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Pharmacodynamic and pharmacokinetics evaluation of ranitidine microspheres on experimental animals

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ABSTRACT

Mucoadhesive microspheres overcome the physiological adversities like short gastric residence time promoting the drug bioavailability. In the present investigation Ranitidine loaded Eudragit RS 100 microspheres were prepared by solvent evaporation technique using disparate drug polymer ratios with Eudragit RS 100 polymer and Ranitidine loaded chitosan microspheres were prepared by coacervation phase separation method. The prime objective was to enhance the absorption and bioavailability by prolonging the gastric residence time. Totally six formulations RES1- RES6 Eudragit RS 100 microspheres and six formulations RCP1 – RCP6 Eudragit RS100 microspheres were prepared and evaluated. RES3 and RCP3 showed nearly 90% drug release after 24 h. Hence the ranitidine loaded Eudragit RS100 microspheres and Chitosan microspheres were further studied for the *in vivo* study for the suitability of the formulation. The bioavailability of ranitidine after oral ingestion is about 50% and is absorbed *via* the small intestine and it may be attributed to permeability of the intestine is relatively slow in nature. Hence the current research was to widen the therapeutic efficacy of ranitidine by formulating microspheres enhance the intestinal permeability as well as bioavailability by performing the anti-ulcer activity.

Keywords: Microspheres, Eudragit RS 100, Chitosan, Bioavailability.

1. INTRODUCTION

Peptic ulcer is a very common disease escalating throughout the world. A peptic ulcer is an erosion or sore in the wall of the gastrointestinal tract. The mucous membrane being delicate in nature which lines the digestive tract erodes and leads to a slow breakdown of tissue. This breakdown yields a burning pain in the upper middle part of the belly (abdomen).

Peptic ulcer disease (PUD) occurs to the following:

- *H pylori* infection
- Drugs
- Lifestyle factors
- Severe physiologic stress
- Hypersecretory states (uncommon)
- Genetic factors

Ranitidine restrain histamine stimulation of the H2 receptor in gastric parietal cells, which consecutively reduces gastric acid secretion, gastric volume, and hydrogen ion concentrations.[1-2] Ranitidine hydrochloride by inhibiting basal gastric acid secretion cause depletion of volume of acid and pepsin secretion. Ranitidine is administered as often in tablet, capsule, syrup and injection but the bioavailability is less. Hence to elevate the peptic ulcer pain the present work comprises of formulating Ranitidine as mucoadhesive microspheres since mucoadhesive microspheres form an integral part of novel drug delivery systems. Mucoadhesive microspheres provides numerous advantages such as effective drug absorption and intensified bioavailability of drugs due to a high surface-to-volume ratio and enormous intimate contact with the mucus layer, and specific targeting of drugs to the absorption site. Mucoadhesive microspheres being adhered to the stomach wall and thereby remain in the gastrointestinal tract for a longer time period. In recent years, mucoadhesive microspheres attention has been greatly focused since drug releases to the need of the body throughout the period of treatment and it provides the active entity solely to the site of action.[3-5]

2. MATERIALS AND METHODS

2.1. Chemicals

Ranitidine USP was obtained from SMS Pharmaceuticals Pvt. Ltd. (Hyderabad, India) as free gift sample. PEG-400 was purchased from BD Pharmaceuticals Ltd. (Kolkata, India), and Tween-80 was purchased from Merck Specialties Pvt. Ltd. (Mumbai, India). All other chemicals used in this study were obtained commercially and were of analytical (AR) grade.

2.2. Preparation of microspheres by solvent evaporation technique

Ranitidine loaded Eudragit Rs 100 microspheres were prepared by solvent evaporation. The ranitidine microspheres were prepared with various ratios of drug and Eudragit RS100 polymer as shown in Table 1 using solvent evaporation technique. The method is a modification of emulsion solvent evaporation technique and involves preparation of o/w emulsion between organic phase consisting of ranitidine and Eudragit RS 100 in dichloromethane (DCM) and aqueous phase, 1% w/v aqueous solution of polyvinyl alcohol (PVA).The dichloromethane solution of ranitidine and Eudragit RS100 was emulsified by using probe homogenizer (Virtis Cyclone IQ, USA).The dichloromethane was completely evaporated by stirring overnight(12 to 16 hrs) at room temperature ($25^0C \pm 2^0C$). The prepared microspheres were recovered by centrifugation for 20 minutes at 15,000rpm [Sorvall Ultracentrifuge, USA]. The precipitate was washed repeatedly with ice cold water to remove the traces of polyvinyl alcohol. Finally, the product was dispersed in cold water and recovered by lyophilisation (Labconco Lyophilisor, USA). Six batches of Eudragit microspheres were prepared keeping organic phase to aqueous phase at 1:5, and varying drug: polymer ratios. The biopharmaceutical parameters of dosage forms can be achieved directly by measuring the drug blood levels as a function of time or indirectly by measuring pharmacodynamic response as a function of time. [6]

2.3. Preparation of ranitidine microspheres by coacervation- phase separation method

The ranitidine loaded chitosan microspheres were prepared by coacervation-phase separation technique using various ratios of drug and polymer. The polymeric solution was prepared by dissolving the chitosan in DCM. Then ranitidine was dissolved in the polymeric solution and sonicated for 5 min. The organic non-solvent liquid paraffin was added to the polymeric solution at the rate of 1ml/min under continuous stirring using mechanical stirrer with 600 rpm. The slow addition of liquid paraffin coacervates the polymer in the mixture. The coacervate phase is then added to the hexane (non-solvent) under gentle stirring to harden the coating layer. The formed microspheres were then centrifuged for 10 min at 10,000 rpm. The precipitate was then collected and washed at least three times with distilled water. The prepared microspheres were lyophilized and stored in container for further studies. Six batches of microspheres were prepared using various ratios of drug: polymer Selected formulations of ranitidine microparticles prepared by Eudragit RS100 and Chitosan were subjected to *in vivo* studies in animal model. The *in vivo* anti ulcer activity and bioavailability study were carried out in rat model. Rats are widely used for *in vivo* bioavailability study as well as anti ulcer activity study, as its physiological structure is similar to human model. Moreover rats are easy to handle and are low in cost. [7]

2.3. Animals

Wistar albino rats weighing 200–250 g of either sex were used for this experiment and were selected at random from animal house of the K.M. College of Pharmacy, Madurai. Institutional animal ethics committee approved the experimental protocol; animals were maintained under standard conditions in an animal house approved by committee for the purpose of control and supervision on experiments on animals (CPCSEA). All animal experiments were approved by Institutional Animal Ethics Committee (IAEC) of R. GOWRI/ACI(2)61830/2011/PhD/IAEC/KMCP/232/2014.

The animals were housed in polypropylene cages and maintained at 24°C ± 2°C under 12 h light/dark cycle, were feed *ad libitum* with standard pellet diet (Golden Feed, New Delhi), and had free access to water.

2.4. *In vivo* anti ulcer activity study

The anti ulcer activity study of the formulation RES3 and RCP3 was carried out in Wistar albino rats weighing 150 to 180 gm. The oral dose of 20 mg/kg was selected for this purpose. The healthy rats were divided into five groups with five animals each. The animals in the test groups were administered 1 ml per 100 gm of rat with necrotizing agent (80% ethanol) orally which is known to produce gastric lesions. The dosage schedule for the study was as:

Group 1: Animals were given the normal saline with the dose of 10 ml / Kg and served as negative control

Group II: Animals were administered with ethanol (80%) orally and served as positive control

Group III: Animals were administered with ethanol 1ml per 100 gm and treated with pure ranitidine 20 mg/kg.

Group IV: Animals were administered with ethanol and treated with formulation RES3 (equivalent of 20 mg ranitidine) and

Group V: the animals administered with ethanol and treated with RCP3 (equivalent of 20 mg ranitidine).

Based on the gastric emptying in fasted rats, formulations were given 30 min before the necrotizing agent. Animals were sacrificed under ether anesthesia 1 hr after treatment with formulation. The stomach was excised and opened along the greater curvature. After washing with normal saline, the gastric lesions were quantified using a magnifying glass. If there is no ulceration, hyperemia, hemorrhagic spots, 1-5 small ulcers, many small ulcers, many large ulcers and stomach full of ulcers then the ulcer index was given as 0, 0.5, 1, 2, 3, 4, 5 and 6 respectively [8]. The ulcer index (U/I) was calculated as follows [9].

$$U/I = \frac{10 \times \text{Total ulcerated area}}{\text{Total mucosal area}}$$

2.5. *In vivo* bioavailability study

Bioavailability study was conducted in adult wistar albino rats of either sex weighing 200 to 250 gm. The animals were divided into three groups of five animals each and fasted overnight before commencing the experiment with free access to water. The pure ranitidine and prepared microspheres formulations (RES3 and RCP3) were administered orally in a dose of 20 mg/kg body weight with the help of cannula after anaesthetizing for a very short period of time with diethyl ether. After administration 0.3 ml blood samples were collected from the retro-orbital plexus into the heparinized tubes at preset time points of 0.5, 1, 2, 4, 8, 12 and 24h. The blood samples were centrifuged at 4000 rpm for 10 minutes and the separated plasma samples were stored at – 20°C until analysis. [10-12]

2.5.1. Estimation of ranitidine in plasma sample by RP-HPLC analysis

The amount of ranitidine in blood samples was measured by the previously described RP-HPLC method with slight modification (Haque et al., 2011). The method was validated prior estimation. The HPLC system (Shimadzu, Japan) consisting of LC-20AT pumps and SIL-20A auto sampler were used. The measurement was carried out at 280 nm using SPD-20A UV-Visible detector. The mobile phase used consists of a mixture of 0.1(M) orthophosphoric acid (pH3.0) and methanol in the ratio of 30:70. The pump flow rate was 1 ml/min and C18 (250mm X 4.6 mm) column was used for analysis. The mobile phase was filtered with nylon membrane filter and degassed prior use.[13]

To 0.1 ml of plasma 50 µL of internal standard ranitidine (50ng/ml) was added in a micro centrifuge tube and volume made to 2 ml with acetonitrile to precipitate the protein. Then the sample was centrifuged at 4000 rpm for 25 min. The supernatant was collected and transferred into an eppendorf tube and was dried under nitrogen air. The residue was dissolved in 200 µL of mobile phase and 10 µL was injected to the HPLC system. The analysis was carried out by RP-HPLC method using flow rate 1.0 ml/min and the measurements were made at 280 nm. The amount of the ranitidine in the sample was determined from the peak area ratio correlated with the standard curve prepared under the same identical condition.

2.5.2. Pharmacokinetic analysis

2.5.2.1. Determination of C_{max} and T_{max}.

The peak plasma concentration (C_{max}) and the time of peak plasma concentration (T_{max}) were determined from the plasma drug concentration vs time plot for the pure drug and prepared microspheres.

2.5.2.2. Determination of elimination rate constant (Ke) and biological half life (t½):

Elimination rate constant (Ke) and biological half life (t½) were determined using the Kinetica software (Thermo Fischer Scientific, Thermo Kinetica version 5.0).

2.5.2.3. Determination of area under curve (AUC):

The area under the time versus plasma concentration curve (AUC) was measured by applying trapezoidal rule. $(AUC)_{0-\alpha}$ was calculated as given below:

$$(AUC)_{0-t} = \int_0^t C(t)\,dt$$

$$(AUC)_{0-\alpha} = (AUC)_{0-t} + C_t / K_{el}$$

2.5.2.4. Determination of relative bioavailability

The relative bioavailability (F_r) of Ranitidine was calculated using the following equation:

$$Fr\ (\%) = \frac{\text{AUC (Ranitidine microspheres)}}{\text{AUC (Pure ranitidine suspension)}}$$

3. RESULTS AND DISCUSSION

3.1. Optimized formulation

In the present study mucoadhesive microspheres using chitosan and Eudragit RS 100 of ranitidine hydrochloride were successfully prepared by solvent evaporation technique. The formulations RES3 and RCP3were selected as an ideal formulation based in vitro drug release tests. Formulation and evaluation of Ranitidine loaded chitosan and Eudragit RS 100 microspheres for controlled release was found to be potential and effective in terms yield, encapsulation efficiency, particle size distribution.

RES3 formulation drug release was slow and extended beyond 12 hr up to 24 hr which could be the constant release of drug which has been loaded near the surface of microspheres. Kinetic results reveals microspheres obeyed Higuchi and Peppas model.RCP3formulations showed slow drug release which extended beyond 12 hr up to 24 hr. The formulation showed rapid drug release after 4 hr and this could be result of polymer erosion in the surface of microspheres and obeyed Higuchi and as well as Peppas model indicating the diffusion drug release mechanism. Hence the optimized RES3 and RCP3 formulations of Ranitidine mucoadhesive microspheres were selected for *invivo* anti ulcer activity study and *invivo* bioavailability study

3.2. *In vivo* anti ulcer activity study

Ethanol is considered a major risk factor for the gastric ulcer. The ethanol penetrates the gastric mucosa in the stomach due to its ability to solubilize the mucous on the membrane. Once the mucous get solubilized the membrane becomes exposed to the proteolytic enzyme pepsin and hydrochloric acid in the stomach causing the damage to the membrane.

The rat model is widely used for anti-ulcer activity as its physiological structure is resembled to the human. In the present study the anti-ulcer activity of the prepared microspheres formulations (RES3 and RCP3) was evaluated in the ethanol induced ulcerative rats by measuring the ulcer index. The ulcer index is given in table 1.

The ulcer index value in the ethanol treated group was 23.92 ± 0.58, whereas, the normal saline treated group showed no ulcer owing to the lower value. The ulcer index of the formulations RES3 and RCP3 was notably reduced as compared to ethanol treated group, but this value is remarkably reduced for the pure drug treated group as compared to the positive control group. The percentage of ulcer protection of the pure ranitidine and the formulations RES3 and RCP3 was given in figure 1.

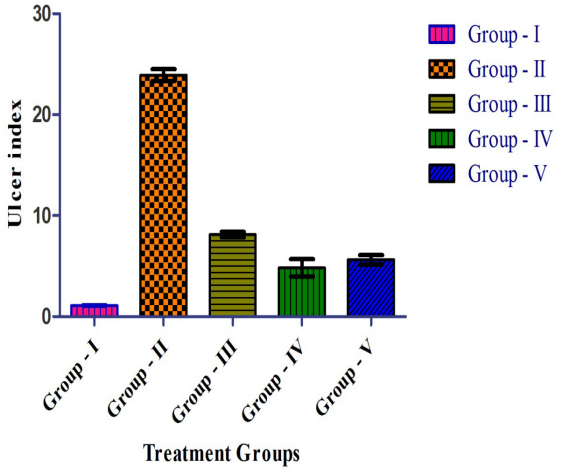

Figure - 1: Anti-ulcer activity of the Ranitidine formulations RES3 and RCP3.

The ulcer protection of the microspheres formulations RES3 and RCP3 were 79.84% and 76.49% respectively as compared to the ranitidine pure drug (66.05%) in ulcer induced rats. The pure ranitidine showed poor activity against mucosal damage as compared to the microspheres. The improve activity of the microspheres was because of sustained release property as well as mucoadhesive property of the RES3 and RCP3, which suggests that ranitidine microspheres strengthens and protects the gastric mucosal barrier

3.3. *In vivo* bioavailability study

The availability of the drug to the biological system and its therapeutic effectiveness is the goal of any dosage form design. The bioavailability study of the two microsphere formulations RES3 and RCP3 were carried out in the rat model. The rat model is widely used for bioavailability study for many drugs. The plasma drug concentration-time profile of ranitidine was constructed following the oral administration of pure ranitidine and ranitidine loaded microspheres at a dose of 20 mg/kg to rats (table 2). The plasma concentration-time profile is illustrated in figure 2 and the pharmacokinetic parameters are listed in table 3.

The plasma concentration time profile of ranitidine pure drug showed the higher peak than that of formulation RES3 and RCP3. The C_{max} value of ranitidine as obtained from the graph was 575.14 ± 55.43 ng/ml with T_{max} value 2 h and for formulations RES3 and RCP3 C_{max} were 206.58 ± 7.71 and 221.52 ± 9.42 ng/ml respectively (Figure 2).

Table - 1: Anti-ulcer activity of the ranitidine formulations RES3 and RCP3 in ethanol induced gastric ulcer in rats

Groups	Induction	Dose	Ulcer Index*
Group – I	Normal Saline	10 ml/Kg	1.090 ± 0.04
Group – II	Ethanol	1ml/ 100gm	23.92 ± 0.58
Group – III	Ranitidine	20 mg/kg	8.12 ± 0.28**
Group – IV	RES3	20 mg/kg	4.83 ± 0.86*
Group – V	RCP3	20 mg/kg	5.63 ±0 .46*

Note: Values express as mean ± SEM; n=5; * *P*< 0.05 versus control, ***P*< 0.01 versus control.

Table - 2: Drug plasma concentration vs. time data following oral administration of pure ranitidine (standard) to rats

Time (hrs)	Plasma concentration in ng/ml vs. Time		
	Pure Ranitidine	RES3	RCP3
0	0	0	0
0.5	269.65 ± 42.31	45.250 ± 8.42	40.250 ± 5.560
1	391.72 ± 56.25	69.250 ± 9.81	79.760 ± 10.03
2	**575.14 ± 55.43**	138.26 ± 13.94	169.50 ± 13.26
4	283.5 ± 33.960	189.250 ± 7.03	197.75 ± 14.23
8	61.790 ± 7.720	**206.58 ± 7.71***	**221.52 ± 9.42***
12	16.430 ± 4.570	97.750± 10.38	112.75 ± 12.37
24	-	22.500± 9.600	29.500 ± 7.920

Note: Data represents as Mean ± SEM; (n=5). * *P*< 0.05 versus pure ranitidine

Table - 3: Pharmacokinetic profile of pure ranitidine and ranitidine loaded microspheres after oral administration in rats.

Pharmacokinetic parameters	Units	Ranitidine Standard	RES3	RCP3
C_{max}	ng/ml	575.14 ± 55.43	206.58 ± 7.71	221.52 ± 9.42
T_{max}	H	2 ± 0	8 ± 0	8 ± 0
$AUC_{(0-24)}$	h x (ng/ml)	2064.07	2272.22	2408.46
Fr	(%)	-	110.07	116.68

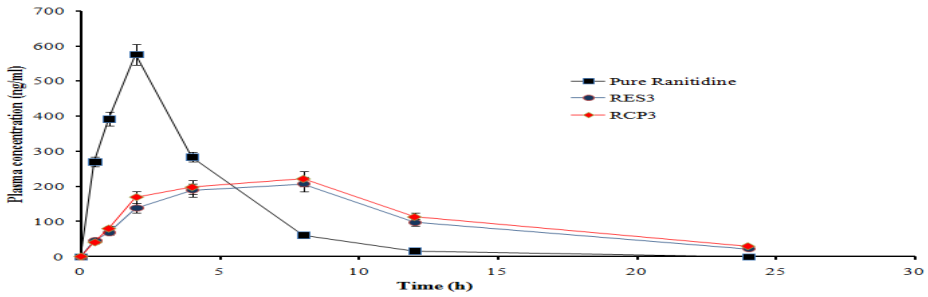

Figure - 2: *In vivo* bioavailability plot of ranitidine and ranitidine loaded microsphere formulations (Plasma concentration in ng/ml vs. Time); Note: The values are calculated from the mean plasma concentration vs. time, for n = 5.

The drug release from the ranitidine microspheres extend up to 24h as the formulations showed sustained release properties as evidenced from the *in vitro* drug release study. The AUC $_{(0-24)}$ of the formulations were higher than the AUC $_{(0-24)}$ of the pure ranitidine, which proves the better bioavailability of the microspheres and better therapeutic effect for the management of gastric and peptic ulcer.

4. CONCLUSIONS

In the present investigation, the utility of microsphere as carrier for oral delivery of ranitidine was studied. The Ranitidine loaded microspheres were prepared by emulsion solvent evaporation method and coacervation phase separation method. The pharmacodynamic study (ethanol-induced ulcer model) revealed that ranitidine microspheres showed lower incidence of mucosal damage when compared with standard drug, both administered orally, indicating the superiority of oral ranitidine microspheres over standard ranitidine. The pharmacokinetic studies reveal that the oral administration of ranitidine microspheres sustained the release of drugs over 24 hrs. The drug release from the microspheres showed controlled drug release mechanism. Enhanced antiulcer efficacy was obtained with reduction in gastric acid secretion. As a consequence of this, decrease in the dose and frequency of administration for drugs is possible to achieve the desired therapeutic activity.

5. REFERENCES

1. Chey WD and Wong BC. American College of Gastroenterology guideline on the management of Helicobacter pylori infection**. American Journal of Gastroenterology.** 2007; 102(8):1808-25.
2. Javid G, Zargar SA, U-Saif R, Khan BA, Yatoo GN and Shah AH. Comparison of p.o. or i.v. proton pump inhibitors on 72-h intragastric pH in bleeding peptic ulcer**. Journal of Gastroenterology and Hepatology.** 2009; 24(7): 1236-43.
3. Nasa P, Mahant S and Sharma D. Floating systems: A novel approach towards gastroretentive drug delivery systems. **International Journal of Pharmacy and Pharmaceutical Sciences.** 2010; 2: 2-7. 7.
4. Arora S, Ali J, Ahuja A, Khar RK and Baboota S. **Floating drug delivery systems: a review.** 2005; 06: 372-390.
5. Al-Saidan SM, Krishnaiah YS, Patro SS and Satyanaryana V. *In vitro* and *in vivo* evaluation of guar gum matrix tablets for oral controlled release of water-soluble diltiazem hydrochloride. **AAPS PharmSciTech.** 2005; 06: E14-E21.
6. Siepe S, Herrmann W, Borchert H, Lueckel B, Kramer A, Ries A. Microenvironmental pH and microviscosity inside pH-controlled matrix tablets: An EPR imaging study**. Journal of Controlled Release**. 2006; 112: 72–8.
7. Carrasquillo KG, Stanley AM, Aponte-Carro J, C, De Jesus P, Costantino HR, Bosques CJ and Griebenow K. Non-aqueous encapsulation of excipient-stabilized spray-freeze dried BSA into poly(lactide-co-glycolide) microspheres results in release of native protein. **Journal of Controlled Release.** 2001; 76: 199-208.
8. Bank S, Zimmerman H, Smolow C and Kranz V. Prevention of duodenal ulcers in the rat using a combination of ranitidine and sucralphate in sub therapeutic doses**. Gut**. 1985; 26(6): 603–606.
9. Boddupalli BM, Ramani R, Subramaniam B, Anisetti RN. *In vitro* and *in vivo* evaluation of hepato protection and anti ulcer activities of piperine gastro retentive micropspheres. **Asian Pacific Journal of Tropical Biomedicine**. 2012; 1237-1240.
10. Dhankar V, Garg G, Dhamija K and Awasthi R. Preparation, characterization and evaluation of ranitidine hydrochloride-loaded mucoadhesive microspheres. **Polimersin Medicine**. 2014; 44(2): 75-81.
11. Wei YM and Zhao L. *In vitro* and *in vivo* evaluation of ranitidine hydrochloride loaded hollow microspheres in rabbits, **Archives of Pharmacal Research**; 2008(10): 1369-77.
12. Mastiholimath VS, Dandagi PM, Gadad AP, Rashmi Mathews and Kulkarni AR. *In vitro* and *in vivo* evaluation of ranitidine hydrochloride ethyl cellulose floating microparticles. **Journal of Microencapsulation**. 2008; 25(5): 307–314.
13. Md. Ashanul Haque, Mohammad Shahriar, Most. Nazma Parvin and Ashraful Islam SM. Validated RP-HPLC Method for Estimation of Ranitidine Hydrochloride, Domperidone and Naproxen in solid dosage form. **Asian Journal of Pharmaceutical Analysis.** 2011; 1(3): 59-63.