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Research Article

EVALUATION OF THE IMPACT OF GREEN TEA PLANT EXTRACT ON INTESTINAL ABSORPTION OF ASPIRIN USING EVERTED SAC TECHNIQUE

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ABSTRACT

In vivo Absorption investigations are time-consuming and expensive in order to find the best medication formulation. Models were employed to research drug permeability and dissolution in order to streamline the search for a recommended formulation. One example of an *in vitro* model is the use of a goat intestinal section (sac) to assess the absorption of related drugs prior to formulation and clinical investigations. The investigation of excipient and additive effects on drug permeability is an intriguing use of this method. In this paper, the impact of epigallocatechin -3-gallate (EGCG), on the intestinal penetration of acetyl salicylic acid was investigated. When Aspirin was kept with green plant extract, the concentration of absorbed Aspirin was 12, 32 ,45, 49 and 51µg/ml after 15, 30, 45, 60 and 75 min respectively; whereas 14 ,16 ,17 ,18 and 23 µg/ml of Aspirin was absorbed when kept alone. The study clearly showed that the green tea extract enhances the absorption of Aspirin from goat intestine and this absorption enhancement activity of the plant is due to polyphenolic content, presence of polyphenols like catechins and epicatechins.

Keywords: Everted sac, Green tea extract, Aspirin, Buffer.

1. INTRODUCTION

One of the most crucial routes for drug administration is oral. In terms of testing parameters and reproducibility, studying a drug's or molecule's absorption qualities is crucial [1].

The dissolution of the drug from the dosage form, the drug's solubility as a result of its physicochemical features, the drug's effective permeability to the intestinal mucosa, and the drug's pre systemic metabolism can all be thought of as significant steps in the oral drug absorption process. Numerous factors may influence the aforementioned procedures, which in turn may influence the pace and volume of oral medication absorption. Three categories can be used to group these variables. The first category refers to a drug's physicochemical characteristics, which include solubility, intestinal permeability, pKa, lipophilicity, stability, surface area, and particle size. Physiological aspects fall under the second group. These include gastrointestinal pH, stomach emptying, small intestine transit duration, bile salt, absorption mechanism, and others. The third group includes dosage form elements including suspension, capsule, pill and solution [2].

The ability of medications to pass through biological membranes is thought to be a crucial component in their absorption and distribution. Poor permeability resulting from structural characteristics as well as membrane-based efflux processes may induce poor distribution throughout the body or poor absorption across the gastrointestinal mucosa [3].

Through various *in vivo* and *in vitro* techniques, including the Using chamber, isolated epithelial cells, and the everted gut sac model, the mechanism of the intestinal absorption of chemical compounds has been researched for many years. Wilson and Wiseman developed the everted sac model in 1954 to investigate intestinal drug delivery. The model has since been developed, being used in the chemical and pharmaceutical industries for a variety of applications, including kinetic mechanism, absorption, metabolism, transport, and so forth [4]. In this technique, the intestinal sac of goat, sheep or rat is everted to expose the mucosal surface in suitable

condition to keep tissue viable. Then the test drug is introduced into mucosal fluid and absorption mechanism is studied and compared. By using this procedure, drugs with low absorption or bioavailability can be identified, and necessary adjustments can be made to improve absorption [5].

The most common application of aspirin is for the treatment of cardiovascular disease, while it is also used for many other purposes. Aspirin has, however, also been suggested as a treatment for a variety of ailments, including diabetes mellitus, cancer prevention, and obstetrics [6]. Since it is a weak acid, the stomach and upper intestinal tract absorb it. However, Aspirin's poor water solubility is what prevents greater absorption of aspirin [7, 8].

Orally administered aspirin requires high and frequent dosages, which is linked to an increased risk of GIT adverse effects since it undergoes substantial presystemic metabolism in the GIT and liver, converting it to salicylic acid [9]. Numerous studies to improve drug absorption have been conducted, and they have revealed the value of several plant extracts and products in doing so. The capacity of plant extracts or naturally occurring components from medicinal plants, such as saponins and flavonoids, to improve bioavailability has also been demonstrated [10-12].

The plant of Theaceae-family; *Camellia sinensis,* is the source of green tea. It is made from the fragile stems, leaves, or buds of Camellia plant species. Worldwide, tea has been consumed as a beverage since ancient times. In terms of antioxidant content and fermentation level, tea comes in three main varieties: green tea, oolong tea, and black tea. Black tea mostly has tannins, whereas green tea mostly has catechins. Notably, green tea is thought to be the main source of catechins. The main components of green tea are polyphenols (about 90%), amino acids (about 7%), theanine, proanthocyanidins, and caffeine (about 3%) [13,14].

Green tea has been included in the category of beverages with functional qualities as a result of growing interest, particularly in relation to its health advantages. Green tea's main ingredients, polyphenols, are what give it its potential pharmacokinetic qualities, antioxidant protection, and other health advantages. The polyphenols are the main components of interest. Flavonoids are the main polyphenols in green tea. Catechins epicatechin (EC), epigallocatechin (EGC), epicatechingallate (ECG), and epigallocatechin gallate (EGCG) are the four main flavonoids found in green tea. The most important active ingredient is thought to be epigallocatechin gallate [15-17]. Leaves and leaf buds contain the most EGCG. The typical number of total polyphenols in dried green tea leaves is between 8 and 12 percent. Gallic acid, quercetin, kaempferol, myricetin, caffeic acid, and chlorogenic acid are other chemicals found in dried green tea leaves that are worth investigating [18].

Green tea is a significant medicinal herb that exhibits positive properties and is helpful for conditions such as weight loss, stress reduction, cold and flu prevention, antiaging effects, hair loss, asthenia, HIV, immunity, food poisoning, cardiovascular diseases, liver diseases, arthritis, Alzheimer's disease, Parkinson's disease, glaucoma, cancer, diabetes, oral health, gargling effect, candidiasis prevention, antibacterial, antiviral, anti-allergic, and antibiotic synergism [19].

Therefore, the present study Aim to investigate effect of green tea extract on intestinal absorption of Aspirin using everted sac technique.

2. MATERIAL AND METHODS

2.1. Drugs and Chemicals

Aspirin and other chemicals like methanol, ferric chloride, calcium chloride, magnesium chloride, disodium hydrogen phosphate, sodium dihydrogen phosphate, sodium bicarbonate, potassium chloride, sodium chloride, glucose was purchased from Loba chemic Pvt Ltd., Mumbai. Analytical grade chemical and solvent was used where ever required.

2.2. Plant materials and extraction procedure

2.2.1. Defatting of tea powder

Leaves were collected from Botanical Garden of Seth Govind Raghunath Sable College of Pharmacy Saswad, Pune. Fifty g of coarsely powdered, air-dried green tea leaves were put in a conical glass flask covering with aluminium foil added enough petroleum ether, and then left to stand for more than two nights. Spot tests on common filter paper were done to certify that the defatting process had been completed. After that, the solvent from the defatted tea powder was filtered off and dried. The dried tea powder was kept in an airtight container till the subsequent extraction method was applied [17].

2.2.2. Soxhlet Extraction

Using 100 mL of ethanol in a conical flask as the solvent, 35g of defatted green tea powder was exposed to a Soxhlet extraction. Until the tea powder was entirely exhausted, the extraction procedure was conducted at a temperature between 60 and 70°C.

2.3. Determination of λ max of Aspirin

100 mg of aspirin was dissolved in 1000 ml of phosphate buffer solution to create a stock solution of 100 g/ml of aspirin (pH 7.4). 1 ml of the stock solution was mixed with 0.5 ml of the 0.0025M $FeCl_3$ solution to increase the buffer solution's volume to 10 ml. The solution's absorbance was screened between 400 and 800 mm using $FeCl_3$ solution as a blank. The X-axis and Y-axis of the graph were wavelength and absorbance, respectively. It was established that aspirin had a maximum absorptivity of λ max. Using the same method, it was also possible to calculate the maximum amount of aspirin that could be taken when green tea extract was present. λ Max of the Aspirin was found to be 530 nm while λ max of Aspirin plus green tea extract was found to be 536nm.

2.4. Calibration response curve of Aspirin

A stock solution of aspirin was dissolved in 1000 ml of buffer solution at a concentration of 100 g/ml, and this solution was used to generate the calibration response curve. A solution containing 0.5 ml of $FeCl_3$ was added to each of the aspirin stock solution aliquots of 1.0, 2.0, 3.0, 4.0, 5.0, and 6.0 ml before being taken individually in 10 ml volumetric flasks. Buffer solution was used to fill the capacity to 10 ml in order to generate working aspirin solutions with concentrations of 10, 20, 30, 40, 50, and 60 g/ml. A UV spectrophotometer, set to 536 nm, was used to gauge each solution's absorbance in comparison to a blank $FeCl_3$ solution. In order to generate a graph, concentration was plotted on the X-axis and absorbance on the Y-axis.

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 Calcium chloride (0.2928g), Magnesium chloride (0.5416g), Disodium hydrogen phosphate (0.7568g), Sodium dihydrogen phosphate (0.1384g), Sodium bicarbonate (4.6664g), Potassium chloride (0.8272g), Sodium chloride (14.712g), Glucose (1.9932g) and Distilled water required to produce 2000 ml.

2.5.2. Everted goat sac technique

The everted sac method was utilised in permeation studies on goat's small intestine. Animals used in experiments were fasted for 24 hours before being put to death and having their guts removed. Two segments of the transport buffer-preserved sacrificed goat intestine, each measuring roughly 15 cm, were found. The intestine's estimated diameter was 0.8 cm. The intestine was everted, knotted at one end, and linked to a cannula at the other end to form a pouch. The tissue was made alive while maintaining a temperature of 37 ± 0.5°C by providing oxygen using an aerator and a buffer solution. After the mucosal side has everted, the serosal side is present inside.

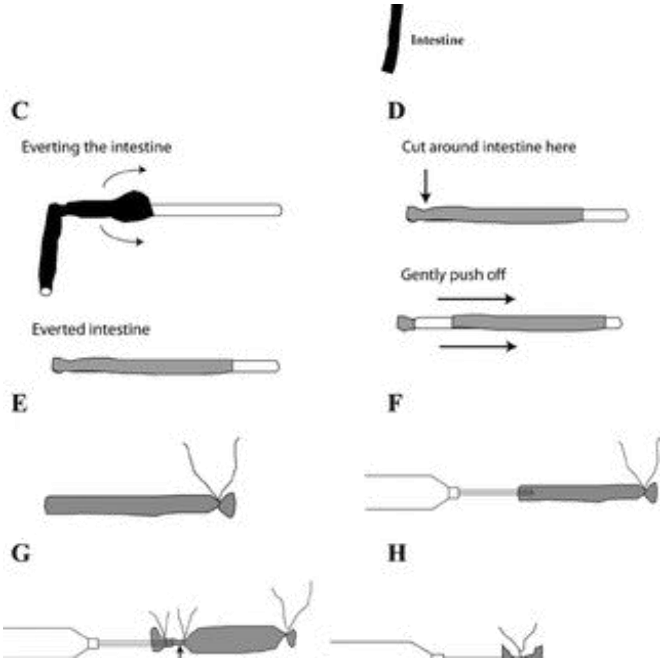

Fig. 1: preparation of everted sac [20]

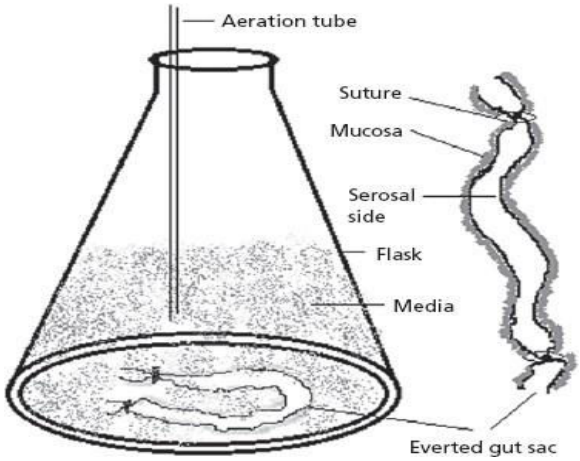

Fig. 2: Outline diagram of the everted gut sac model [21]

2.5.3. Preparation of Aspirin-buffer and Aspirin with green tea extract - buffer solution

The aspirin-buffer solution was made by dissolving 2 g of

aspirin in 2000 ml of buffer solution. A 1% w/v solution of green tea extract was added to 250 ml of buffer solution after 2 g of aspirin had been dissolved in it, bringing the combined volume of the buffer solution and aspirin to 2000 ml.

2.6. Experimental procedure

The buffer solution was used in two organ baths. The two different everted intestines were connected and put in the organ bath as previously mentioned. Consistent temperature and oxygen flow was maintained. The stirrer was placed for agitation in order to produce an effect resembling a peristaltic moment. 1.5 L of aspirin buffer solution was given to one organ bath, and 1.5 L of aspirin plus green tea extract buffer solution to the other. Placing plain buffer within the intestinal sac in a manner that the buffer solution contains both the medications present on the mucosal side of the exterior and plain buffer present on the interior (serosal side). Intestine samples (1 ml each) from the serosal side were taken five times at intervals of 15 min. The obtained samples were combined with 0.5 ml of $FeCl_3$ solution, and the volume was increased to 10 ml by adding phosphate buffer solution. Waited for 2/3 of a minute till the violet hue was observed. The sample was then examined at 530 nm using a blank solution of $FeCl_3$. Using a calibration curve, the amount of aspirin absorbed, was evaluated.

3. RESULT AND DISCUSSION

The λ max of aspirin at 530 nm was determined as 0.493 (Fig. 4). In the same study, aspirin had the λ max of 0.700 at the wavelength of 536nm when green tea extract was present (Fig. 3). 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. 4.

Green tea extract's effects on aspirin's intestinal absorption are shown in Table 1 and Table 2. Table 1 displays the concentration of aspirin absorbed without green tea extract, while Table 2 displays the concentration of aspirin absorbed with green tea extract. After a 75 minute test period, it was found that aspirin absorption was always boosted by the presence of green tea plant extract. Aspirin absorption was 14, 16, 17, 18 and 23μg/ml after 15, 30, 45, 60, and 75 min, respectively. Aspirin absorption was 18, 48, 66, 73, and 75μg/ml when green tea extract was present, respectively.

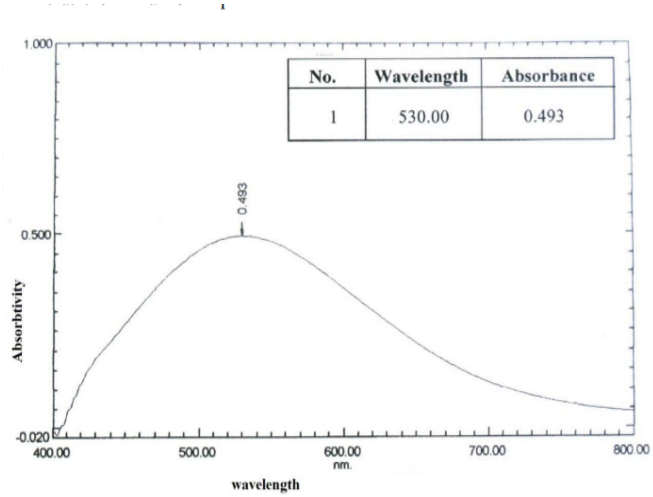

Fig. 3: Wavelength vs absorbance graph to determine the λ max of Aspirin

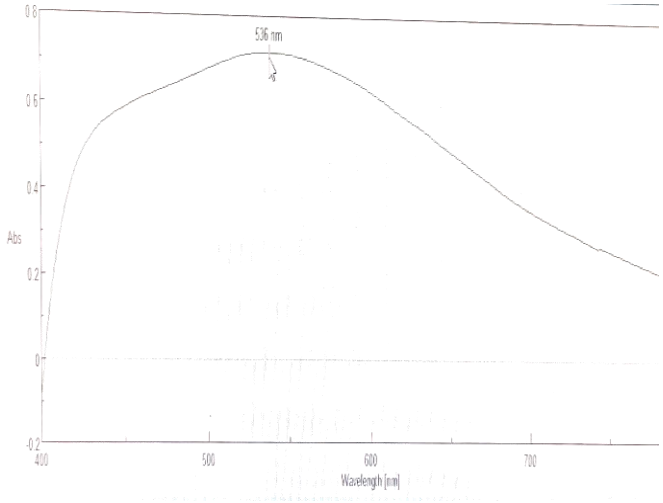

Fig. 4: λ max of Aspirin in presence of Green tea leaves extract

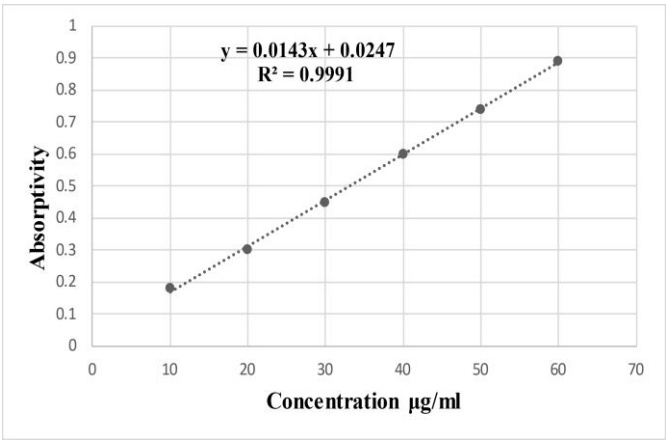

Fig. 5: Calibration response curve of Aspirin

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 green tea extract by Everted Sac technique

Time (min)	Absorbance	Concentration (µg/ml)
15	0.18	12
30	0.48	32
45	0.66	45
60	0.73	49
75	0.75	51

4. CONCLUSION

The findings of the present study showed that the polyphenol present in green tea extract improved the extract's capacity to promote absorption. This study may have provided guidance on how to boost bioavailability using medication combinations that are similar to those used in clinical practise.

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Conflict of Interest

All authors hereby declare that they do not have any financial, academic, commercial, personal, political, employment related, stock ownership related, honoraria or paid expert testimony related, patent related, grants or other funding related or any other conflict with any people, company, organizations or consultancies that could inappropriately influence (bias) our submission. The authors wish to confirm that there are no conflicts of interest associated with this publication. The authors report no declarations of interest.

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