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ORIGINAL RESEARCH ARTICLE

Effect of Gokhru Plant Extract on Intestinal Absorption of Aspirin using Everted Sac Technique

S. ASHOK REDDY[1], SAIKAT SEN*[2], RAJA CHAKRABORTY[3], B. PARAMESHAPPA[2]

[1]Department of Pharmaceutical Chemistry, SAFA College of Pharmacy, Kurnool, A.P.
[2]Department of Pharmacology and [3]Pharmaceutical Chemistry, C.E.S. College of Pharmacy, Kurnool, A.P.

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ABSTRACT

Permeability through biological membranes is a key factor in the absorption and distribution of drugs. Aspirin on oral absorption undergo extensive presystemic metabolism, therefore it requires high and frequent dosing which is related with an increased risk of GIT side-effects. The present study aimed to investigate effect of Gokhru plant (*Tribulus terrestris* Linn.) extract on intestinal absorption by everted sac technique using goat intestine. When Aspirin kept with Gokhru plant extract, the concentration of absorbed Aspirin was 9, 14, 16, 18, 24 µg/ml after 15, 30, 45, 60 and 75 min respectively; where as 8, 10, 13, 15, 20 µg/ml of Aspirin absorbed when kept alone. The study clearly showed that the Gokhru extract enhance the absorption of Aspirin from goat intestine and the absorption enhancement activity of the plant may due to presence of saponin in the extract.

Key words: Aspirin, Gokhru, Absorption; Everted sac technique

INTRODUCTION

The oral route is the one of the most important method for administering drugs. Study of absorption properties of molecules/drugs are essential and are found to be problematic for certain molecules with respect to testing parameters and reproducibility [1]. Permeability through biological membranes is considered as a key factor in the absorption and distribution of drugs. Absorption of drug from the solid dosage forms after oral administration depends on release of the drug substance from the product, the dissolution or solubilisation of the drug under certain physiological condition and permeability across the gastrointestinal tract. Poor permeability because of structural features as well as membrane-based efflux mechanisms, can lead to poor absorption across the gastrointestinal mucosa or poor distribution throughout the body [2, 3, 4]. *In vitro* everted sac technique is suitable for screening the permeability parameter of certain drug substances. In this technique, the intestinal sac of goat, sheep or rat is everted to expose the mucosal surface in suitable condition to keep the tissue viable. Then the test drug is introduced into mucosal fluid and absorption mechanism is studied and/or compared. Drugs with poor absorption/bioavailability can be assessed by this method and appropriate modifications can make to enhance absorption [5, 6, 7, 8, 9].

Aspirin (acetyl salicylic acid), is a most common and widely used as analgesic and antipyretic drug that is safe at therapeutic dose range for a number of treatments. The drug is also useful in acute rheumatic fever, rheumatoid arthritis, osteoarthritis and post myocardial infraction. It is a weak acid and absorbed from stomach and upper intestinal tract. But poor water solubility of Aspirin is the limiting factor for absorption [10, 11, 12]. Orally administered Aspirin is undergo extensive presystemic metabolism in the GIT and liver, converting it into salicylic acid and therefore, orally administered Aspirin requires high and frequent dosing which is associated with an increased risk of GIT side-effects [13]. Different study had been carried out to enhance the absorption of different drugs and reported that different plant extracts/products are useful to enhance the absorption of drug. Plant extract or natural compound from medicinal plant like saponins, flavanoids are also proved effective to increase the bioavailability [14, 15, 16, 17].

***Corresponding Author: SAIKAT SEN**, **Email:** saikat.pharm@rediffmail.com, **Contact No:** +91-9032011182

Tribulus terrestris Linn. (Family: Zygophyllaceae) commonly known as Gokhru is a important traditional medicinal plant of India. Gokhru is important for its medicinal value in Ayurvedic, Siddha and Unani medicinal system. Different parts of the plant are found useful for diuretic, demulcent, anti-inflammatory, anabolic, spasmolytic, muscle relaxant, hypotensive, hypoglycemic activity. This plant is also useful in strangury, calculus affections, urolithiasis, crystalluria, urinary discharges, pruritus-ani, as a tonic in sexual inadequacy; also as a supporting medicine in cough and asthma. Phytochemical study of the plant showed the presence of saponin, flavanoids and carboline alkaloids [18, 19]. Therefore the present study aim to investigate effect of Gokhru plant extract on intestinal absorption of Aspirin using everted sac technique.

MATERIALS AND METHODS

Drugs and Chemicals:

Aspirin and other chemicals like methanol, ferric chloride ($FeCl_3$), calcium chloride, magnesium chloride, disodium hydrogen phosphate, sodium dihydrogen phosphate, sodium bi carbonate, potassium chloride, sodium chloride, glucose were purchased from Loba Chemie Pvt Ltd., Mumbai. Analytical grade chemical and solvent was used where ever required.

Plant materials and extraction procedure:

The leaves of *Tribulus terrestris* were collected in October 2009 from Kurnool district of Andhra Pradesh. Plant material thus collected and were dried under shade and then made into a coarse powder using dry grinder, passed through sieve no. 40 and stored in an air tight container at 25°C. Air dried powdered material (50 g) was transferred into a Soxhlet apparatus containing 200 ml of methanol and extracted for 18 hrs. The extract was concentrated in vacuum under reduced pressure using rotary flask evaporator. It was further concentrated and dried in the desiccators for further studies.

Determination of λ max of Aspirin:

Dissolve, 100 mg of Aspirin in 1000 ml of phosphate buffer solution (pH 7.4) to make stock solution of 100 µg/ml. From that stock solution 1ml was mixed with 0.5 ml of 0.025 M $FeCl_3$ solution and makeup the volume upto 10 ml with buffer solution. The absorbance of this solution was screened from 400 to 800 mm, taking $FeC1_3$ solution as blank. A graph was plotted by taking wavelength in X-axis and absorbance in Y-axis. Maximum absorbtivity was identified as λmax of Aspirin (Figure - 1). The λmax of Aspirin in presence of Gokhru extract was also been identify using the same procedure (Figure - 2). The λmax of Aspirin was found 530 nm in both cases.

Calibration response curve of Aspirin:

Calibration response curve was plotted by taking 100 µg/ml of Aspirin stock solution prepared by dissolving the l00 mg of Aspirin in l000 ml of buffer solution. Aliquots of 1.0, 2.0, 3.0, 4.0, 5.0, 6.0 ml stock solution of Aspirin has taken separately in 10 ml volumetric flasks and 0.5 ml of $FeCl_3$ of solution was added to each. The volume was make up to 10ml with buffer solution to prepare 10, 20, 30, 40, 50, 60 µg/ml working solutions of Aspirin. The absorbance of each solution was determined by U.V. spectrophotometer at 530 nm, taking $FeCl_3$ solution as blank. A graph was plotted by taking concentration on X-axis and absorbance on Y-axis.

In vitro absorption studies by everted sac modification method:

Preparation of transport buffer solution

Buffer solution was prepared by using the following formula[19]

Calcium chloride	-	0.132 g
Magnesium chloride	-	0.2438 g
Disodium hydrogen phosphate	-	0.3406 g
Sodium di hydrogen phospha	-	0.0624 g
Sodium bi carbonate	-	2.1002 g
Potassium chloride	-	0.3726 g
Sodium chloride	-	6.6716 g
Glucose	-	0.8970 g

Distilled water- Required to produce 900 ml.

Everted goat sac technique

Permeation study was carried out using goat small intestine by everted sac technique. Experimental animal was fasted for 24 h and then sacrificed to isolate the intestine from the animal. Sacrificed goat intestine preserved in transport buffer was cut into 2 pieces each about 15 cm; approximate diameter of intestine was 0.8 cm. One end of the intestine was tied up and everted; other end of the intestine is connect to a cannula to form a pouch. The tissue was made alive by supply of oxygen with the help of aerator and buffer solution; the temperature maintained at 37± 0.5ºC. After eversion the mucosal side came out and serosal side is present inside [20].

Preparation of Aspirin-buffer and Aspirin with Gokhru extract - buffer solution

Aspirin-buffer solution was prepared by dissolving 2 g of Aspirin 2000 ml buffer solution. Aspirin with Gokhru extract - buffer solution was prepared by dissolving 2 g of Aspirin in a 250 m1 of buffer solution, to this 250 ml of 1% w/v solution of Gokhru extract was added, the

resulting solution is made up to 2000 ml with buffer solution.

***Experimental procedure*:**

Two organ baths were taken containing buffer solution. Attached and placed the two different everted intestines as describe above in organ bath. Oxygen supply and temperature was maintained. The stirrer was placed for the agitation to get the effect similar to peristaltic moment. About 1.5 L of Aspirin buffer solution was placed in one organ bath and Aspirin with Gokhru extract -buffer solution in another organ bath. Plain buffer placed inside the intestinal sac, so that buffer solution contains the drug present in outside (mucosal side) and plain buffer present in the inside (serosal side).

One ml of sample form each intestine (serosal side) were collected 5 times at the interval of 15 min. The collected samples were mixed with 0.5 ml $FeCl_3$ solution, and volume was made upto 10 ml with phosphate buffer solution; wait for 2/3 min till the violet colour developed. Then the sample was analyzed at 530 mn by taking $FeCl_3$ solution as blank. The concentration of Aspirin absorbed was analyzed using calibration curve.

RESULTS AND DISCUSSION

The λ max o f th e Aspirin was found 530 nm showed absorbtivity of 0.493 (**Figure - 1**). Same study was performed for Aspirin in presence of Gokhru extract and was found highest absorbtivity of 0.894 at the same wavelength (**Figure - 2**). The study reveals that the λ max of Aspirin remain same in presence or without presence of Gokhru extract. The calibration curve of Aspirin found linear and thus it obeys Beers Lamberts law. Resultant graph showed in the (**Figure – 3**).

The effect of Gokhru plant extract on intestinal absorption of Aspirin is tabulate in (**Table -1**) and (**Table – 2**). Table - 1 represents the concentration of Aspirin absorbed without Gokhru plant extract and Table - 2 represents the data of Aspirin concentration absorbed in presence of Gokhru extract. The sampling is continued for 75 min and found that the concentration of Aspirin absorbed is more in presence of Gokhru plant extract in all case. After 15, 30, 45, 60 and 75 min the amount of absorbed Aspirin were 8, 10, 13, 15, 20 µg/ml respectively, where in presence of Gokhru plant extract Aspirin absorbed was 9, 14, 16, 18, 24 µg/ml respectively.

The study clearly suggests that the Gokhru extract enhance the absorption of Aspirin from goat intestine. Earlier investigation showed that saponin and flavanoids are major chemical constituent of the plant.. The saponin is an important phytoconstituents and important for its absorption enhancement activity. Therefore the absorption enhancement effect of Gokhru may due to presence of saponin in the extract. Though a details study required to explore the proper mechanism and identify chemicals responsible for its absorption enhancement activity.

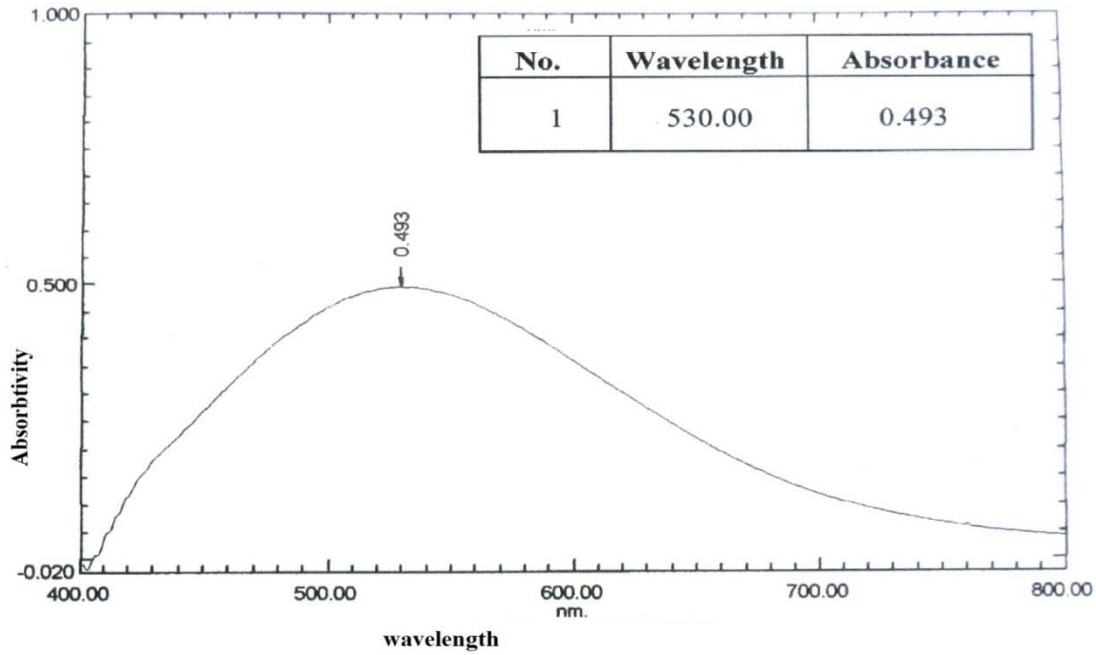

Figure - 1: Wavelength vs absorbance graph to determine the λmax of Aspirin

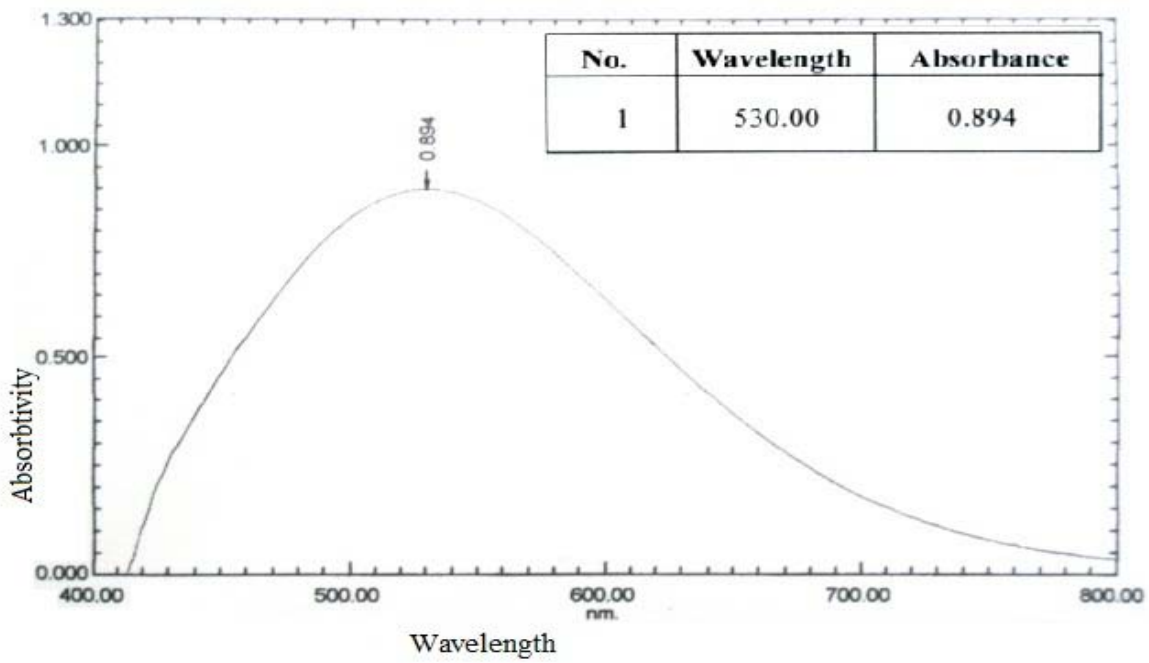

Figure - 2: λmax of Aspirin in presence of Gokhru extract

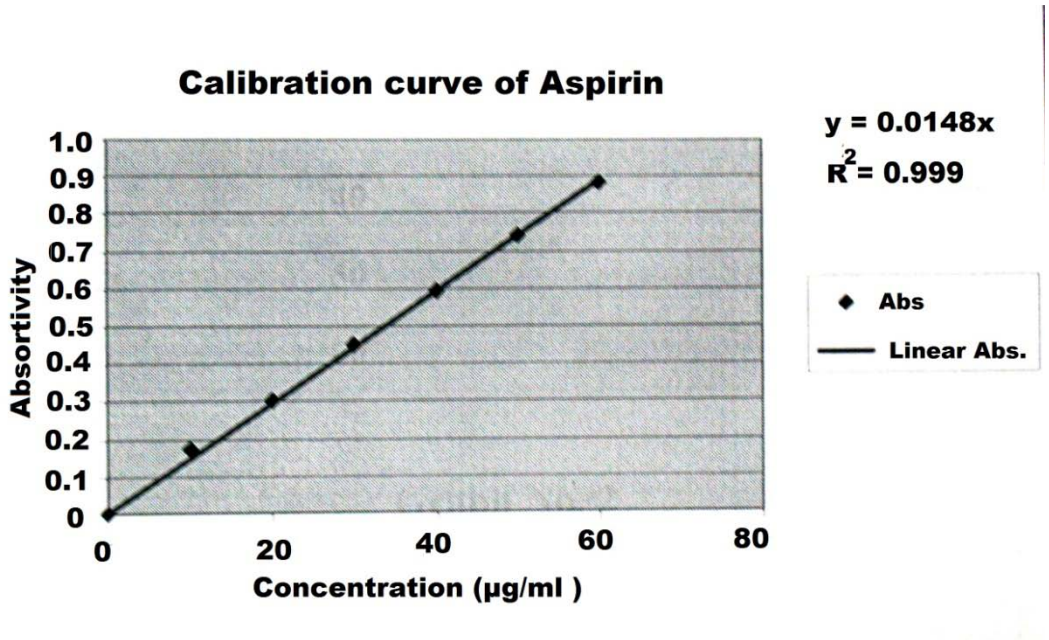

Figure -3 : Calibration response curve of Aspirin

Sl. No	Time (min.)	Absorbance	Concentration (µg/ml)
1	15	0.10	08
2	30	0.15	10
3	45	0.20	13
4	60	0.25	15
5	75	0.30	20

Table - 1: Absorption study of Aspirin by Everted sac technique using goat intestine

Sl. No	Time (min.)	Absorbance	Concentration (µg/ml)
1	15	0.13	09
2	30	0.18	14
3	45	0.23	16
4	60	0.28	18
5	75	0.34	24

Table - 2: Absorption study of Aspirin in presence of Gokhru by Everted sac technique

CONCLUSION

The present study showed that the Gokhru extract absorption enhancement activity. This study may pave the way to extend this type of approaches to similar combinations of drugs used in clinical practice so as to improve bioavailability.

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