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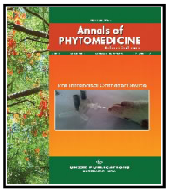

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In vivo evaluation of optimised acetohydroxamic acid floating microballoons

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Abstract

Enhanced gastric residence time is exhibited by floating dosage forms. *In vitro* studies and animal model of retention potential is different due to their development. The main content of this research about the determination of the intragastric behaviour of acetohydroxamic acid floating microballoons in rabbit stomach by x-ray study and estimate the bioavailability of this drug in rabbit plasma by calculating pharmacokinetic parameters and comparision of these results with marketed formulation. Based on the evaluation of floating and dissolution behavior, formulation (AHF5) which showed complete release within 12 h and superior entrapment efficiency can be optimized by the formulation which proceeded as in vivo studies. From the radiographic images, it is showed the formulation was there buoyant at the stomach upto 6 h which indicates the formulation distribution. At unfed condition, the formulation is still buoyant at 3 h in the stomach due to fasting condition, the migrating myoelectric contraction by the pressure of contents in stomach by duodenum. In healthy albino rabbits, the *in vivo* pharmacokinetic studies were performed. The parameters of pharmacokinetic studies of the floating acetohydroxamic acid microballoons are differentiate with Lithostat tablets. The result are showed that AHF5 bioavailability has increased when correlate with marketed formulation. Relative bioavailability has found to be 124.9 with respect to formulation in market was found to be 124.9 because of prolonged residence time in gastric of floating acetohydroxamic acid microballoons.

1. Introduction

Gastroretentive drug delivery systems control the residence time of drugs in the stomach. Enhanced gastric retention might benefit for drugs which are absorbed in the stomach region. Drug substances are retained and float on stomach because the density of drug delivery system is low.

Microballoons are non-effervescent approach in GRDDS. These microballoons are favourable buoyant systems and has good floating properties as the central hollow space in microsphere. Thus, chronic gastritis, gastroesophageal reflux diseases are treated with sub-mucosal tissue of *Helicobacter pylori* from the stomach (Avachat and Dhamne, 2002).

Acetohydroxamic acid (AHA) acts as a main role in the *H. pylori* of chemotactic motility by inhibition of cytoplasm of bacteria. The drug (AHA) has a molecular mass of 75.07 which inhibits the urease activity of *H. pylori*. AHA can be freely diffusible by inhibiting over the 95% activity of urease after 10 min.

Based on the evaluation of floating and dissolution behaviour formulation (AHF5) which showed complete release with in 12 h and superior entrapment efficiency was selected as optimized formulation and further proceeded for *in vivo* studies (Arora *et al.*, 2005).

The solvent evaporation method is used in formulating the floating microballoons with eudragit RS 100 polymer (500 mg), methanol and dichloromethane with 15 ml each acetohydroxamic acid dose is 250 mg in the formulation. By continuous stirring with magnetic stirrer for 15 min, the drug and polymer is dissolved in organic solvent. Then add the organic phase to that aqueous phase. The stirring was continued under 3 blade propellers at 500 rpm for 6 h at 40°C unless the smell of organic solvent fade. After the filtration of solution, the collected microballoons are washed with distilled water to remove the remnants of PVA. Then dried at the room temperature. The microballoons prepared with above combination are found stable from the *in vitro* studies and proceeded for *in vivo* evaluation.

2. Materials and Methods

2.1 In vivo floating behaviour

The formulation of floating optimized microballoons were studied in healthy rabbits (weight 400-600 g) for *in vivo* floating behaviour with the approval of Institutional Animal Ethical Committee of University College of Pharmaceutical Sciences, Kakatiya University, Warangal, India (Registration No. IAEC/22/UCPSC/2018). This study was conducted for monitoring the radiological activity. Polypropylene cage stand is maintained under standard conditions where the animals can be housed individually. Total 12 albino rabbits are six in each group. Before initiating the study, all the rabbits were fasted for 12 h. To make sure the absence of radio opaque material in the stomach first x- ray has withdrawn for all the rabbits. In the

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time of study, rabbits are not supposed to eat food but water were contributed. By incorporating 500 mg of barium sulfate into polymeric solution radiopaque microballoons were prepared and similar procedure is followed for preparation of optimized formulation. Optimized microballoons formulation are administered by one group and the other group was administered with pure drug solution prepared with same amount of drug with radio opaque material. The x-ray photographs at time interval of gastric region are taken for monitoring the microballoons of floating behaviour (Barge and Awasthi, 2017).

2.2 The evaluation of optimized microballoons in *in vivo* pharmacokinetic parameters

The pharmaceutical product development is a fundamental dosage form for best interpretation of the *in vitro* and *in vivo* studies. In case, the oral controlled drug delivery system the *in vitro* studies may not provide complete information about *in vivo* studies because of several unpredictable physiological factors which affects the absorption and drug release. At present investigation, floating microballoons were developed to release the drug through upper part of the GIT resulting in the improved bioavailability compared to conventional dosage forms. The *in vivo* studies were evaluated on healthy albino rabbits procured from 2-2-185/55, Harsha homes, Mahaveera enterprises, Hyderabad-500013 and compared with pure drug suspension and marketed formulation.

2.2.1 Evaluation of pharmacokinetic optimized formulation

The evaluation of floating behaviour and dissolution behavior, formulation that showed complete release with in 12 h and superior entrapment efficiency and stability were choose to carry off with the pure drug solution and marketed formulation is compared with *in vivo* performance (Christmann *et al*., 1997). These tests are performed in albino rabbits. The given below formula of drug dose:

Human equivalent dose (mg/kg) = Animal dose (mg/kg)* Human Km value/Animal Km value.

2.2.2 Dose calculations-acetohydroxamic acid microballoons

Marketed formulation: Lithosat tablets 250 mg.

Dose employed for *in vivo* study: 12.89 mg/kg.

2.2.3 Experimental design

For single-dose crossover study, the 6 healthy rabbits accepted an open label, balanced, randomized, 3-period, 3-treatment, 3-sequence each within a washed out period at seven days, so that the results are performed in six albino rabbits.

2.3 *In vivo* study protocol

2.3.1 Subject selection

For physical examination, selected a healthy weight of 1.5-2.5 kg six rabbits.

2.3.2 Administration of drug

Before administration of dose, the animals are fasted overnight. Later assemble, the zero hour blood sample in the morning a standardized diet was given to animals. The albino rabbits were kept at a particular holder as their head protruding outside (Desaiand Botton, 1989). Formulations are administered through the oral route by introducing mouth gag in the middle of their two jaws helding in position by holding the upper jaw and lower jaw with the left hand. An I.V. cannula, (Hindustan syringes and amp; Medical devices, Faridabad, India) 20-G Kitkath were inserted in one marginal ear vein before drug administration for blood collection.

2.3.3 Blood sample collection

Marginal ear veins for every rabbit, 0.5 ml venous blood samples are collected in AcCuvet tubes (Quantum Biologicals Pvt. Ltd., Chennai, India) which carrying the K 3 EDTA. Samples are gathered at 11 predetermined time intervals (0-24.00 h). Immediately, the plasma were separated apart through the centrifugation process at 7500 rpm for 15 min from the blood samples then it was stored in a frozen conditions at –20°C and labeled with subject code number, study date and collection time prior to the analysis (Kawashima *et al*.,1992). In each sample of rabbit plasma, the concentration of drug was measured by HPLC method.

2.3.4 Pharmacokinetic parameters

The peak plasma concentration (C max), at time were C max occur (T max), (AUC), rate of elimination constant (Kel), half-life (t½), absorption (Ka) and (MRT) are calculated at every case by using the data as Kinetica TM 2000 software.

3. Results

3.1 Floating behavior of *in vivo* performance

The formulation of optimized floating microballoons prepared and tested in healthy albino rabbits for *in vivo* floating behaviour. An initial, 3 h and 6 h radiographic images was shown in Figure 1. The formulation was remained buoyant for upto 6 h which is observed in the images indicates that the formulation remains buoyant in the stomach (Mulugeta Fentie, *et al*., 2015).

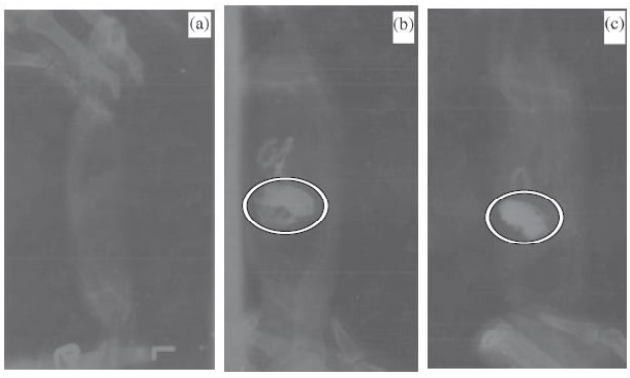

Figure 1: Formulation at the gastric region of rabbit in X-ray images : (a) before dosing (initial), 3 h after dosing and 6 h after dosing.

3.2 *In vivo* pharmacokinetic results

3.2.1 Treatments

The healthy male albino rabbits are divided into 3 groups. A single dose is received in three different periods on each group of each for the above 3 treatments was washed out period (Rouge *et al*., 1996). 1week between each treatment in a random order and the administration of the treatments were shown in the Table below .

Table 1: Selected optimized formulations for study of three way crossover treatment

	1-Sequence		2-Sequence		3-Sequence
1-Group	Pure drug solution	Wash out	Marketed formulation	Wash out	Optimized formulation
2-Group	Optimized formulation		Pure drug solution		Marketed formulation
3-Group	Marketed formulation		Optimized formulation		Pure drug solution

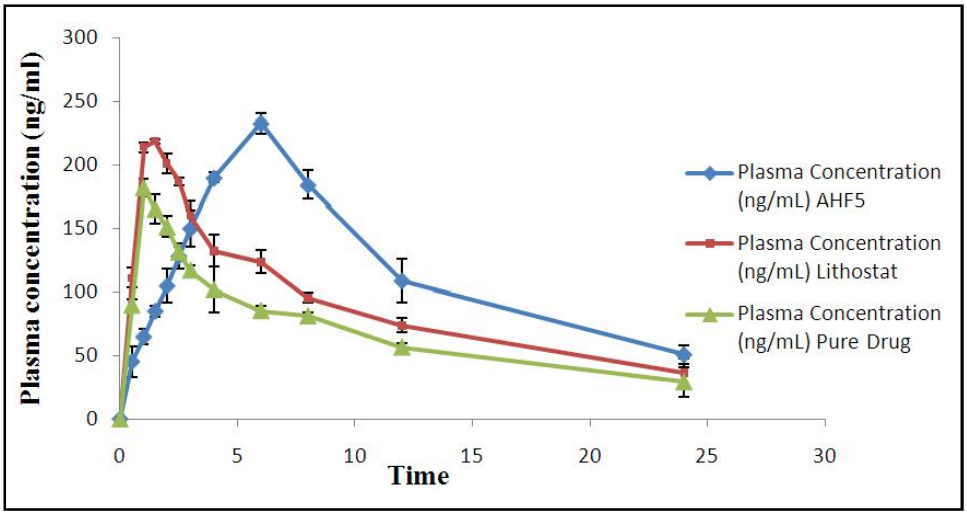

Figure 2: Plasma concentration time profile of acetohydroxamic acid formulations.

Table 2 : Pharmacokinetic parameters of acetohydroxamic acid

Parameter	Unit	AHF5	Marketed	Pure drug
Elimination rate constant	1/h	0.083	0.059	0.061
t1/2	h	8.38	11.62	11.18
Tmax	h	6	1.5	1
Cmax	ng/ml	232.82	218.66	182.13
Clast_obs/Cmax		0.217	0.167	0
AUC 0-t	ng/ml*h	2805.9608	2124.948	0.161
AUC 0-inf_obs	ng/ml*h	3418.66	2736.03	2117.82
AUMC 0-inf_obs	ng/ml*h^2	48941.13	42882.11	33049.84
Relative BA of AHF5 with marketed	124.9			
Relative BA of AHF5 with pure drug	161.4			

4. Discussion

The pharmacokinetic study was performed in healthy rabbits. Time profile was obtained through the study as shown in Figure 2. Some of the pharmacokinetic parameters are estimated as Tmax, C max, AUC and relative bioavailability are given in Table 2. In the results, oral bioavailability AHF5 is increased when it is compared with pure drug and market formulations. The relative bioavailability of optimized formulation and marketed drug products were found as 161.4 and 124.9, respectively.

5. Conclusion

In the present work, AHF5 is used for *in vivo* evaluation in albino rabbits which has shown from the x-ray images that the formulation was remained buoyant upto 6 h which indicates that the formulation is retained in the stomach. Pharmacokinetic parameters were estimated through results optimized formulation (AHF5) oral bioavailability was raised when it was correlated with the marketed formulations.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

References

Arora, S.; Ali, J. and Ahuja, A. (2005). Floating drug delivery systems: A review. AAPS Pharma. Sci. Tech., **19**:72-90.

Avachat, M and Dhamne, A. (2002). Gastric floating system. Patent, 02:102-415. December 27.

Bagre, A. and Awasthi, S. (2017). Clarithromycin loaded floating eudragit microsphere for anti *H. pylori* therapy: *In vitro* and *In vivo* assessment. Journal of Chemicals and Pharmaceutical Research, **9**(4):270-276.

Choe, S.Y.; Neudeck, B.L.; Welage, L.S.; Amidon, G.E.; Barnett, J.L. and Amidon,G.L. (2001). Novel method to assess gastric emptying in humans: The pellet gastric emptying test. Eur. J. Pharm. Sci., **14**:347-353.

Christmann,V.; Rosenberg, J.; Seega, J. and Lehr, C.M. (1997). Simultaneous *in vivo* visualization and localization of solid oral dosage forms in the rat gastrointestinal tract bymagnetic resonance imaging (MRI). Pharm. Res., **14**:1066-1072.

Desai, S. and Bolton, S. (1989). Floating sustained release therapeutic compositions. US Patent, **4**:814-179.

Kawashima,Y.; Niwat. and Takeuchi, H. (1992). Hollow microspheres for use as a floating controlled drug delivery system in the stomach. J. Pharm. Sci., **81**:135-40.

Mulugeta Fentie; Anteneh Belete and Tsige Gebre-Mariam (2015). Formulation of sustained release floating microspheres of furosemide from ethylcellulose and hydroxypropyl methylcellulose polymer blends. J. Nanomed. Nanotechnol., 6:1.

Rouge, N.; Buri, P. and Doelker, E. (1996). Drug absorption sites in gastrointestinal tract and dosage forms for site delivery. Int. J. Pharm., **13**(6):117-139.

Umamaheshwari.; Jain, R.B.; Bhadra, S. and Jain, N.K. (2003). Floating microspheres bearing acetohydroxamic acid for the treatment of *Helicobacter pylori*. J. Pharm. Pharmacol., **55**:1607-1613.

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