IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

Pharmacological activity of Bottle Guard *(Lagenaria Siceraria)* Fruit Extract on Permeation of ARBs In Ussing Chamber Across Transepithelial Cell Membrane

[1]Pali Khobragade[*], [2]Vipin Sharma

[1, 2,] Shri Rawatpura Sarkar Institute of Pharmacy, Kumhari, Durg C.G 490042

Abstract

This research work investigate the effect of *Lagenaria siceraria* fruit extract (LSFJ) Ussing Chamber across transepithelial membrane to investigate ions transport the relationship between drug intake and a clinical response is highly complex. Deviations between drug responses within or between individuals may be ascribed product bioavailability, drug pharmacokinetics and particular concentration-effect relationship. Membrane integrity was evaluated in the terms of resistance. At the 0 milli volt current measured as a short circuit current. Regular samples were withdrawn in 15 minutes - 90 minutes. Current at 0 mV and at 10 mV was noted. After 90 min. Membrane was replaced by fresh membrane It has proved by this study that Ussing Chamber is a useful technique to evaluate permeation and interaction of other material on permeation. Sweet and bitter both types of Lagenaria have effects on permeation of model drugs (**Angiotensin II receptor blockers**) and variations occur with concentration, time and subject. There are some impressive results have come which will be later used to discover the applications of bitter *Lagenaria* and sweet *Lagenaria* in modifications of drug effects or we can say modification of pharmacodynamic and pharmacokinetic of drug materials.

Key word: Transepithelial Membrane, Bioavailability, Bottle Guard, Short-circuit current, ARBs

INTRODUCTION

During the past 20 years, a great deal has been published on the subject of drug absorption. Despite this, much is still unknown. [1] When faced with a discovery candidate with excellent intravenous activity yet low oral activity, absorption enhancers are often explored. However, their relatively frequent use in discovery does not imply acceptance for the marketed formulation. [2] Only a few countries (e.g. Japan and Sweden) have marketed products utilizing the absorption enhancer approach to drug delivery [3]. Yet enhancers may be useful as tools to increase development candidate's systemic exposure in toxicology studies, and/or to identify the rate-limiting 'barrier(s)' in oral absorption [4]. Drugs can be defined as agents that modify normal biological responses and thus produce pharmacological effects. These are frequently dependent on the transfer of drugs across one or more cellular membranes, whose structure and physicochemical properties govern the rate and extent of drug transfer [5]. A drug is only considered to be absorbed once it has entered the blood capillaries so "Absorption is defined as transport of unchanged drug from site of administration to the site of action". A fundamental premise associated with the use of a Therapeutic agent is that for any given patient, the Clinical response can be predicted on the basis of the selected drug product, dose, and dosing regimen. This tenet provides the foundation for concepts of prescribability and switch ability. [6] Prescribability refers to an assumed relationship between a therapeutic outcome and the rate and extent of drug exposure. [7] A physician will prescribe a particular product in accordance with assumptions pertaining to this relationship. Generally, the process of drug movement from intake (e.g. oral delivery systems) to its site of action can be schematically presented as follows (Figure 1). As depicted in Figure 1, the relationship between drug intake and a clinical response is highly complex, potentially affected by a host of intrinsic and extrinsic variables [8]. Accordingly, deviations between drug response within or between individuals may be ascribed either to product bioavailability (i.e., the rate and extent of drug absorption), drug pharmacokinetics (which includes the metabolism, distribution, and elimination of a compound), or the particular concentration-effect relationship [9]. A summary of the most important factors influencing the rate and extent of intestinal absorption is given below.

Physicochemical Factors	Physiological Factors	Formulation Factors	Biochemical Factors
Aqueous solubility,Molecular size and weight,Aggregation / complexation, Charge (pKa),H-bonding potential, Molecular surface area, Drug hydrophobicity, Crystal lattice energy	Stomach emptying tim, Intestinal ,uotility Membrane permeability,Intestinal pH, Disease state, Blood flow,Luminal, content and composition, (Including bile salts)	Dosage form Drug release Absorption enhancers	Metabolism Efflux transporters Active uptake transporters

Table 1: Overview of the main factors influencing the extent and rate of intestinal drug absorption

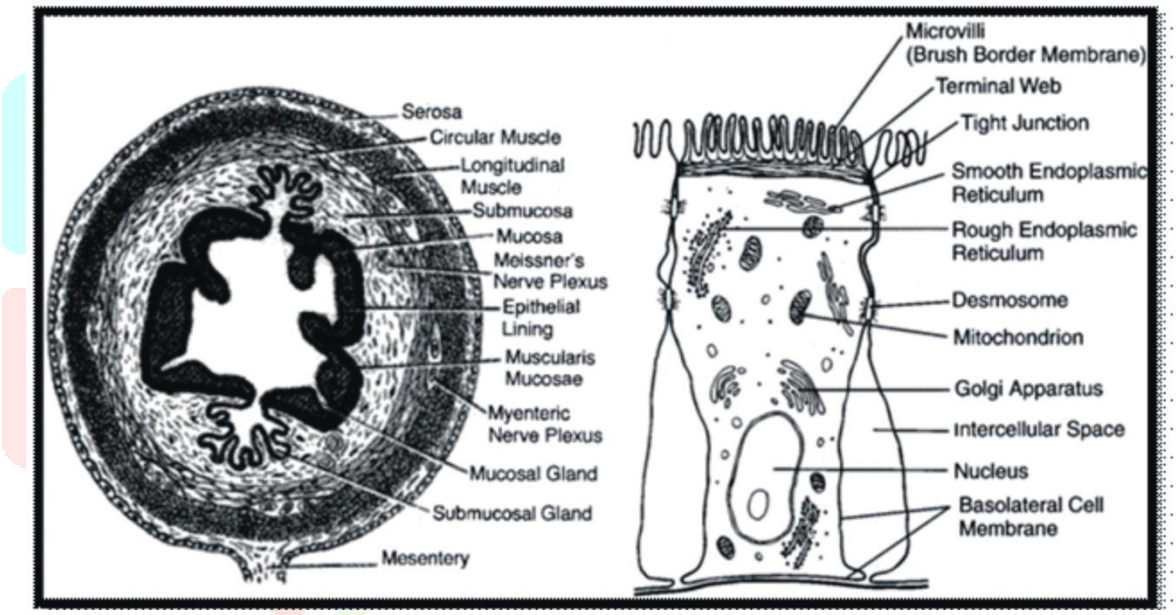


Fig. 1 (A) cross-sectional view of the small intestine. (B) Gastrointestinal (GI)

Volume of Ingested Material	As volume increases initially an increase then a Decrease. Bulky material tends to empty more slowly than liquids
Type of Meal	
Fatty food	Decrease
Carbohydrate	Decrease
Temperature	Food Increase in temperature, increase in emptying rate
Body Position	Lying on the left side decreases emptying rate. Standing *versus* lying (delayed)
Drugs	
Anticholinergics (e.g. atropine), Narcotic (e.g. morphine, alfentanil), Analgesic (e.g. aspirin)	Decrease
Metoclopramide, Domperidone, Erythromycin, Bethanchol	Increase

Table 2 Factors affecting gastric emptying [11]

Fig 2 *Lagenaria siceraria* plant & fruit

Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Cucurbitales
Family	Cucurbitaceae
Genus	Lagenaria
Species	L. siceraria

Table 3 Taxonomical Classification: [12, 13]

MATERIAL AND METHOD

Physiological salt solution (Tyrode's solution): Tyrode's solution is a solution that is roughly isotonic with interstitial fluid and used in physiological experiments and tissue culture. It resembles Lactated Ringer's solution, but contains magnesium, a sugar (usually glucose) as an energy source and uses bicarbonate and /or HEPES as a buffer instead of lactate. Some variations also include phosphate and sulfate ions. It is typically gassed with 95% Oxygen 5% Carbon Dioxide when used for cell culture applications and physiology

experiments. With the addition of extra potassium salt, it can be used to prepare a cardioplegic solution. Physiological sodium chloride solution, an aqueous solution of sodium chloride having an osmolarity similar to that of blood serum.

S.no.	Chemical	Quantity
1.	Sodium chloride	8 gm.
2.	Glucose	2 gm.
3.	Sodium bi-carbonate	1 gm.
4.	Potassium chloride	0.2 gm.
5.	Magnesium chloride	0.1 gm.
6.	Calcium chloride	0.2 gm.
7.	Sodium hydrogen phosphate	0.05 gm.
8.	Distilled water	Up to 1000 ml

Table 4 Composition of Tyrode's` Solution

a) Drugs

ARBs (**Angiotensin II receptor blockers**) Candesartan, Telmisartan,. Gift samples were procured from Reddy's Pharmaceutical Ltd. Hyderabad.

b) Animals

Cockerel intestine bought from the nearest slaughter house from freshly prepared cock/hen were used in tyrode's solution.

c) Equipment and Assembly

There are numerous things are used to perform the practical's which are following:

USSING CHAMBER

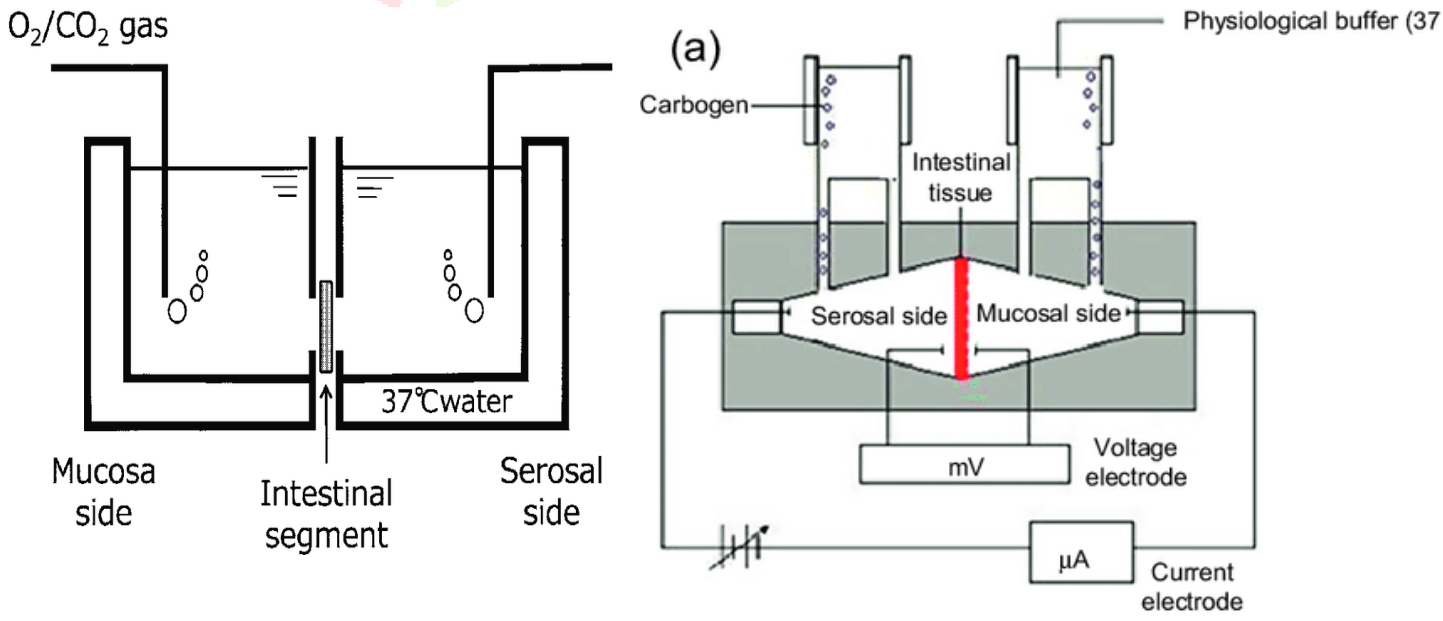


Fig 3 Schematic drawing of Ussing-chamber

It consist of the following-

Reservoirs: These are generally made of glass or plastic material, having capacity of 250 ml. with controlling valve for monitoring the flow rate of the solution go through the pipe to the chamber.

Chambers: These are the heart of the instrument and made up of good quality glass having bore size of 1 cm^2 area, this bore is used to mount the intestine between the chambers. A chamber have four tubes which are used as inlet and outlet, in which one is used for inlet for PSS, one for outlet for PSS, two for electrodes from ammeter & voltammeter.

Collecting reservoirs:

These are generally made up of borosilicate glass having 50 ml capacity used for collecting the sample from the instrument.

Electrical assembly

It consists of power pack, and multimeters.

Power pack

It is an instrument which generates electric current from 0-5 volts, and current 0-20 ampere.

Voltammeter

It is an instrument of capacity 200 mill volts to 20 volts which is used to measure the voltage generated by the intestine.

Ammeter

It is an instrument having capacity of 20 micro amperes to 20 amperes & used to measure the current.

METHOD

Cockerel intestine was bought from the slaughter house. 1cm jejunum segment was excised and placed in tyrode's solution (pH -7.4) in an atmosphere of a 95% O_2/5% CO_2 gas mixture. The epithelium was than exposed via longitudinal incision along the mesenterium, and underline muscle layer was removed. Finally the epithelium was mounted on to an using chamber having a tissue surface area of 0.196cm^2. Tyrode's solution was added to both the donor (mucosal) and receiver (serosal) chambers. After 30 minutes pre-incubation drug solution (dose/250ml) and tyrode's solution were added to the mucosal and serosal side respectively. After permeation study was started samples are withdrawn at 15 minute regular interval up to 90 minutes and samples are analyzed in U.V. spectroscopy at respective wavelength. In permeation study P_{app} from the mucosal to serosal and serosal to mucosal sides was basically evaluated. In this study experiments were conducted in two groups. The permeation study of drugs was conducted as plain drug and in combinations thereof. **Estimation of membrane integrity:** Membrane integrity was evaluated in the terms of resistance. At

the 0 milli volt current measured as a short circuit current measured in micro ampere, this current shows the permeation of ions from the epithelial barrier and at the 10 milli volt current is measured in micro amperes.

Data analysis: the apparent permeability coefficient per unit membrane surface area (P_{app} / cm^2sec) was calculated according to the following formula-

$$P_{app} = dM/dtn \text{ X } 1/AC_0$$

Here: dm/dt is the steady state appearance rate of drugs to the opposite side (opposite to drug side) in microgram/second. C_0 is the initial drug concentration on the opposite side (µg/ml). And A is the area of the membrane(cm^2).

EXPERIMENTAL WORK

Preparation of calibration curves

Tyrode's solution was prepared by using the given formula. Stock solution of 100µg/ml was prepared for drugs in tyrode's solution. Dilutions were prepared from 10-100 µg/ml. Absorbance of the prepared dilutions were analyzed under U.V. spectroscopy at the respective wavelength of each drug.

Permeability testing

A 2 cm. jejunum segment was excised and placed in tyrode's solution. Epithelium was then exposed via longitudinal incision along the mesenterium, and underline muscle layer was removed. The epithelium was mounted on to an using chamber. Carbogen tyrode solutions are allowed to flow at a rate of 1 ml/minute from either side of ussing chamber to stabilize the membrane. After stabilization of membrane for 30 min., one side of ussing chamber solution was replaced with drug solution (dose of drug / 250 ml.). Regular samples were withdrawn in 15 minute- 90 minute. Current at 0 mV and at 10 mV was noted. After 90 min. membrane was replaced by fresh membrane. Drug solution was supplied from serosal side and whole practical repeated as prior.

Transepithelial electrical resistance (TEER) Ohm/ cm^2

TEER was evaluated by taking reading of ammeter at 10 mV.

Short circuit current (SCC) µA/ cm^2

Short circuit current was measured by taking the reading of ammeter at 0 mV in voltammeter.

COLLECTION OF *Lagenaria Siceraria* FRUITS

The fresh fruits of *Lagenaria siceraria* (sweet & bitter both) were collected in the month of December to January from the local market & the wild region of Raipur (C.G.), India, and authenticated by the authority of the botanical survey of India (BSI), Tamilnadu Agricultural University, Coimbatore, Tamilnadu, India.

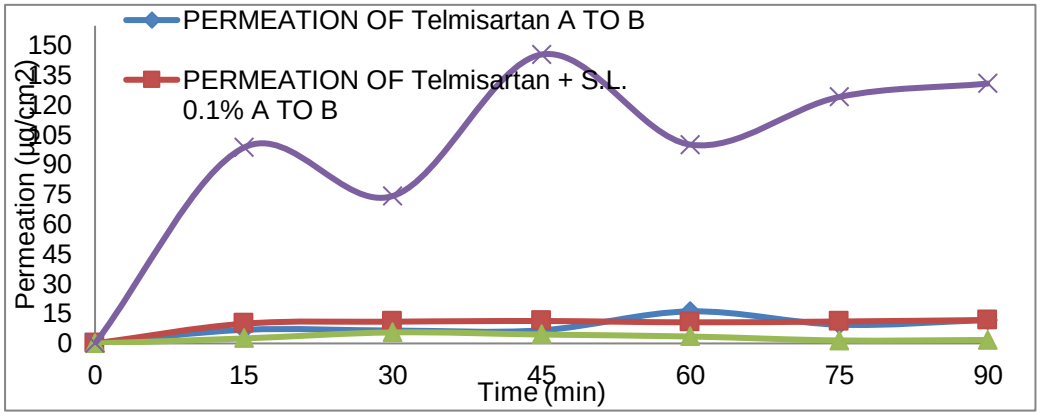
PREPARATION OF FRUIT EXTRACT

The fresh *Lagenaria siceraria* cut into pieces and dipped in ethanol over night. Then the pieces of *Lagenaria siceraria* (500g) were extracted with 90% ethanol using soxhlet apparatus. The solvent was evaporated under vacuum which yielded semisolid mass. The extracts were stored in tight containers in desiccators, until further use.

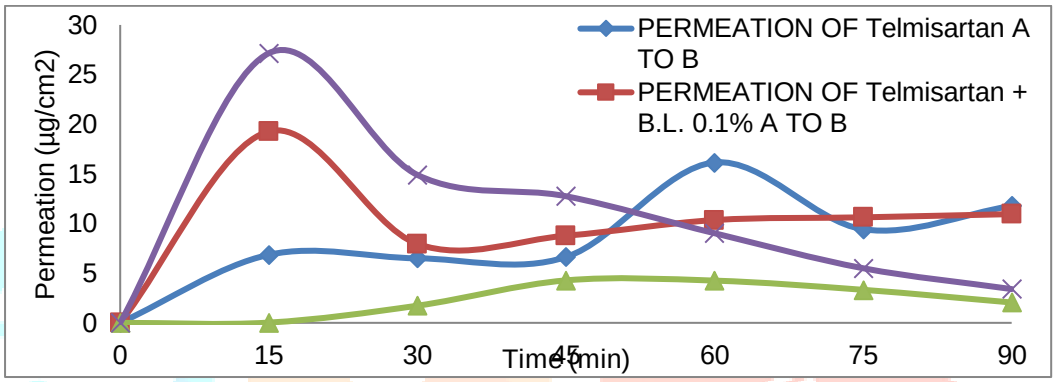
S/no.	DRUGS	A to B	B to A	A to B	B to A	P RATIO	AUC A - B	AUC B - A
1	TELMISARTAN	6.85	17.07	10.15	15.11	0.67	771.56	1236.18
2	CANDESARTAN	0.068	13.15	35.19	84.34	0.41	261.32	668.39

S/no.	DRUGS	A to B	B to A	A to B	B to A	P RATIO	AUC A - B	AUC B - A
1	**TELMISARTAN**	6.85	17.07	10.15	15.11	0.67	771.56	1236.18
2	TELMISARTAN + S.L. 0.1%	9.92	8.37	11.62	10.73	1.03	896.25	829.87
3	TELMISARTAN + S.L. 0.2%	2.5	5.2	3.41	4.12	0.82	276	336.37
4	TELMISARTAN + S.L. 1.0%	98.62	2541.38	119.31	1446	0.08	9120.93	118243.12
5	TELMISARTAN + B.L. 0.1%	19.27	14.4	12.03	12.08	0.95	936.75	934.12
6	TELMISARTAN + B.L. 0.2%	0.02	3.07	2.77	1.58	1.75	219.37	126.56
7	TELMISARTAN + B.L. 1.0%	27.15	25	12.88	14.68	0.87	1065.37	1180.8
8	**CANDESARTAN**	0.068	13.15	35.19	84.34	0.41	261.32	668.39
9	CANDESARTAN+S.L. 0.1%	2.94	1.41	27.02	10.99	7.13	215.06	90.42
10	CANDESARTAN+S.L. 0.2%	0.83	0.17	13.78	5.93	2.32	116.34	49.59
11	CANDESARTAN+S.L. 1.0%	69.28	196.87	649.69	1325.13	0.49	5058.98	10573.59
12	CANDESARTAN+B.L. 0.1%	0.35	0.012	6.91	3.06	2.25	54.32	25.54
13	CANDESARTAN+B.L. 0.2%	2.88	1.45	23.66	5.74	4.11	197.48	48.18
14	CANDESARTAN+B.L. 1.0%	0.71	2.19	2.54	7.31	0.34	19.45	61.87

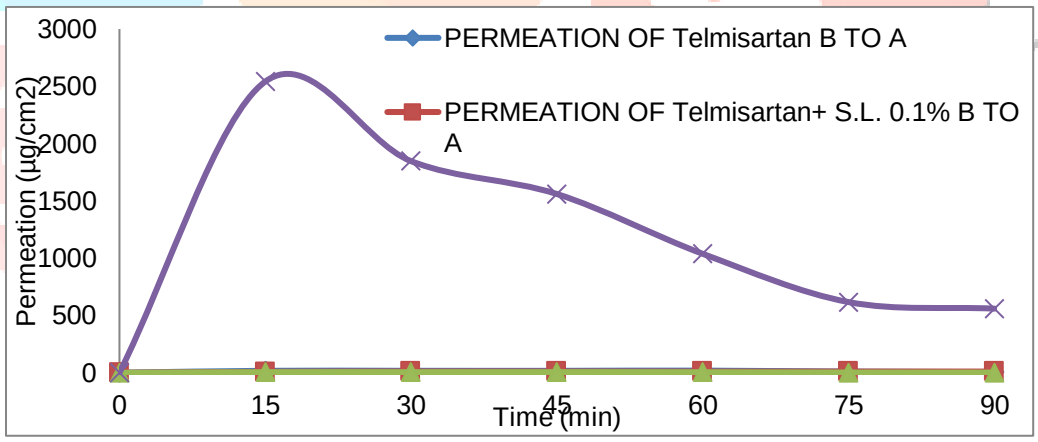
Table 5 Apparent permeability and AUC of both drugs with combinations



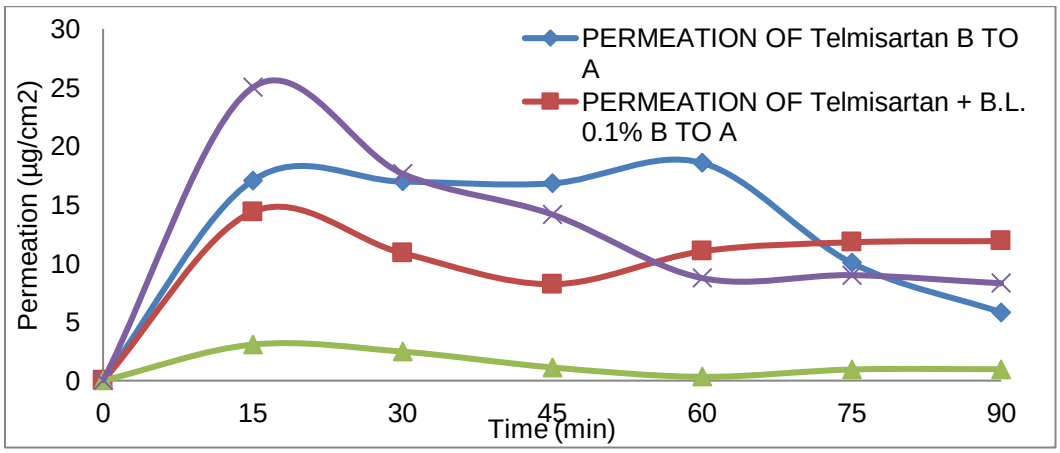
Permeation of Telmisartan with various % of *Lagenaria siceraria* (Sweet) A-B side



Permeation of Telmisartan with various % of *Lagenaria siceraria* (Bitter) A-B side



Permeation of Telmisartan with various % of *Lagenaria siceraria* (Sweet) B-A side



Permeation of Telmisartan with various % of *Lagenaria siceraria* (Bitter) B-A side

SUMMARY AND CONCLUSION

This study is based on utility of Ussing Chamber experiment in the permeation study and their application to evaluate the effects of Bitter and Sweet *Lagenaria* on permeation of the selected model drug. *Lagenaria* commonly known as bottle guard and used as daily consumed food material and bitter *Lagenaria* commonly found in wild areas and assumed as toxic to eat. So effects of them on permeation of model drugs were evaluated in this research work. It has proved by this study that Ussing Chamber is a useful technique to evaluate permeation and interaction of other material on permeation. Sweet and bitter both types of Lagenaria have effects on permeation of model drugs and variations occur with concentration, time and subject. There are some impressive results have come which will be later used to discover the applications of bitter *Lagenaria* and sweet *Lagenaria* in modifications of drug effects or we can say modification of pharmacodynamic and pharmacokinetic of drug materials.

REFERENCE

1. Edward L. Le Cluyse", Steven C. Suttonb "In vitro models for selection of development candidates. Permeability studiesto define mechanisms of absorption enhancement" *Advanced Drug Delivery Reviews* 23 (1997) 163-183.
2. BLUK100-Calvey November 27, 2007, "Drug Absorption, Distributionand Elimination".pg: 1-22
3. PHAR 7633 Chapter 11 "Physiological Factors Affecting Oral Absorption", http://www.boomer.org/c/p4/c11/c11.pdf.
4. Rakesh Tiwle "An Exhaustive Review On Solubility Enhancement For Hydrophobic Compounds By Possible Applications Of Novel Techniques." Science Alert –Trends Research In Applied Science And Research. 7(8): 596-619; 2012
5. Mayersohn M (1996) Principles of drug absorption. In: Banker GS, Rhodes CT (Eds) Modern Pharmaceutics, 3rd ed., Marcel Dekker, New York, pp 21–74.
6. Fiese EF (2003) General pharmaceutics—The new physical pharmacy. *J Pharm Sci* 92:1331–1342.
7. Balimane PV, Chong S, Morrison RA (2000) Current methodologies used for evaluation of intestinal permeability and absorption. *J PharmacolToxicol Methods* 44:301–312.
8. Stipanuk MH (2000) Digestion and absorption of macronutrients. In: Stipanuk MH (Ed) Biochemical and Physiological Aspects of Human Nutrition. WB Saunders Company, Philadelphia, pp 75–152.
9. Rakesh Tiwle, D.K. Sanghi, Phase Solubility Study of Hydrophobic Drug Domperidone Using A Novel Technique Inclusion Complex With β-Cyclodextrine. International Journal of Pharmaceutical Research and Analysis. Vol 4 / Issue 2 / 2014 / XX-XX.
10. Pade V, Stavchansky S (1997) Estimation of the relative contribution of the transcellular and paracellular pathway to the transport of passively absorbed drugs in the Caco-2 cell culture model. *Pharm Res* 14:1210–1215.
11. Artursson P, Ungell AL, Löfroth JE (1993) Selective paracellular permeability in two models of intestinal absorption: cultured monolayers of human intestinal epithelial cells and rat intestinal segments. *Pharm Res* 10:1123–1129.
12. Artursson P, Palm K, Luthman K (2001) Caco-2 monolayers in experimental and theoretical predictions of drug transport. *Adv Drug Deliv Rev* 46:27–43.
13. Tukker JJ (2002) Characterization of transport over epithelial barriers. In: Lehr CM (Ed) Cell Culture Models of Biological Barriers. In-vitro Test Systems for Drug Absorption and Delivery. Taylor and Francis, London, pp 52–61.