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ORIGINAL RESEARCH ARTICLE

A toxicity study of *Pippali* (*Piper Longum Linn*.) Fruits

Megha Pathak[1]*, Hitesh Vyas[2], Mahesh Vyas[3] and B. Ravishanker[4]

[1]M.D. (Ayu), Consulting Ayurved Physician, Pune (M H), India
[2]Asst. Prof. Department of Basic Principles, IPGT & RA, Jamnagar, Gujarat, India
[3]Associate Professor, Department of Basic Principles, IPGT & RA, Jamnagar, Gujarat, India
[4]Ex. Head, Department of Pharmacology, IPGT & RA, Jamnagar, Gujarat, India

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ABSTRACT

Pippali(*Piper longum* Linn.) fruits, one of the important drugs of Ayurvedic pharmacopoeia has been used in approximately 324 compound formulation, as one of the ingredient. It is also widely used as spices or as a food ingredient in Indian kitchen. In spite of its frequent use, the classical texts of Ayurveda advocates not to use this drug in higher dose and also for a long time period. In order to assess the safety of the drug, an acute and sub-chronic toxicity studies of *Pippali* fruit was carried out on Wistar strain albino rats. It was observed that at the dose of 3750mg/Kg, at five times higher dose than therapeutic equivalent dose, there was no mortality in acute toxicity study. And in sub-chronic toxicity study even at three time higher dose than therapeutic equivalent dose i.e 2250mg/kg, no serious toxicity was observed on different parameters studied.

Key words: *Pippali*, Acute toxicity, Sub-Chronic Toxicity.

INTRODUCTION

Pippali (*Piper longum* Linn.) fruits, one of the important drugs of Ayurvedic pharmacopoeia, has been used as the ingredient of many compound formulations. It is also widely used as spices or as a food ingredient in Indian kitchen. In spite of its frequent use, the classical texts of Ayurveda advocates not to use this drug in higher dose and also for a long time period [2]. From the present literature review it revelled that *Pippali* fruits, in its powder form, has not been evaluated for its toxic effect when consumed in higher dose. Hence, in the present study an attempt has been made to assess the possible adverse effects if any by carrying out acute and sub acute toxicity study on albino rats.

MATERIALS AND METHODS

Animals:

Wistar strain albino rats of either sex weighing between 160 – 220g body weights were used for experimental study. The animals were obtained from the animal house attached to Pharmacology laboratory, I.P.G.T & R.A., Gujarat Ayurved University, Jamnagar. The animals were exposed to natural day and night cycles under ideal laboratory conditions in term of ambient temperature (22 ± 2^0C) and humidity (50-60%). They were fed with Amrut brand rat pellet feed supplied by Pranav Agro Industries and tap water given ad libitum.

The experiment was carried out in accordance with the direction of the Institutional animal ethics committee (IAEC) after obtaining its permission (Approval number IAEC; 09-10/05MD 07).

Test drug:

Fruit of *Pippali* (*Piper Longum* Linn.) after proper identification by pharmacognosist, were passed through mess no 60 with the help of a mechanical grinder.

Dose fixation and schedule:

The dose calculation was done on the basis of body surface area ratio using the table of Paget and Barnes [3].

Acute toxicity study:[3]

In acute toxicity study the drug was given in five dose levels, i.e. TED (Therapeutic equivalent dose) – 750mg/kg, TED×2, TED×3, TED×4 and TED×5 i.e. up to 3750mg/kg for higher dose level. This is to confirm with the concept of the dose limit test as suggested by OECD guidelines[4]. Twenty four animals were taken in 6 divided

***Corresponding Author:** Dr. Megha Pathak**, Email:** drmeghapathak@gmail.com

groups and each group comprising 4 animals (**Table 1**).

Table 1: Grouping for acute toxicity study

Group No.	Group	No. of animals	Dose (mg/kg)
1	Control (water)	4	Q.S.
2	TED (750 mg/kg)	4	750
3	TED× 2 (1500mg/kg)	4	1500
4	TED× 3 (2250 mg/kg)	4	2250
5	TED× 4 (3000 mg/kg)	4	3000
6	TED× 5 (3750 mg/kg)	4	3750

Frequency and duration of dose – once only

The test formulation was suspended in distilled water and administered orally with help of suitable of gastric catheter sleeved on to a syringe.

Sub-Chronic toxicity study:

It refers to harmful effects of long term exposure to test drug. In this study eighteen animals were taken and divided in to three groups, each group comprising 6 animals (3 males and 3 females). Body weight and behavioural pattern, food consumption pattern and water intake of each animal from all the group was recorded before starting drug administration (Pre-acclimatization phase) (**Table 2**).

Table 2: Distribution of animals and dose during sub-chronic toxicity study

S. No	Group	No. of animals	Dose (mg/kg)
1	TED (Group A)	6	750
2	TED× 3 (Group B)	6	2250
3	Control (vehicle) (Group C)	6	Q.S.

The drug was dissolved in tap water and administered orally once daily for 45 consecutive days through gastric catheter sleeved to syringe. Body weight and food consumption patterns were observed weekly. On the 46th day the animals were weighed again and sacrificed by over dose of ether anaesthesia. Blood was collected immediately by jugular vein puncturing in two different types of ampoules, one containing dilution fluid for cell counter and other in plain bulb for biochemical investigations. Further the rats were dissected and organs were separated and kept in normal saline (0.09%) carefully. All the organs were weighed with a monoplane balance and transferred immediately to a glass bottle containing 10% formalin. These samples were sent to the laboratory to carry out histopathological studies.

Parameters studied:

1. Ponderal changes: Body weight at every 8 days, weight of important organs like Thymus, Heart, Liver, Spleen, Kidney, Testis, and Uterus were recorded.
2. Effect on food intake: Quantity of food intake was recorded weekly.
3. Haematological parameters: R.B.C count, W.B.C count, Lymphocyte count, Granulocytes count, Eosinophil count, Monocyte count, and Hemoglobin were recorded.
4. Biochemical Parameters: Blood sugar, S. total cholesterol, blood urea, S. creatinine, S. triglyceride, , S. Alkaline phosphates, S.G.O.T, S.G.P.T, Total protein, S.bilirubin, S. albumin, S. globulin and A/G ratio.
5. Autopsy: Examination of the viscera and general animal profile at the time of the sacrifice (involving external examination of the animal's - different parts like head, body, limbs, nature of discharges from natural orifices like mouth, nose, vagina, anus, penis etc. Internal examination of buccal cavity; abdominal cavity and its organs, pelvic cavity with organs)
6. Histopathological studies: Histopathology of important organs like brain, spleen, thymus, lymph node, heart, lungs, liver, intestine, kidney, testis, uterus and ovary has been carried out.

Statistical analysis:

The data generated during the study were subjected to student's 't' test for unpaired data to assess the statistical significance. A 'P' value less than 0.05 is considered as statistically significant.

RESULTS AND DISCUSSION

Acute toxicity study:

No mortality and behavioural changes were observed when the drug *Pippali* was studied after a single administration up to five dose levels with 3750mg/kg as the maximum dose.

In this study, the animals in both the test drug groups did not manifest any signs of toxicity up to 5 times (3750mg/ kg) human therapeutic equivalent dose. No exitus (death) was observed so no figure for median lethal dose (LD_{50}) could be provided. No significant gross change in behaviour could be observed; so, in the light of the above observations it can be interpreted that *Pippali* is considered to be a safe drug for acute administration in multiples of the indicated therapeutic dose. At the therapeutic dose level the test drug is unlikely to produce any acute drastic toxic effects.

Sub-Chronic toxicity study:

No behavioural changes (including cage side behaviour) were observed in both the treated groups. No mortality was observed in any of the group viz. TED and TED × 3.

In this study *Pippali* was administered at two dose level, viz., at therapeutic dose (750mg/kg) and

three times the equivalent to human therapeutic dose (2250 mg/kg).

Key to the abbreviations used in all the tables:

TED- *Pippali* administered at Therapeutic Equivalent Dose i.e. 750 mg/kg

TED × 3- *Pippali* administered at three times higher than TED

NSI: Non-significant increase **SI:** Significant increase

NSD: Non-significant decrease **SD:** Significant decrease

NSE: No significant Effect

Table 3: Consolidated statements depicting the activity profile of test preparations on body weight and weight of different organs

S. No	Parameter	TED	TED × 3
1	Body weight	NSI	NSI
2	Weight of Thymus	NSI	NSE
3	Weight of Heart	NSD	NSD
4	Weight of Liver	NSI	NSI
5	Weight of Kidney	NSD	NSD
6	Weight of Spleen	NSI	NSI
7	Weight of Testes	NSD	NSD
8	Weight of Uterus	NSD	NSD

Analysis of the ponderal changes indicate that out of the 9 parameters recorded that is weight of eight organs and body weight no significant changes were observed with respect to any organ in both the group.

As the observed changes were not up to statistically significant level and also do not have corroborative support in the histopathological study it can be suggested that the test drug at the dose level studied has no significant on ponderal parameters studied indicating it has no organ degeneration producing potential.

Table 4: Consolidated statements related to the effect of test preparations on important biochemical parameter in albino rats

S. No	Parameter	TED	TED × 3
1	Blood sugar	NSI	NSI
2	Blood urea	NSI	NSI
3	S. creatinine	NSI	NSI
4	S.G.O.T	NSD	NSD
5	S.G.P.T.	NSI	NSD
6	T. protein	NSI	NSI
7	Albumin	NSI	NSI
8	Globulin	NSI	NSD
9	A:G ratio	NSI	NSI
10	Alkaline Phosphatase	NSD	NSD
11	S. Bilirubin (T)	NSE	NSI
12	Uric Acid	NSD	NSD

Total thirteen parameters were studied. Test drug at both the dose level did not affect the any of the biochemical parameters studied to significant extent after 45 days of administration. This again clearly indicates that long duration of administration of *Pippali* at higher doses do not physiological activity disruption potential.

Table 5: Consolidated statements related to the effect of test preparations on different hematological parameters in albino rats

S. No	Parameter	TED	TED × 3
1	WBC count	NSI	SI
2	Neutrophils (%)	NSD	NSI
3	Lymphocytes (%)	NSI	NSD
4	Eosinophils (%)	NSD	NSD
5	Monocytes (%)	NSD	NSE
6	RBC count	NSI	NSI
7	Hb (g%)	SI	SI
8	PCV (%)	NSI	NSI
9	MCV	NSI	SI
10	MCH	NSI	SI
12	MCHC	SI	NSI
13	Platelet count	NSI	NSI

WBC- related parameters:

Five parameters were studied. Only one change that too of moderate intensity was observed at TED × 03 dose levels, is total WBC count. It may be due to mobilization of neutrophil. Since it is not remarkable and may be due to mild to moderate inflammatory changes observed in the jejunum it can be considered to have no significant toxicity concern.

RBC- related parameters:

Test drug did not affect the total RBC count level to significant level at both the dose level, where as significant increase in haemoglobin content was observed at both the dose levels. There was increase in MCV and MCH this may be due to increased Hb level. Since there was no corresponding increase in RBC count it is difficult to suggest the exact mechanism underlying this observation. However, what is certain is the fact that this elevation is beneficial and does not fall under adverse effects.

Overall the analysis of the hematological parameters do not reveal any significant toxicity potential in the test drug at the dose level studied and for the duration administered.

Table 6: Summary of the data on histopathological changes of the organs recorded during toxicity study

S. No	Organs	TED	TED× 3
1	Pituitary	NSC	NSC
2	Thymus	NSC	NSC
3	Lymph node	NSC	Moderate increase in cellullarity
4	Heart	NSC	NSC
5	Liver	Mild fatty changes in 2 animals	Mild fatty changes in 2 animals

6	Spleen	Increased white pulp proportion	Increased white pulp proportion
7	Kidney	NSC	NSC
8	Stomach	NSC	NSC
9	Jejunum	Mild inflammatory changes in one rat. Others normal	Mild to moderate inflammatory changes
10	Lung	NSC	NSC
11	Testis	NSC	NSC
12	Ovary	NSC	NSC
13	Uterus	NSC	NSC

NSC – No significant changes

Lymph node: Microscopic examination of sections of lymph nodes obtained from TED dose treated groups showed normal cytoarchitecture. In sections obtained from TED x 3 dose treated group features of increased cellularity was observed. Photomicrographs of representative sections of lymph node from different groups have been shown in Fig A to F in Plate 3.

Jejunum: Mild to moderate inflammatory changes were observed in jejuna sections obtained from TED x 3 administered groups. In TED administered group mild inflammatory changes were observed in one rat while in the remaining rats the cytoarchitecture was almost normal. Photomicrographs of representative jejuna sections from different groups have been shown in Fig A to F in Plate 7.

Liver: Microscopic examination of liver sections from control group showed normal cytoarchitecture. The sections from two animals treated at TED and TED × 03 dose level of *Pippali* show mild fatty changes in comparison to control rats. Representative photomicrographs from different groups can be found in Fig A to F in Plate 8.

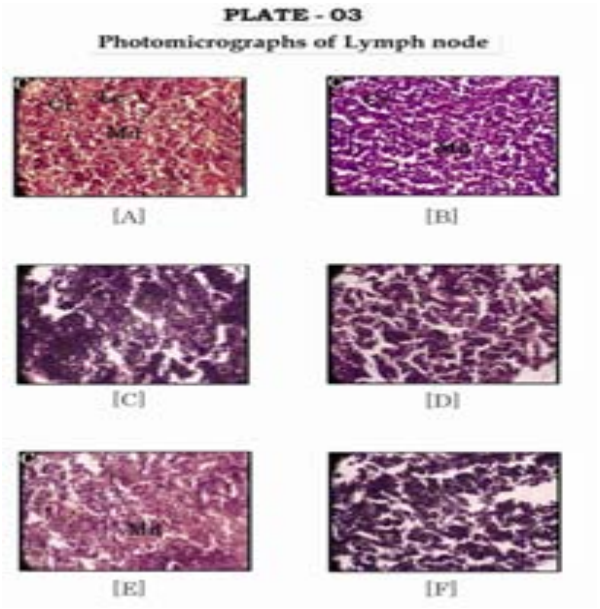
Spleen: Microscopic examination of spleen sections from control group showed normal cytoarchitecture. In sections from TED and TED × 03 dose test drug administered groups increase in white pulp proportion was observed. Photomicrographs of representative sections of spleen from different groups have been shown in Fig A to F in Plate 9.

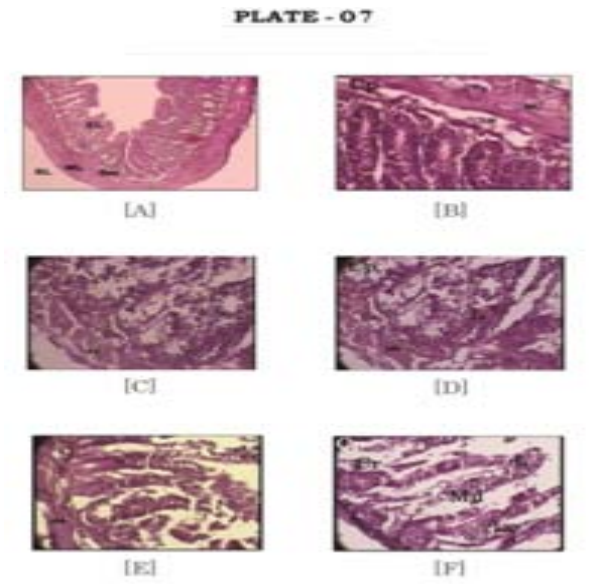
Out of 13 organs and organ parts studied the changes were observed in liver, spleen, lymph node and jejunum**.** In liver mild fatty changes were observed in both the groups; in jejunum mild inflammatory changes at lower dose and moderate inflammatory changes at higher dose level were observed. Considering that changes are of low magnitude- it can be suggested that these changes are not serious in nature. In lymph node at higher dose level increased cellularity was observed and in spleen increased proportion of white pulp was observed these two changes

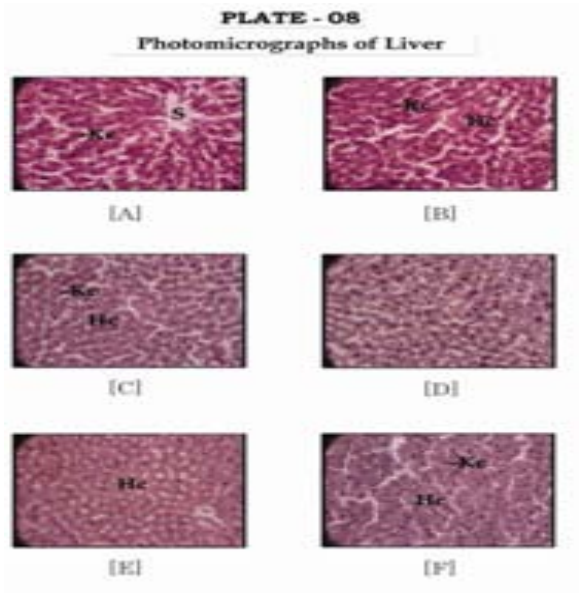
These two changes do not fall under the category of toxic changes. Hence overall analysis of histopathological study also does not reveal any serious toxicity potential of the drug even after prolonged administration.

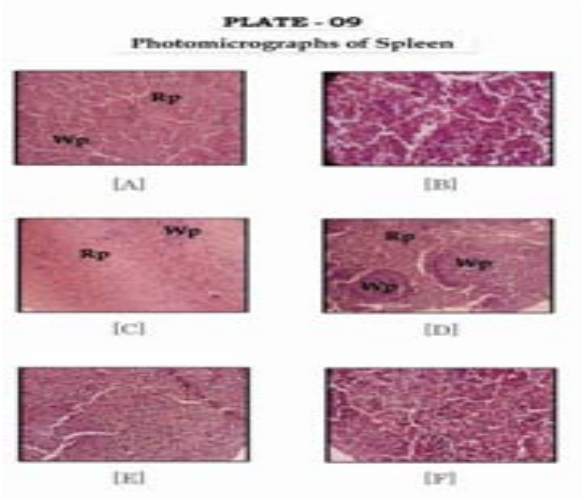
Another point to be noted is that in most of the studies reported on animals the studies have been conducted by employing different extracts like petroleum ether, ether, benzene at a relatively higher dose level. Hence their correlation cannot be done directly. It is necessary to take in to consideration the extract yield, its chemical profile and the route of administration. In one of the study it has been mentioned that Piperine is acutely toxic to the mice, rats and hamsters. The LD_{50} values ranged for a single i.v., i.p., s.c., and i.m. administration of piperine to adult male mice were 15.1, 43, 200, and 400mg/kg body weight respectively. This does not have much implications for the therapeutic utility of *Pippali* since it is not administered by parenteral routes and even then piperine content is only 0.29 to 0.38%. This amounts to a large fraction which is not likely to be attained by oral administration of normal human dose. Thus the maximum human dose of 10g would contain 25 to 30 mg of piperine. 15 mg piperine in mice is equivalent to approximately 117 mg of piperine in human beings- a large amount not likely to be administered by IV route. 400 mg is equivalent

to 3104 mg in human beings. The amount of root powder which should be consumed to obtain this dose is approximately 1 kg! Thus normal therapeutic dose is not likely to produce any serious toxic effect.









CONCLUSION

Acute toxicity: Acute toxicity study has been carried out in albino rats receiving the test drug 5 dose level maximum at up to 5times higher (3750mg/kg) then the therapeutic equivalent dose(750mg/kg). No mortality was observed in any group.

Sub-Chronic toxicity: It has been carried out in two groups with different dose i.e. therapeutic equivalent dose (750mg/kg) and three times higher than therapeutic equivalent dose (2250mg/kg) for duration of 45days. The data generated in the study show that the *Pippali* Churna up to the dose of TED×3 do not produce any change in the important biochemical parameters studied. In haematological parameters, WBC increases of moderate extent and in histopathological examination mild fatty changes in the liver and moderate inflammatory changes in the small intestine were observed. It can be suggested that at the dose level studied for considerable longer duration *Pippali* has no serious toxicity potential.

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