ScienSage

Journal of Advanced Scientific Research

Available online through https://sciensage.info

ISSN
0976-9595
Research Article

EFFECT OF ORANGE PEEL EXTRACT ON INTESTINAL ABSORPTION OF ASPIRIN USING EVERTED SAC TECHNIQUE

Komal Dattatraya Pol*[1], Pradnya Nilesh Jagtap[1], Sumit Kailas Musle[2], Shweta Shivaling Bobade[3], Ankita Mahadeo Kadam[4],Vaishnavi Pradeep More[5], Pratibha Pradip Deshmukh[6], Ashwini Mahadev Kunjir[7]

Department of Pharmacology, Pune District Education Association's Seth Govind Raghunath Sable College of Pharmacy, Saswad, Pune, Maharashtra, India

**Corresponding author: polkomal94@gmail.com*

Received: 23-11-2022; Accepted: 31-01-2023; Published: 31-03-2023

 https://doi.org/10.55218/JASR.2023140307

ABSTRACT

Drug distribution and absorption are significantly influenced by the biological membrane's permeability. Since aspirin requires high and frequent doses and undergoes substantial presystemic metabolism during oral absorption, there is a higher risk of GIT adverse effects. The purpose of the current study was to use the everted sac technique on goat intestine to examine the impact of orange peel (Citrus Aurantium Dulcis) extract on intestinal absorption. When kept with orange peel extract, the concentration of absorbed aspirin was 20, 26, 30, 38, and 50µg/ml after 15, 30, 45, 60, and 75 minutes, compared to 14, 16, 17, 18 and 23 µg/ml when kept alone. According to the study, aspirin is absorbed more readily from goat intestines when orange peel extract is used. Citrus peel contains a lot of phenolic chemicals, including flavonoids and phenolic acids. In addition, limonene, citral, neohesperidin, naringin, rutin, rhamnose, eriocitrin, and Vitamin-C etc. are found in the peel. They might be in charge of the plant's capacity to boost absorption.

Keywords: Everted sac, Orange peel, Aspirin, Buffer, Absorption.

1. INTRODUCTION

A number of factors can affect the systemic availability of pharmacological compounds given orally. While some of them have systemic causes, others are related to medicines [1-2]. Product technology and material science have researched and altered drug-related properties such as solubility, partition coefficient, ionic charge of molecule, penetrability, particle size and shape, salts, isomers, polymorphs, and stability in great detail [3-5]. But altering systemic elements like membrane transporters, intestinal enzymes, membrane permeability, areas or sites of absorption, methods of absorption, etc. can be difficult. The oral route is one of the most important drug administration techniques. The investigation of a drug's or molecule's absorption properties has been shown to be challenging in terms of testing parameters and consistency [6]. The mechanisms driving medication interactions, drug metabolism, and drug absorption are all highly complex processes that necessitate in-depth study. The intestinal epithelium has a number of carriers and pumps that aid in intestinal absorption in addition to passive absorption. Drug substrates are moved by one type of pump (influx transporter) from the intestinal mucosa to the serosal side, and by another pump (efflux transporter) from the serosal side to the mucosal side [7]. The potential of drugs to cross biological membranes is believed to be a key factor in their distribution and absorption. The medication's solubilisation or dissolution under particular physiological conditions, the permeability of the gastrointestinal system, and the drug's release from the solid dosage form after oral administration all affect how well the drug is absorbed. Poor permeability caused by structural features and membrane-based efflux mechanisms may occur in inadequate gastrointestinal mucosal absorption or inadequate body distribution. Some materials' permeability parameter using the everted sac method, pharmacological substances can be evaluated in vitro." [8] "To maintain the tissue, the intestinal sac of a goat, sheep, or rat is everted in this method to display the mucosal surface in a healthy state. Following administration of the test chemical into the mucosal fluid,

the absorption process is investigated and/or compared [8-9]. Aspirin (acetyl salicylic acid), the most widely used analgesic and antipyretic drug, is safe when used within the therapeutic dose range for a number of medical disorders. The drug is also beneficial for osteoarthritis, rheumatoid arthritis, acute rheumatic fever, and post-myocardial infarction. The stomach and upper intestine system absorb it because it is a moderate acid. The reduced water solubility of aspirin, however, acts as a barrier to absorption [9]. Since aspirin undergoes significant pre-systemic metabolism in the GIT and liver, where it is converted to salicylic acid, oral administration of aspirin necessitates high and frequent dosages, which is associated with an elevated risk of GIT side-effects [10]. The effectiveness of different plant extracts and products in enhancing drug absorption has been demonstrated in numerous researches to improve drug absorption. Additionally, demonstrated to increase bioavailability are plant extracts or the naturally occurring saponins, alkaloids, and flavonoids found in medicinal plants [9,11,12].

Fig. 1: *Citrus Aurantium* Dulcis (Orange) Peel [17]

An orange is a fruit of various citrus species in the family Rutaceae. Orange peel is the fresh or dried outer layer of the pericarp on ripe or almost ripe fruits of Citrus aurantium. Orange peel extract is a botanical ingredient derived from the peel of the orange. *Citrus xaurantium* is its botanical name. Other names include *Citrus Aurantium* Dulcis Peel Extract, Orange Peel Extract, and Touhi Ekisu (JPN) [13]. Citrus seeds and peel both contain high levels of phenolic compounds, including flavonoids and phenolic acids; the peel has a higher concentration of these components than seeds [16]. Plants contain a class of natural compounds known as flavonoids, which have variable phenolic structures. When radicals oxidise flavonoids, they produce a more stable, less reactive radical. These include flavones (such as flavone, apigenin, and luteolin), flavonols (such as quercetin, kaempferol, myricetin, and fisetin), flavanones (such as flavanone, hesperetin, and naringenin), and others. Numerous flavonoids have been linked to hapatoprotective effects, including catechin, apigenin, quercetin, naringenin, rutin, and venoruton [15]. Orange peel also contains good amounts of provitamin A, folate, riboflavin, thiamine, vitamin B6, and calcium. Plus, it's rich in plant compounds called polyphenols, which may help prevent and manage many chronic conditions, such as type 2 diabetes, obesity, and Alzheimer's disease [13]. Therefore, the current study's goal is to examine, using the everted sac technique, the influence of orange peel extract on the intestinal absorption of aspirin.

2. MATERIAL AND METHODS

2.1. Drugs and Chemicals

Aspirin, calcium chloride, magnesium chloride, ferric chloride, disodium hydrogen phosphate, sodium dihydrogen phosphate, sodium bi carbonate, potassium chloride, sodium chloride, and glucose were purchased from Loba chemie Pvt. Ltd, Mumbai. Analytical grade chemical and solvent was used where ever required.

2.2. Plant materials and extraction procedure

About 20 kg of fresh oranges were collected from local market of Saswad, Pune in their commercial maturity. All fruits were of eating quality and without blemishes or damage. On arrival at the laboratory, the orange fruits were immediately washed using distilled water and peeled. The obtained peels were air dried in a ventilated oven at 40°C for 48 h, then finely ground using a coffee grinder and passed through a 24 mesh sieve size to produce the orange peel powder. Dried Orange peel powder (5 g) was boiled in water (100 mL) for 10 min. After cooling, the sample was centrifuged (5000 rpm for 10 min) and the clear solution was recovered in a conical flask, and then rinsed to 100 mL with water [14].

2.3. Determination of λ max of Aspirin

A stock solution of 100µg/ml of aspirin was made by dissolving 100 mg of aspirin in 1000 ml of phosphate buffer solution (pH 7.4). To make the volume of the buffer solution up to 10 ml, 1 ml of the stock solution was combined with 0.5 ml of the 0.0025M $FeCl_3$ solution. Using $FeCl_3$ solution as a blank, the absorbance of this solution was screened from 400 to 800 mm. By placing wavelength on the X-axis and absorbance on the

Y-axis, a graph was created. Aspirin's maximum absorptivity was determined as λmax. Using the same procedure, the λmax of aspirin present while orange peel extract was present was also determined. The λmax of Aspirin was found 530 nm for Aspirin and Aspirin with Orange peel extract.

2.4. Calibration response curve of Aspirin

The calibration response curve was constructed using a stock solution of aspirin dissolved in 1000 ml of buffer solution at a concentration of 100 µg/ml. Aliquots of 1.0, 2.0, 3.0, 4.0, 5.0, and 6.0 ml of aspirin stock solution were taken individually in 10 ml volumetric flasks, and each received 0.5 ml of a solution containing FeCl3. To prepare working solutions of aspirin containing 10, 20, 30, 40, 50, and 60µg/ml, the volume was made up to 10 ml with buffer solution. Using a blank solution of FeCl3, the absorbance of each solution was measured using a UV spectrophotometer at 530 nm. By placing concentration on the X-axis and absorbance on the Y-axis, a graph was created.

2.5. *In vitro* absorption studies by everted sac modification method

2.5.1. Preparation of transport buffer solution

Buffer solution was prepared by using the following formula

1. Calcium chloride - 0.2928 g
2. Magnesium chloride - 0.5416 g
3. Disodium hydrogen phosphate - 0.7568 g
4. Sodium di hydrogen phosphate - 0.1384 g
5. Sodium bi carbonate - 4.6664 g
6. Potassium chloride - 0.8272 g
7. Sodium chloride - 14.712 g
8. Glucose - 1.9932 g
9. Distilled water - Required to produce 2000 ml.

2.5.2. Everted goat sac technique

Goat small intestine was used in permeation research utilizing the everted sac method. An experimental animal was fasted for 24 hours before it was killed and its gut was separated. The transport buffer-preserved sacrificed goat intestine was divided into two segments, each measuring around 15 cm; the intestine's estimated diameter was 0.8 cm. The intestine was everted and knotted at one end, and the other end was connected to a cannula to create a pouch. By providing oxygen to the tissue with the aid of an aerator and a buffer solution, the tissue was brought alive while maintaining a temperature of 37 ± 0.5°C. The serosal side is present inside after eversion of the mucosal side.

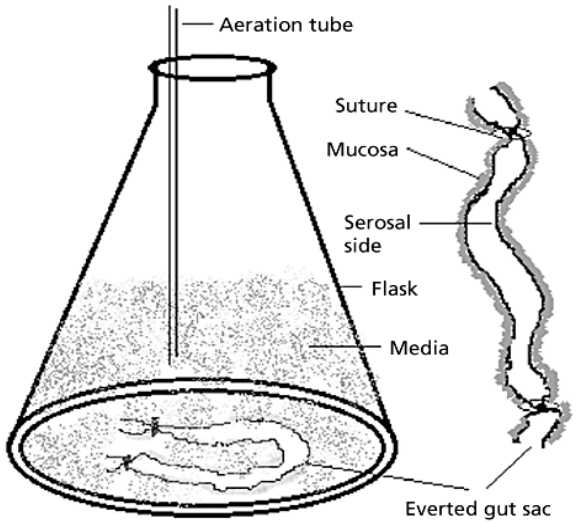

Fig. 2: Outline diagram of the everted gut sac model [7]

2.5.3. Preparation of Aspirin-buffer and Aspirin with Orange peel extract - buffer solution

Aspirin (2 g) was dissolved in 2000 ml buffer solution to create the aspirin-buffer solution. For preparation of Aspirin with Orange peel extract - 2 g of aspirin were dissolved in 250 ml of buffer solution, and then 250 ml of a 1% w/v solution of orange peel extract were added, bringing the total volume of the aspirin and buffer solution to 2000 ml.

2.6. Experimental procedure

Two organ baths with buffer solution were taken. The two distinct everted intestines as described above were attached and placed in the organ bath. Temperature and oxygen flow were kept constant. To create an effect akin to a peristaltic moment, the stirrer was put for agitation. One organ bath received 1.5 L of aspirin buffer solution, and the other received 1.5 L of aspirin with orange peel extract buffer solution. Placing plain buffer within the intestinal sac in such a way that the buffer solution includes both the drug present on the mucosal side of the outside and plain buffer present on the interior (serosal side). 1 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 up to 10 ml with phosphate buffer solution; wait for 2/3 min till the violet colour developed. The sample was then analysed at 530

nm by taking $FeCl_3$ solution as blank. The concentration of aspirin absorbed was analysed using calibration curve.

3. RESULTS AND DISCUSSION

The λ max of aspirin at 530 nm was determined to be 0.493. (Fig.5). In the same study, aspirin had the λ max of 0.710 at the same wavelength when orange peel extract was present (Fig. 6). According to the study, the λ max of aspirin doesn't change in the presence of orange peel extract. Aspirin's calibration curve was determined to be linear and as a result, it follows Beers Lambert's law. The final graph appeared in the (Fig. 7). The impact of orange peel extract on aspirin intestinal absorption given in Table 1 and Table 2. The concentration of aspirin absorbed without orange peel extract is shown in Table 1, while the concentration of aspirin absorbed in the presence of orange peel extract is shown in Table 2. Following a 75-minute sample period, it was discovered that the presence of orange peel extract always increased the amount of aspirin that was absorbed. After 15, 30, 45, 60, and 75 min, aspirin absorption was 14, 16, 17, 18 and 23μg/ml, respectively. When orange peel extract was present, aspirin absorption was 16, 21, 27, 36 and 45 μg/ml, respectively. The study clearly indicates that orange peel extract improves aspirin absorption from goat intestine. An earlier analysis revealed that the main chemical components in plants are flavonoids. The flavonoid is a crucial component of plants and has the ability to increase absorption. Orange peel's ability to increase absorption may therefore be a result of the flavonoid content of the extract. However, a thorough investigation is needed to pinpoint the molecules responsible for its absorption-enhancing effect and examine the correct mechanism.

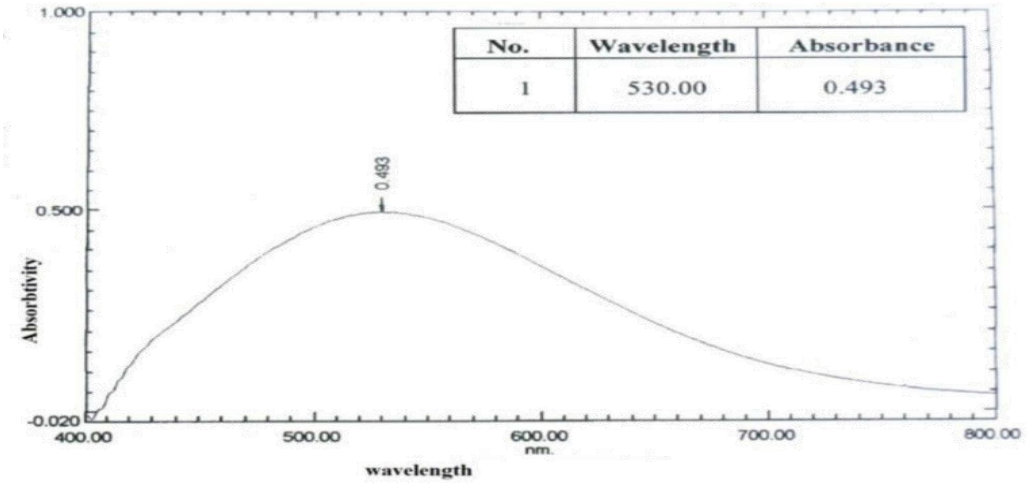

Fig. 3: Wavelength vs absorbance graph to determine the λmax of Aspirin

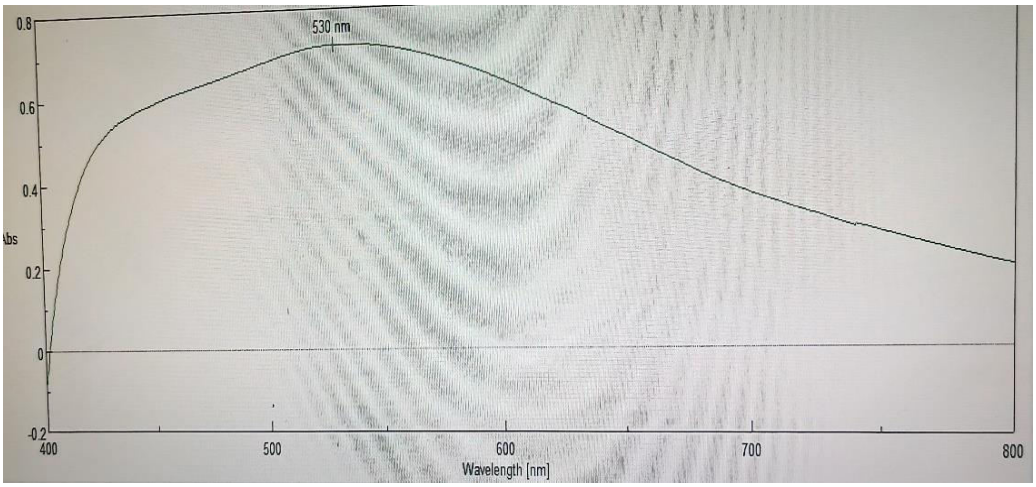

Fig. 4: λmax of Aspirin in presence of Orange peel extract

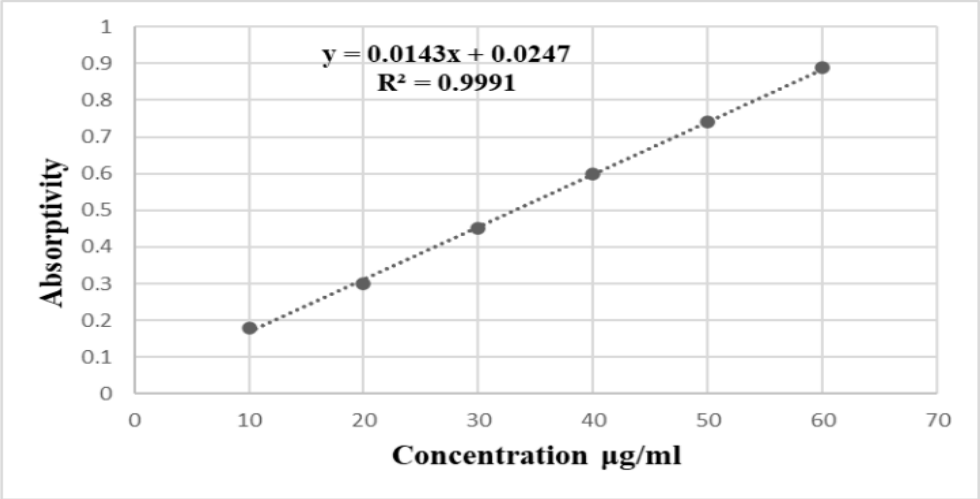

Fig. 5: Calibration response curve of Aspirin

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

Time (min.)	Absorbance	Concentration (µg/ml)
15	0.21	14
30	0.25	16
45	0.28	17
60	0.28	18
75	0.32	23

Table 2: Absorption study of Aspirin in presence of Orange peel by Everted sac technique

Time (min.)	Absorbance	Concentration (µg/ml)
15	0.26	16
30	0.31	21
45	0.42	27
60	0.56	36
75	0.67	45

4. CONCLUSION

The results of the current investigation demonstrated that the flavonoids included in orange peel extract enhanced the extract's ability to increase absorption. This study may have shown how to use similar drug combinations used in clinical practice to similar types of approaches to increase bioavailability.

5. ACKNOWLEDGEMENT

We are thankful to PDEA's Seth Govind Raghunath College of Pharmacy, Saswad, Pune, Maharashtra, India for providing laboratory facility for this research work.

Conflict of Interest

All authors mentioned above declare that, they have no conflict of interest.

Source of Funding

Self-funding

6. REFERENCES

1. Levine RR. *Dig Dis Sci*, 1970; **15**:171–188.
2. Song NN et al. *Asian J Drug MetabPharmacokinet.,* 2004; **4**:167–176.
3. Yen FL et al. *J Agric Food Chem.,* 2010; **58**:7376–7382.
4. Xia D et al. *Pharm Res.,* 2010; **27**:1965–1976.
5. Figueiras A et al. *AAPS PharmSciTech.,* 2010; **11**:233–240.
6. Kwon Y. Handbook of essential pharmacokinetics, pharmacodynamics and drug metabolism for industrial scientists. 1st ed. New York:Kluwer Academic Publishers; 2002.
7. Mohd AA, Fahad IJ, Abdullah MM. *Journal of Pharmacy and Pharmacology*, 2012; **64**:326-336.
8. Reddy AS, Sen S, Chakraborty R, Parameshappa B. *International Journal of Pharmaceutical & Biological Archives,* 2011; **2(1)**:549-553.
9. Volpe DA. *The AAPS Journal,* 2010; **12**:670-678.
10. Ammar HO, Ghorab M, El-Nahhas SA, Kamela R. *Asian J Pharmaceutical Sci.,* 2007; **2**:96-105.
11. Chen W, Lu Z, Viljoen A, Hamman J. *Planta Med.,* 2009; **75**:587-595.
12. Kang MJ, Cho JY, Shim BH, Kim DK, Lee J. *J Med Plants Res.,* 2009; **3**:1204-1211.

13. Citrus Aurantium Dulcis (Orange) Peel Extract. *https://ecostore.com/nz/ingredients/used-with-care/citrus-aurantium-dulcis-orange-peel-extract, 07/10/2022.*
14. Olufunmilayo SO, Rebeccah OO, Sule OS, Aline AB, Margareth LA**.** *Antioxidants***,** 2015**; 4:498-**512
15. Tapas AR, Sakarkar DM, Kakde RB. *J. Pharm. Res.,* 2008; **7**:1089–1099.
16. Sawalha SMS, Arráez RD, Segura CA. *Food Chem.,* 2009; **116**:567–574
17. Sneha PW. no more! Reap the benefits of orange peel.*https://www.theweek.in/authors.html?author=Sneha-Pillai* March 30, 2017.