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Effect of *Piper longum* Linn on the oral bioavailability of Phenytoin

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Abstract
Background: Bioavailability is the part of an orally administered dose which reaches in the systemic circulation after metabolism. Ayurveda Indian system of medicine uses herbal combination *Trikatu* in most of the formulation. *Piper longum* Linn is an ingredient in the *Trikatu* as considered as a bioavailability enhancer. The antiepileptic drug, Phenytoin is having a narrow therapeutic range with side effects. By incorporating *P.longum* with Phenytoin, the absorption of Phenytoin was studied.
Objectives: To study the oral bioavailabilityof *P.longum* Linn with Phenytoin.
Materials and Methods: In the control group of six rats, 0.5 ml of suspension containing 10 mg Phenytoin was given for 5 days orally. In an experimental group of six rats, 0.5 ml of suspension containing 10 mg Phenytoin was given along with 200 mg powder of *P.longum* Linn for 5 days orally. On the 6th day, the blood sample was collected by using capillary tubes by retro-orbital puncture. HPLC was performed to assess the Phenytoin concentration in the blood serum in both groups.
Results: Estimation of Phenytoin in blood serum, the illustrated area under the curve was more in rats in which *P.longum* Linn was given along with Phenytoin as compared to rats in which Phenytoin was given alone.
Conclusion: The study demonstrates that *P.longum* Linn may be increasing the absorption of Phenytoin. This study provides the insight for possibility of decreasing the dose of Phenytoin, if administered with *P. longum* Linn.

Keywords: *P. longum* linn, phenytoin, oral bioavailability

1. Introduction
Bioavailability can be determined as the quantity of the drug that reaches in the systemic circulation. "The term bioavailability is used to indicate the fraction of an orally administered dose that reaches the systemic circulation as an intact drug, taking an account absorption & local metabolite degradation" [1].

Bioavailability of orally administered drug is the ratio of the calculated area under curve against area calculated for intravenous administration of the particular drug. When the drug is administered through oral route only some extent of the drug appears in plasma. By drawing the plasma concentration of the drug against time, the bioavailability can be determined. The representation is done by drawing the curve, known as area under the curve (AUC) [2].

Oral Bioavailability depends upon properties of the drug, excipients added in the formulation, which is responsible for the dissolution of the drug in the intestine, decomposition of the drug, pH of the drug and its compatibility, Drug passes through first pass mechanism, which metabolizes the drug in some proportion. Hence drug entering in the systemic circulation will be less. A bioenhancer when combined with the particular drug is capable of increasing the bioavailability and bioefficacy of the drug [3]. The term bioavailability enhancer was first used by Indian scientists at the Regional Research laboratory, Jammu (RRL, now known as Indian Institute of Integrative Medicine). In 1979, scientific authentification of Piperine as the bioavailability enhancer was done [4]. The action of bio-enhancers was first mentioned by Bose in 1929. Use of *P. longum* Linn along with *Adhatoda vasika* leaves, increased the antiasthmatic activity, was documented by Bose [5]. Piperine was discovered by Hans Christian Orsted, in 1819. He had separated & isolated it from the fruits of Piper nigrum [6]. Candila Phrama Launched the formulation named Risorine which contains 200 mg rifampicin and 300 mg isoniazid. In this combination 10 mg Piperine was added. By the addition piperine in the formulation bioavailability of rifampicin was increased by 60%. The dose of rifampicin was decreased so the extra load of the drug on the body was reduced Piperine when added with various drugs give synergestic effect thus by reduction in the dose by means of which side effects can be reduced. As, Piperine can influence drug levels of a number of drugs, care should be taken when using with other drugs whose levels are influenced by it. It can potentiate the efficacy of drugs hence, dose reduction is required to prevent toxicity [7].

In Indian Traditional system Ayurveda, most of the formulations contain the combination of *Piper longum* Linn, *Piper nigrum* Linn & *Zinziber officinale* jointly known as *Trikatu*. C.K. Atal, the Director of the Regional Research laboratory, Jammu, concluded that *Trikatu* increases the efficacy of formulations. On this basis, a research team led by Usha Zutshi studied these ingredients and found that Piperine increased the bioavailability of many drugs [8].

Phenytoin is the drug used in Epilepsy. Epilepsy usually begins in childhood results in problem in education, maintenance of social relationships, services and the development of a sense of socially lacking [9]. Epilepsy is among the disorders that are associated with psychological

and social problems [10]. However, it was observed that with the help of antiepileptic drugs there is a better control of epileptic patients under seizure. Carbamazepine, ethosuximide, phenobarbital, phenytoin, and valproate are commonly used conventional antiepileptic drugs. Phenytoin has a good antiepileptic effect, but when given for long duration untoward effects are observed. Phenytoin causes neurotic & pschychological changes. Biochemical alteration is also found in the patients. Many experts avoid the long-term use of Phenytoin because of its dangerous side effects [11]. It was also noted that the bioavailability of all drugs will not increase when given with Piperine [12].

Piperine is constituent of *P.longum* Linn & *P.nigrum* linn. Both the pipers has been used as a food condiment. Piperine present in the drug can interfere with the levels of drugs in the plasma. It can potentiate the drug level and efficacy. Thus, monitoring the drug levels in the plasma is important related to drugs which are having narrow therapeutic range like phenytion. Hence the study was planned with the vision to assess the effect of food condiment *P. longum* Linn when given with phenytoin.

2. Materials & Methods

2.1 Plant material collection & identification

Mature fruits of *P. longum* Linn (Fig. 1A) were collected from the medicinal plant garden (*Bhavamishra Vatika*) situated at Mahatma Gandhi Ayurved College, Hospital & Research centre, Sawangi (M), Wardha, Maharashtra state, India. The identification & authentification was done by Taxonomist by the department of Bio & Herbal division, Mahatma Gandhi Institute of Rural Industrialization, Wardha. The voucher specimen (No. 13 /DG/MGACH&RC) was kept in *Dravyaguna* department of Mahatma Gandhi Ayurved College, Wardha, India.

2.2. Method of preparation of churna

The collected fruits of *P. longum* Linn were shade dried and after proper drying were subjected to prepare powder. The pulverization of fruits was done at Dattatrya Ayurved Rasashala, Salod (H), Wardha, Maharashtra, India. While preparation of powder the standard operating procedure mentioned in API was followed [13].

2.3. Animals

This was an open study conducted on healthy wistar rats with protocol approval No. DMIMSDU/IAEC/2016-17/12.The "resource equation" was applied for sample size calculation [14]. Twelve healthy wistar rats of either sex of body weight between 200-250 Gms were included in the study. The wistar rats with weight less than 200gms & more than 250gms were excluded from the study. The animal study was conducted at Animal House, JNMC, Sawangi (M), Wardha situated in Maharashtra. Before one week, the rats were maintained under the room temperature (25°C ± 2°C) and humidity was (60% ± 5%). For feeding standard diet and distilled water were used. Before experiments, the rats were kept on fasting overnight.

2.4. Bioavailability study

Both the test drug and standard drug are available in oral dosage form, the oral route was chosen. Phenytoin sodium suspension containing 10 mg/ ml was purchased from the medical shop situated in the campus of Acharya Vinoba Bhave Rural Hospital, Sawangi (M), Wardha, Maharashtra, India. For HPLC, Acetonytrile & Methanol (HPLC grade) was purchased from the market of Merck Company. HPLC grade water was purchased from the market, which is of Milli-Q-Academic (Millipore, USA).

In the standard group consisting of six wistar rats 0.5 ml of suspension containing 10 mg Phenytoin was given to the rats for 5 days per oral. In an experimental group consisting of six wistar rats 0.5 ml of suspension containing 10 mg Phenytoin was given along with 200 mg powder of *P.longum* Linn for 5 days. The dose of *P.longum* Linn was calculated as per Burner's conversion factor 1964, human dose * 0.018 for 200gm of rats [15]. On the 6th day, the blood sample was collected by using capillary tubes. The blood was collected by retro-orbital puncture. The blood was collected into a heparinized tube and centrifuged at 1300 g for 10 minutes to collect the serum. The analysis was done by high-performance liquid chromatography (HPLC) procedures.

2.5. Assessment of Bioavailability in animals

Blood samples were estimated for the level of Phenytoin by high-performance liquid chromatography (HPLC) at Central Research Laboratory by using the standard protocol, at Jawaharlal Nehru Medical College, Sawangi (M), Wardha. Estimation of Phenytoin was done from the serum of animals by reverse-phase high-performance liquid chromatography. 100 ul of serum which was collected by centrifuging was mixed with 200 ul, of acetonitrile, and subjected for analysis. The calibration curve was plotted against blank serum of known quantities of Phenytoin. The serum samples of wistar rats, in which the only Phenytoin was given & the samples in which Phenytoin with *P.Longum* Linn powder was given, were run for 10 minutes. The peaks were detected for quantification at 255 nm.

2.6. Statistical analysis

The serum Phenytoin level was presented as mean & standard error. Statistical analysis was done by using unpaired t-test. The SPSS 17.0 version software was used and $p<0.05$ was considered as the level of significance.

3. Results

3.1. Serum estimation of Phenytoin

The results of the study were made based on graphical representation from HPLC. The analysis was done by high-performance liquid chromatography (HPLC) procedures. The graphical representation shows that the rats in which *P.longum* Linn was given along with Phenytoin show maximum peak height of Phenytoin, as compared to rats in which the only Phenytoin was given. The result is depicted in the chromatogram of the test group & controlled group (Fig.2A) The level of serum Phenytoin concentration was compared in the test group & the controlled group shows the average difference of 0.982µgm /ml. In the test group, the level of Phenytoin on average was 1.363 µgm. However, in the controlled group, the level of Phenytoin on average was 0.381 µgm. The values are depicted in table 1. Statistical analysis was done by using unpaired t-test. The SPSS 17.0 version software was used and $p<0.05$ is considered as the level of significance. The statistical analysis shows a significant concentration of serum phenytoin in the test group ($p<0.05$). The analytical depiction is given in table 2.

Discussion

Serum concentration of therapeutic drugs is affected due to herbal supplements. This mechanism may vary with each herb & drug. Herbal products directly or indirectly interfere by alteration in the pharmacokinetics of co-administered drugs [16]. Piperine is a major constituent of *P. longum* Linn & *Piper nigrum* which is responsible for bio enhancing effect [17]. When piperine was given along with nimesulide showed synergistic effect [18]. The serum levels of some nutritional substances, such as coenzyme Q10 and beta-carotene were increased when given with Piperine. Many drugs under the categories of cardiovascular, respiratory, CNS, GIT antibiotics, and anticancer shown bioenhancement effect [19]. Some drugs belonging to the categories of antibiotics and anti-cancer showed bio enhancement due to addition of piperine. With the addition of piperine, the synergism effect was found in antitubercular drug, anti-epileptic drug & several other classes of drugs [20]. For bio enhancing effect of piperine some mode has been given. Increases the gastrointestinal absorption, flow of drugs will be reduced from site of action, inhibition of intracellular penetration, reduction in the metabolism of the drug are some possibilities explored regarding bioavailability of Piperine [21].

Considering the bioavailability enhancing effect of Piperine, which is the constituent of *P. longum* Linn, this study was planned to assess the effect of *P. longum* Linn in augmentation of bioavailability of Phenytoin. The antiepileptic drug Phenytoin is recommended for a longer duration to control epileptic seizers and ultimately results in another side effect [22]. It is needed that the dose should be reduced. The synergistic effect of herb along with Phenytoin can promote the biological activity of Phenytoin, thus can results in the development of evidence of bioavailability of *P.longum* Linn when given with Phenytoin. The increased concentration of Phenytoin in serum might be due to various facts. *P.longum* Linn might be responsible for increase in absorption of Phenytoin. May be accountable for decreasing the metabolism of Phenytoin. There may be the possibility of increase in free plasma concentration of Phenytoin due to *P. longum* Linn [23, 24].

Conclusion

The present study demonstrates that the *churna* formulation of *P. longum* Linn has a significant role in increasing the concentration of Phenytoin in serum. These findings need further investigations to access its importance in epileptic patients undergoing Phenytoin. Thus it can be stated that *P.longum* Linn combined with Phenytoin promotes the biological activity of Phenytoin, which may result in the reduction of the dose of the target drug. It can be stated that employing bioavailability enhancer effect of *P. Longum* Linn with Phenytoin therapeutic efficacy can be achieved in comparative lesser doses. This study can open a new avenue in drug development. More studies are needed to validate the fact.

Fig 1: A Mature fruits of *Piper longum* Linn

Table 1: Level of serum Phenytoin in controlled & experimental group

Animal No.	Level of serum Phenytoin in controlled group	Animal No.	Level of serum Phenytoin in experimental group
1	0.66µgm/ml	1	1.02µgm/ml
2	0.519µgm/ml	2	1.5µgm/ml
3	0.193µgm/ml	3	1.06µgm/ml
4	0.419µgm/ml	4	1.5µgm/ml
5	0.29µgm/ml	5	1.9µgm/ml
6	0.207µgm/ml	6	1.2µgm/ml
Average	0.381µgm/ml	Average	1.363µgm/ml

Table 2: Statistical analysis in controlled & experimental group

Groups	N	Mean	SD	Standard error mean	t value	P (significance)
Controlled	6	0.381	0.186	0.076	5.033	.004
Experimental	6	1.363	0.335	0.137		

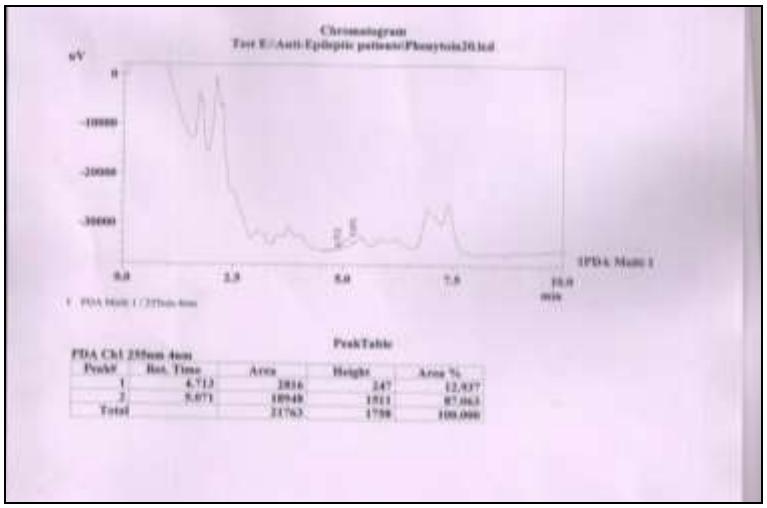

Fig 2: A Chromatogram showing Phenytoin absorption in controlled & experimental group

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