86±0.22</td>
<td>26.072±0.39</td>
<td>38.399±0.53</td>
<td>22.724±1.856</td>
<td>2.129±0.002</td>
</tr>
<tr>
<td>R</td>
<td>7.03±0.15</td>
<td>21.058±0.55</td>
<td>28.626±0.43</td>
<td>49.207±2.672</td>
<td>2.105±0.007</td>
</tr>
<tr>
<td>F1</td>
<td>6.42±0.11</td>
<td>19.922±0.54</td>
<td>27.067±0.33</td>
<td>54.438±2.735</td>
<td>2.079±0.023</td>
</tr>
<tr>
<td>F2</td>
<td>7.12±0.11</td>
<td>22.696±0.73</td>
<td>29.427±0.56</td>
<td>27.434±1.164</td>
<td>2.101±0.008</td>
</tr>
</tbody>
</table>

- Results were expressed as mean±SE for each 6 rats.
- * Significance difference versus control at P < 0.05.
- Groups have the same letter mean non-significant while groups have different letters means significant at P < 0.05.

Table (4): Effect of different formulae on some serum biochemical parameters as therapeutic agents (post-treatment) against standard ranitidine treatment.

<table>
<thead>
<tr>
<th>Treatment Parameters</th>
<th>TP mg/dl</th>
<th>ALT U/L</th>
<th>AST U/L</th>
<th>ALK U/L (37°C)</th>
<th>TAC mM/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>7.46±0.21</td>
<td>20.743±0.66</td>
<td>30.029±0.43</td>
<td>44.382±1.621</td>
<td>2.059±0.001</td>
</tr>
<tr>
<td>U</td>
<td>6.28±0.19</td>
<td>30.704±0.29</td>
<td>55.756±0.49</td>
<td>20.616±1.047</td>
<td>2.129±0.002</td>
</tr>
<tr>
<td>R</td>
<td>6.70±0.15</td>
<td>23.530±0.63</td>
<td>57.416±0.48</td>
<td>28.444±0.754</td>
<td>2.033±0.003</td>
</tr>
<tr>
<td>F1</td>
<td>7.15±0.12</td>
<td>23.673±0.92</td>
<td>45.062±0.45</td>
<td>46.558±2.417</td>
<td>2.053±0.007</td>
</tr>
<tr>
<td>F2</td>
<td>6.85±0.12</td>
<td>23.035±0.61</td>
<td>41.461±0.60</td>
<td>34.494±2.954</td>
<td>2.075±0.002</td>
</tr>
</tbody>
</table>

- Results were expressed as mean±SE for each 6 rats.
- * Significance difference versus control at P < 0.05.
- Groups have the same letter mean non-significant while groups have different letters means significant at P < 0.05.

Table (5): Severity of histopathological reaction in stomach of different treatments as protective agents (pre-treated) . Histopathology Alterations

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Ulceration (mucosa)</th>
<th>Necrosis (mucosa)</th>
<th>Inflammation Reaction (submucosa)</th>
<th>Haemorrhage (mucosa&amp; submucosa)</th>
<th>Degree Of Severity</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>U</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>R</td>
<td>−</td>
<td>++</td>
<td>+++</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>F1</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>F2</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>++</td>
</tr>
</tbody>
</table>

- Results were expressed as mean±SE for each 5 rats.
- +++ = Sever effect; ++ = Moderate effect; + Mild effect & − Nil

Table (6): Severity of histopathological reaction in stomach of different treatments as therapeutic agents (pre-treated) . Histopathology Alterations

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Ulceration (mucosa)</th>
<th>Necrosis (mucosa)</th>
<th>Inflammation Reaction (submucosa)</th>
<th>Haemorrhage (mucosa&amp; submucosa)</th>
<th>Degree Of Severity</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>U</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>R</td>
<td>−</td>
<td>++</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>F1</td>
<td>−</td>
<td>−</td>
<td>++</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>F2</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
</tbody>
</table>

- Results were expressed as mean±SE for each 5 rats.
- +++ = Sever effect; ++ = Moderate effect; + Mild effect & − Nil
Photomicrograph (1) Stomach of control rats showing normal histopathological structure the mucosa (mu), submucosa (sm), muscularis (ml) and serosa (s). H&E (A =X16); (B =X40).

Photomicrograph (2) Stomach of ulcerated rats (Pre-treated) showing necrosis (nmu) and hemorrhage (h) in mucosa with oedema (O) and few inflammatory cells infiltration (m) in submucosa. H&E (A =X16); (B =X40).

Photomicrograph (3) Stomach of ranitidine treated rats (Pre-treated) showing inflammatory cells infiltration (m) and congestion in blood vessel (v) of the sub mucosa. H&E (A =X16); (B =X40).

Photomicrograph (4) Stomach of formula (1) treated rats (Pre-treated) showing intact mucosa (mu) with oedema (O) in the sub mucosa. H&E (A =X16); (B =X40).

Photomicrograph (5) Stomach of formula (2) treated rats (Pre-treated) showing ulceration with haemorrhage (h) in superficial mucosal layer (umu), lamina propria and submucosa. H&E (A =X16); (B =X40).
Photomicrograph (6) Stomach of ulcerated rats (Post-treated) showing ulceration and haemorrhage (umu) in mucosa with dilation in blood vessels (v), oedema (O) and few hemorrhage (h) in submucosa. H&E(A=X16); (B=X40).

Photomicrograph (7) Stomach of ranitidine treated rats (Post-treated) showing necrosis and desquamation of the superficial mucosa (nmu) with inflammatory cells infiltration(m), oedema (O) and mild congestion in blood vessel (v) in the sub mucosa. H&E(A=X16); (B=X40).

Photomicrograph (8) Stomach of formula (1) treated rats (Post-treated) showing intact mucosa (mu) with odema(O), hemorrhage (h), congested blood vessel (v) and inflammatory cells infiltration(m) in sub mucosa. H&E (A=X16); (B=X40).

Photomicrograph (9) Stomach of formula (2) treated rats (Post-treated) showing intact mucosa (mu) with odema(O) in the sub mucosa and few inflammatory cells infiltration (m) in sub mucosa. H&E (A=X16); (B=X40).
Histochemical findings: Detection of mucous

Plate. 1: Stomach tissue of albino rat treated with different formulae using alcian blue; (Con) Stomach tissue of control albino rat, showing mucous localized in diffuse manner all over the mucosal epithelial cells (arrow) X: 40.; (U pre-) Ulcerated rat, showing absence of the mucus from the mucosal epithelium (arrow) X:40. (U post-) Ulcerated rat showing very few amount of mucus in the mucosal epithelium (arrow) X:40. (R pre-) Ranitidine treated rats, showing very few amount of mucus in the mucosal epithelium (arrow) X:40; (R post-) Showing localization of mucus in the epithelium of the deep mucosa (arrow), X: 40.; (F1 pre-) formula 1 treated animals showing blue color of mucus in the desquamated mucosal epithelium as well as in the underlying intact one (arrow), X: 40.; (F1 post-) Showing localization of mucus in the intact mucosal epithelial cells (arrow); (F2 post-) Showing localization of mucus in the deep intact glandular mucosal structure (arrow), X: 40.

REFERENCES:


تقييم صياغات دوائية جديدة لأحد مضادات القرحة على نموذج تجريبي للقرحة

نشوة اسماعيل زكى 1، لبنى عبد المنعم محمد حسنين 2، عبير مصطفى خطاب 3

1- شعبة الفسيولوجي - الهيئة القومية للرقابة والبحوث الدوائية
2- شعبة تقييم الهرمونات - الهيئة القومية للرقابة والبحوث الدوائية
3- شعبة الصيدلانيات - الهيئة القومية للرقابة والبحوث الدوائية

قرحة المعدة من الأمراض الشائعة في العصر الحديث، وهي تحدث نتيجة عدم التوازن بين العصارة المعدية الهاضمة والعوامل المختلفة التي تحمي جدار المعدة من التأثير البديع لهذه العصارة. وتتعد القرحة اما عن بفعل العوامل، أو مثبط للعصارة المعدية، أو مواد تحمي العضلات.

تعد المقاطع المعدية تأثير غير مباشر على تراكم العصارة المعدية، ولذلك تنظر في تأثيرها على تراكم العصارة المعدية بفترة ممثثين لما يمثل إلى امداد التأثير المتشابك للعقار وزيادة فاعليته. وقد أجريت هذه الدراسة لدراسة قوة الإصابة الحيوي وتحديد التأثير الواقعي (قبل) و العلاجي (بعد).

عقار الرانيتيدين في صورة إستامات متممة على وموجد جيوشتي. لقد تم استخدام اثاث جرائ الالعاء التي تزن 200-220 جم (عدد 75 جرذة تلقير قوة الإصابة) و 70 جرذة تلقير التأثير والعلاج، ومستمدة هذا المجموعة عشان اتريبيت وعلاجية ووقائية ودعاية تأثير الاضطرابات المريض للرانيتيدين. حيث يركب (F1) من خليط من الكيتوزان مع هيدروكسي بروبيل ميثيل سيلولوز بنسبة 1:9 بينما يحتوي F2 على خليط من صوديوم كربوكسي ميثيل سيلولوز مع هيدروكسي بروبيل ميثيل سيلولوز بنسبة 1:9.

تم استخدام الإناث الجرذان البالغة التكافئ 200-220 جم (عدد 75 جرذة للتقدير قوة التأثير) و 70 جرذة لتقدير التأثير والعلاج. كل المجموعات تم تقسيمها عشوائيا إلى تجربتين ووقائيتين وعلاجية تأثير التحضيرات المختارة للرانيتيدين، حيث يركب (F1) من خليط من الكيتوزان مع هيدروكسي بروبيل ميثيل سيلولوز بنسبة 1:9 بينما يحتوي F2 على خليط من صوديوم كربوكسي ميثيل سيلولوز مع هيدروكسي بروبيل ميثيل سيلولوز بنسبة 1:9.

كل تجربة بها خمس مجموعات وكل مجموعة بها 6 جرذة. كل الجرعات تأخذ عن طريق الفم. المجموعة (1) اعتبرت بحثا المجموعة الضابطة والجموعة رقم (2) بثامرة مجموعة القطرة الضابطة حيث تلقى الإيثانول (5 مجم/كم من وزن الجسم). المجموعات (3) تلقى عقار الرانيتيدين (27 مجم/كم في وزن الجسم) بينما تلقى المجموعة (4) و (5) الحجم المختارة للرانيتيدين بالتدر. وقد تم قدر كل من مؤثر القرحة والإدمان الهيدروجيني وحمضية الكلية وحمضية المعدة في كل مجموعة الفحص، والجذوبات الاصطناعية في جميع المجموعات، وحمضية المعدة في كل مجموعة الفحص.

الدراسة أن الصياغة F1 أكثر فعالية من الصياغة F2 كعقار وقائي وعلاجي حيث قل موثر القرحة والإدمان الهيدروجيني وحمضية المعدة في مجموعة الإيثانول. وقد اظهر أيضاً نقص معني في مستوى كلا من F1 و F2، حيث قدر أن هذه النتائج تؤثر على النتائج بالدراسات الهستوباثولوجية والهستوكيميائية. وقد وجد أن كمية الرانيتيدين المحتقة في العلاج بعد أربع ساعات هي الأكثر في حالة F1 نسبتها قصيرة إلى تعتبر أكثر دقة في تجربة عند F2، F1.

ت+'العلاج الفعال من الرانيتيدين منفرداً ولكن F1 أكثر فعالية وعاجلياً يجب تشغيل استخدام F2 كعقار أكثر منه كوقائي.