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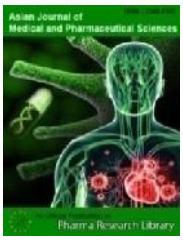

Research Article **Open Access**

Antiulcer Activity of *Symphytum Officinale* Linn Root on Wistar Albino Rats

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A B S T R A C T

The effect of Methanolic root extract of *Symphytum officinale* Linn was investigated in rats to evaluate the anti-ulcer activity by using Aspirin plus pyloric ligation model on experimentally induced gastric ulcer. The parameters taken to assess anti-ulcer activity were volume of gastric secretion, PH, free acidity, total acidity and ulcer index. The results indicate that the methanolic extract significantly ($P< 0.001$) decreases the volume of gastric acid secretion, PH, free acidity, total acidity and ulcer index with respect to control.

Keywords: *Symphytum officinale* Linn, Anti-ulcer, Anti-secretory

A R T I C L E I N F O

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1. Introduction

Peptic ulcer is the most common gastrointestinal disorder in clinical practice. Considering the several side effects (arrhythmia's, impotence, funaecomastia, haematopoeitic changes) of modern medicine [1], indigenous drugs possessing fewer side effects should be looked for as a better alternative for the treatment of peptic ulcer. There is evidence concerning the participation of reactive oxygen species in the etiology and pathophysiology of human diseases, such as neurodegenerative disorders, inflammation, viral infections, autoimmune pathologies and digestive system disorders such as gastrointestinal inflammation and gastric ulcer [2]. The study assumes

significance in the context that prolonged use of synthetic anti-ulcer drugs leads to adverse drug reactions and a search for new anti-ulcer agents that retain therapeutic efficacy and are devoid of adverse drug reaction is warranted. A study of the efficacy of methanolic extract of *Symphytum officinale* Linn root (MERSO) in gastric ulcer with aspirin plus pylorus ligation induced ulcer was undertaken in a rat model. *Symphytum officinale* Linn (common name: Comfrey) is a herb belongs to the family boraginaceae. It contains wide range of chemical constituents justifying its medicinal importance for various diseases traditionally. Roots and leaves contains 0.8% and 0.4% allantoin. Other constituents include poly phenolic acids, pyrrolizidine alkaloids, triterpenic saponosides, rosmarinic acids, tannins, amino acids, phytosterols, and saccharides. The pyrrolizidine alkaloids: lycopsamine, intermedine (monoesters of retronecine), their acetylated derivatives and symphytine. Traditionally, roots or leaves were taken internally against lung disorders, gastritis, and stomach ulcer and bleeding. Externally for the treatment of inflammation, cuts, bruises, sprains, sores, eczema, broken bones, pulled ligaments and muscles, arthritis, and boils. It is also used as analgesic, astringent, demulcent, diuretic, expectorant, hemoptysis [3, 4]

2. Experimental

Materials

Collection of plant material

The roots of *Symphytum officinale* Linn was collected in the month of March 2013 from Tirumala hills, Tirumala, Chitoor Dt, A.P, India. It was authenticated by Dr.K. Madhava chetty, Department of botany, Sri Venkateswara University, Tirupati, A.P, India.

Chemicals

All the chemicals used for the study are of analytical grade.

Selection of experimental animals

Wistar albino rats of either sex (150-200 gm) were used in the study. Animals were housed individually in polypropylene cages in a ventilated room under ambient temperature of 22 ± 2° C and 45-65 % relative humidity, with a 12 hour light followed by 12 hour dark. All the animals were acclimatized for at least 7days to the laboratory conditions prior to experimentation. Tap water and food pellets were provided ad libitum. Food pellets was with held overnight prior to dosing. All rats were handled and maintained strictly as per guidelines of "Guide for the care and Use of Laboratory animals".(Institute of Laboratory Animals Resources, National Academic Press 1996: NIH Publication number # 85-23, revised 1996).

Methods

Extraction of *Symphytum officinale* Linn roots

Symphytum officinale Linn. roots was shade dried and made into coarse powdered which was passed through a# 40 mesh sieve to get uniform particle size and was extracted using methanol by continuous hot percolation process using soxhlet apparatus [5].

Antiulcer activity

Aspirin plus pylorus ligation induced ulcer method

In this model Wistar albino rats were divided into five groups of six animals in each. Group I: Control group received normal saline 10ml/kg body weight, Group II: Standard group received ranitidine 50mg/kg body weight, Group III, IV&V received MERSO 250, 500 & 1000mg/kg body weight. All the doses of test drug and standard are administered per orally. All the animals were received 200mg/kg of aspirin once daily for three days. The various groups were treated with vehicle, standard drug and extract. On the fourth day, pylorus part was ligated, following 18 hr fasting. The animals were anaesthetized using thiopentone sodium (35mg/kg.i.p), the abdomen was opened and pylorus ligation was done without causing any damage in its blood supply. The abdomen was then sutured, at the end of 4hrs of ligation the animals were sacrificed by cervical dislocation. The abdomen was opened and their stomachs were dissected out. Gastric juice collected in to centrifuge tubes was centrifuged at 1000 rpm for 10 min and volume was noted. The pH of the gastric juice was recorded by digital pH Meter. The gastric content was subjected for analysis of free and total acidity. The stomachs were washed with 5ml of water with help of disposable syringe. The stomachs were opened along its greater curvature, pinned on a cork plate and inner surface examined for ulceration with a simple microscope for determination of volume and pH of gastric juice and free and total acidity [6,9]. A score for the ulcer was made as follows.

Table 1

S. No	Stomach colour	Ulcer score
1	Normal color	0
2	Red colour	0.5
3	Red spots	1
4	Hemorrhagic streaks	1.5
5	Ulcer >3mm but<5mm	2
6	Ulcer >5mm	3

Mean ulcer score for each animal was expressed as ulcer index. The percentage of ulcer inhibition was determined as follows:

$$\text{Percentage inhibition of ulcer} = \frac{\text{control mean ulcer index - Test mean ulcer index}}{\text{control mean ulcer index}} \times 100$$

3. Results and Discussion

Lamivudine was found to be soluble in Water, Benzene, Glacial acetic acid, Sodium hydroxide, hydrochloric acid and acetone at room temperature. The λ max was found to be 270nm. The λ_{max} of Lamivudine pure drug and in tablets were shown in fig 2 and 3 respectively. The Molar Absorptivity was found to be 7.5×10^{-6} by using the formula (e= A / c l), (A= absorbance, c = sample concentration in moles/liter &l = length of light path through the sample in cm). The Sandell's sensitivity (Sandell's sensitivity = mol. wt / molar absorvitity) was found to be 2.96×10^{7} µg cm^{-2}.The Accuracy results were shown in table 1. The precision was carried out for 10µg/ml. The intraday precision for 10µg/ml was found to be 1.493% RSD, while it gave a value of 1.49366% RSD at interday (intermediate precision). The accuracy of the method was calculated as percent bias. The Intraday

Precision and Inter-day precision of Lamivudine tablets were shown in table 2 and 3 respectively. The spectrum of standard solution of Lamivudine in water was given in Fig. 2 and 3.The λ_{max} was found to be 270 nm. The calibration curve was prepared by plotting concentration (in µg/ml) on the abscissa and absorbance in ordinate axis in the range of 10-100µg/ml. Good linearity was obtained in the concentration range considered. The linearity equation was developed by using least square regression analysis. The Limit of detection and Limit of quantitation was calculated according to the ICH guidelines. The Beer-Lamberts limit was found to be10-100µg/ml (table 4). Calibration curve of Lamivudine was plotted (shown in fig. 4) and the linearity, regression, LOD and LOQ were shown in table 5. The LOD was found to be0.675µg/ml and the LOQ was found to be 2.25µg/ml. The acid, base, heat and peroxide degradation spectrum of Lamivudine tablets were summarized in table 6.

Table 2: Intraday Precision of Lamivudine tablets

S. No	Conc. (µg/ml)	Absorbance		
		Day-I	Day-II	Day-III
1	10	0.548	0.523	0.509
2		0.544	0.528	0.505
3		0.547	0.521	0.513
4		0.566	0.536	0.517
5		0.545	0.539	0.514
6		0.543	0.534	0.528
Average		0.549	0.530	0.514
SD		0.009	0.007	0.008
% RSD		1.569	1.378	1.534

Table 3: Inter-day precision of Lamivudine tablets

S. No	Conc. (µg/ml)	Absorbance		
		Day-I	Day-II	Day-III
1	10	0.548	0.523	0.509
2		0.544	0.528	0.505
3		0.547	0.521	0.513
4		0.566	0.536	0.517
5		0.545	0.539	0.514
6		0.543	0.534	0.528
Average		0.549	0.530	0.514
SD		0.009	0.007	0.008
% RSD		1.569	1.378	1.534
Limit% RSD must be less than 2%.				

Table 4: Calibration (Linearity) of Lamivudine tablets

Concentration (µg/ml)	Absorbance
1	0.129±0.001
5	0.287±0.001
10	0.507±0.001
15	0.691±0.002
20	0.887±0.002
Values are in mean ±S.D: number of trials (n)=3	

Table 5: Slope, Linearity, LOD and LOQ of Lamivudine tablets

Slope	Linearity (R^2)	LOD (µg/ml)	LOQ (µg/ml)
0.04x+0.0923	0.9992	0.675	2.25

Table 6: Degradation studies of Lamivudine tablets

Parameter	Result
Acid degradation	0.545±0.001
Base degradation	0.464±0.001
Peroxide degradation	0.543±0.001
Heat degradation	0.523±0.001
Values in mean±S.D; N=3	

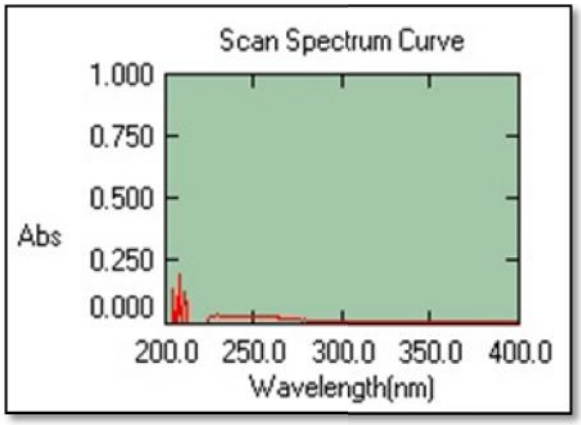

Figure 2: λ_{max} of Lamivudine pure drug

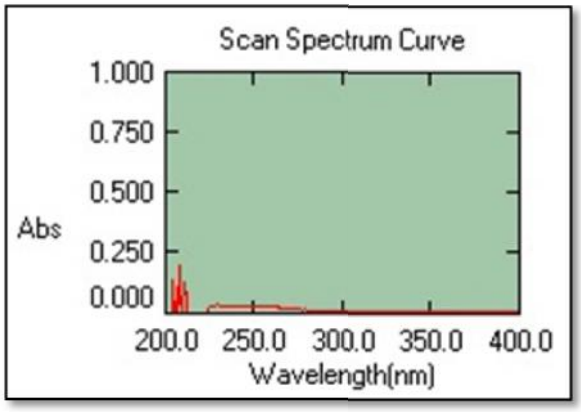

Figure 3: λ_{max} of Lamivudine tablet powder

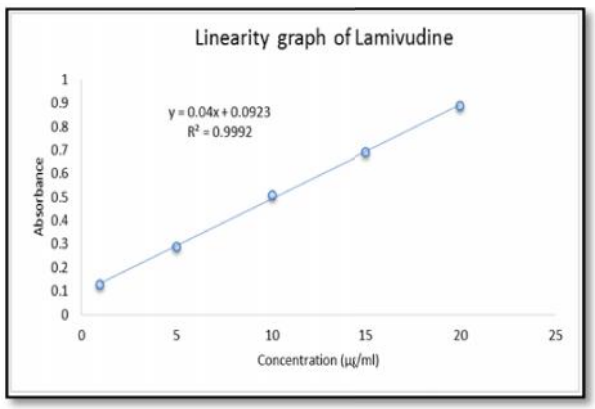

Figure 4: Calibration curve of Lamivudine

Table 1: Accuracy results of Lamivudine tablets

Conc. Level	Sample Weight (mg)	Absorbance	Amount added	Amount found	% Recovery	Mean % Recovery
50%	15.55	0.275	4.969	4.964	99.888	99.403
		0.276	4.969	4.982	100.251	
		0.274	4.969	4.946	99.524	
		0.272	4.969	4.910	98.798	
		0.271	4.969	4.892	98.435	
		0.274	4.969	4.946	99.524	
100%	31.1	0.537	9.939	9.693	97.527	98.616
		0.526	9.939	9.495	95.529	
		0.566	9.939	10.217	102.794	
150%	46.65	0.835	14.908	15.072	101.098	101.643
		0.836	14.908	15.090	101.220	
		0.866	14.908	15.632	104.852	
		0.836	14.908	15.090	101.220	
		0.826	14.908	14.910	100.009	
		0.838	14.908	15.126	101.462	

4. Conclusion

In this study simple, fast and reliable UV spectrophotometric method was developed and validated for the determination of Lamivudine in tablet formulation. The method was applied directly to the analysis of pharmaceutical dosage forms without the need for separation such as extraction steps prior to the drug analysis. As these proposed methods have the lowest LOD value and wider linear range so these methods were more sensitive methods. From the results obtained, we concluded that the suggested methods showed high sensitivity, accuracy, reproducibility and specificity. Moreover, these methods were simple and inexpensive and they can be employed for the routine quality control analysis of Lamivudine in pharmaceutical formulations.

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