**Research Article** **CODEN: IJRPJK** **ISSN: 2319 – 9563**

**International Journal of Research in Pharmaceutical and Nano Sciences**

Journal homepage: **www.ijrpns.com**

# ACUTE AND SUB ACUTE TOXICITY STUDY ON SIDDHA DRUG ATTHI PINCHUENNAI

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**ABSTRACT**

Varicosities of the veins in the anal canal are known as haemorrhoids. The perverted activities such as sedentary life style, western dietary habits, strange sleep habits which plays as important role to bring out this disease. In siddha system, lot of herbal and herbominaral drugs were prescribed for haemorrhoids. Atthi Pinchu Ennai (APE) is one of the poly herbal formulation quoted in 'Brhamamuni Vaithiya Soothiram 390' indication for Rattha Moolam (Haemorrhoids). The present study aims to carry out acute and sub-acute toxicity of APE. Adult both sexes of Wister rats weighing 150-200 gm (8 -12 weeks) were used. For acute studies different doses of APE were administer orally to rats once daily for one week as per Organization for Economic Cooperation Development (OECD) 423. In sub-acute toxicity study was carried out OECD 407 for 28 days in different doses as 315mg/kg, 1575mg/kg. and 3150 mg/kg body weight. Haematological, biochemical parameter, histopathological studies were performed for all animals. The study conclude that on oral administration of 3150 mg/kg body weight of APE to wister rats there was no change in behaviors movements and no characteristic clinical sign of toxicity and no mortality was observed.

**KEY WORDS**

Haemorrhoids, Atthi Pinchu Ennai (APE) and Toxicity.

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## INTRODUCTION

Internal haemorrhoids are varicosities of the internal haemorrhoidal plexes[1]. Internal haemorrhoids (Greek: *haima* = blood, *rhoos* = flowing; synonym: piles, Latin: *pila* = a ball) are symptomatic anal cushions and characteristically lie in the 3, 7 and 11 o'clock positions[2]. It is common in both men and women those who have exposed to sedentary life and western diet habit have a greater

incidence. The prevalence of symptomatic hemorrhoids ranges from 4.4% in the general population to 36.4% in general practice [3]. Clinical features are spurting out of blood occur during defecation, mucous and pus discharge, pain and burning sensation in anal region anaemia and loss of weight. These are correlated with features of Ratha moolam, which is one among 21 type of Moola noi mentioned in Yugi Vaidhya Chinthamani[4].

Hemorrhoids that fail to respond to medical management may be treated with rubber band ligation, sclerosis and hermotherapy. Rubber band ligation has been demonstrated to be the most effective method to treat symptomatic internal hemorrhoids that have failed conservative management[5-8]. Complications of this procedure include vasovagal response, anal pain, bleeding from early dislodgment and pelvic sepsis[9]. Atthi Pinchu Ennai (APE) is prepared as per 'Birhamamuni Vaithiya Soothiram 390' indication for Rattha Moolam[10]. Tender fruits (*Ficus recemosa*) are used as astringent, stomachic, astringent to bowel, styptic, tonic, useful in the treatment of leucorrhoea, blood disorder, burning sensation and fatigue[11]. The structure of *Plumbago zeylanica* active principle compound is similar to that of vitamin K[12].

Piper species have been used in traditional medicine for intermittent fevers, Neurological, Respiratory problem and also recommended for gastrointestinal disorder including dyspepsia, flatulence, constipation and hemorrhoids[13]. *Aconitum heterophyllam* exhibit antipyretic, anti-inflammatory, analgesic and astringent activities [14]. Most of the ingredients have Astringent property which helps in arrests of bleeding in that disease. A pre-clinical toxicity study is mandating in determining a safety dose for human trial[15]. Prior to initiation of human trial the safety of the drug is to be proved[16]. The present preclinical study aimed to evaluating acute and sub-acute toxicity of APE. This study reveals vital information about efficacy and safety of APE.

## MATERIAL AND METHODS

### Source of Drugs

The required raw drugs are procured from a well reputed indigenous drug shop. The raw drugs will be authenticated by the concerned pharmacognosist at SCRI, Chennai, Tamil Nadu, India

### Ingredients

VetPalai Arisi (*Wrightia tinctoria*), Omam(*Trachyspermum ammi*), Kodiveli *(Plumago zeylanica*), Milagu (*Piper nigram*), Perunkayam (*Ferula asafoetida*), Thippili Moolam (*Piper longum*), Sirukansori (*Tragia involucrata*), Nerunjil (*Tribulus terestriis*), Athividayam (*Acconitum heterophylum*), Kadugu (*Brassica juncea*), Kadugurogini (*Picrorhiza scrophulariflora*), Anai thippili (*Scindapsus officinalis*), Peru marapattai (*Stercullia foetida*), Vellai venkayam(*Allium cepa*), Vilvam leaf (*Aegle marmelos*) – each 5.1 g ( 1 Kazhanchu), Atthi unripe fruit (*Ficus recemosa*) - 175 g ( 5 Palam), Nallennei (*Sesamum indicum oil*) - 1050 g.

### Standard Operating Procedure of APE

The raw drugs (listed from 1 to13) were powdered and grinded with wet drugs (listed from 14 to 16). The mixture is added with Nallennei (*Sesamum indicum* oil) then it heat till it attains the consistency of Mezhugu. After that the oil is filtered and stored, this oil used internally for treatment Rattha moolam

### Experimental Animals

Wister rats of either sex weighing 150-200gms (8-12 weeks) were obtained from Animal house department, King Institute, Guindy, Chennai. Rats were housed in individually in poly propylene cages and fed with standard rodent pellet obtained and water ad libitum. The animals were subjected to a 12:12 hrs light:dark cycle under standard laboratory conditions at a temperature of 20 - 24ºC with relative humidity of 30% -70% .

The experimental protocol for Trail Drug APE was approved by the Institutional Animal Ethical Committee (IAEC Approval No: 1545/ PO/ ALL/ CPCSEA/1-7/2013 dated 26.03.2013) of Sairam Advanced Centre for Research, Poonthandalam, West Tambaram, Chennai, Tamil Nadu.

**Experimental Design**

**Acute Oral Toxicity (LD 50 Determination)**

Three female nulliparous and non –pregnant rats were used for acute oral toxicity study according to OECD guideline 423[17]. Depending on the mortality and /or morbidity of the animals a few steps may be necessary to judge the toxicity of the text substance. This procedure has advantage over other methods because of minimal usage of animals while allowing for acceptable data. The method was defined doses (5, 50, 300, 2000 mg/kg body weight and the results allow a substance to the ranked and classified[18].

The starting dose of Atthi Pinchu Ennai was administered orally 2000 mg/kg body weight of different groups of rats and absorbed for toxicological study. The animals were observed individually after dosing the first 30 mins, Periodically during the first 24 hr. with special attention given, during the first 4hr. and daily thereafter, for 14 days. Observations included changes in skin, fur, eyes, mucous membrane (nasal), autonomic (salaivation, lacrimation, perspiration, pile erection, urinary incontinence and defecation) and central nervous system (drowsiness, gait, tremors convulsions) changes respectively. Mortality if any was determined over a period of 2 weeks.

**Sub-Acute Oral Toxicity**

To assess the nature of the toxic effects in the trial drugs. In this study equal number of males and females are used according to Organization for Economic Cooperation Development (OECD) guideline 407[19].

The doses are generally selected on the basis of the information obtained in acute toxicity studies. The trail drug APE was nontoxic and no behavioral changes was observed up to the dose level of 2000 mg/kg body. In the literature, therapeutic dosage for APE in human is 17.5 gm. On the basis of body surface area ratio between rat and human, the doses selected for the study were 315mg/kg(X), 1575mg/kg(5X) and 3150mg/kg(10X) body weight. The dose administered orally for 28 days

**Observation and examination**

Body weight, water consumption, food consumption of the animals was evaluated weekly. Appearance, behavior and any abnormality were recorded

Three dose level of the test drug were used in sub-acute toxicity study for 28 days. On 29$^{th}$ day the animal was anaesthetized and blood was collected by retro orbial puncture. Haematological parameters were evaluated. Serum was separated and biochemical parameters estimated. Animals were sacrificed and organs like liver, heart, lung, spleen, brain, kidney, were removed and weighed. The organs were kept in 10% formalin and used for histo pathological analysis.

**RESULTS**

**Statistical Analysis**

All of the data were expressed as mean ± SEM. Statistical significance between more than two groups were using one way ANOVA followed by Dunnet's multi comparison test. Calculations were done using GraphPad Instat -3 version softwere. The significance level was set at p value $\leq 0.05$ for all tests.

**DISCUSSION**

1. The results of acute toxicity study of APE revealed decreased motor activity, lacrimation, diarrhoea and writhing present whereas no mortality and other abnormal signs and behavioral changes in rats at the dose of 2000 mg kg/ body weight administered orally (Table No.1).
2. All animals from control and all the treated dose groups survived throughout the dosing period of 28 days for sub-acute toxicity study.
3. The results for body weight determination of animals from control and different dose groups show comparable body weight gain throughout the dosing period of 28 days (Table No.2).
4. The results of haematological investigations such as RBC, PCV, HB, WBC, TC, and Platelets count (Table No.3) conducted on day 29, revealed no significant changes in the values

when compared with those of respective controls.

5. Results of Biochemical investigations conducted on 29th day and recorded in Table No.4, revealed the following significant changes in the values of different parameters studied when compared with those of respective controls. SGOT, SGOT, Bilirubin, Total Cholesterol, slightly increased in lower dose administration compared with median and higher dose group whereas within normal limits other parameter were within the normal limits.
6. Urine analysis data (Table No.5) of control group and treated group of animals did not reveal major abnormalities
7. Group Mean Relative Organ Weights (% of body weight) are recorded in Table 6. Comparison of organ weights of treated animals with respective control animals on day 29 was found to be comparable with respective control group.
8. Gross pathological examination of animals in control as well as the treated groups did not reveal any abnormalities.
9. Histopathology: The vital organs such as liver, heart, Spleen and kidneys were removed from the test groups at the end of the study and carefully observed macroscopically to find any observable gross lesions compared with the control group and did not reveal any abnormal macroscopic changes in (Figure No.1).
10. Microscopically, these organs of the test groups revealed normal histological appearance when compared with the control group.

**Table No.1: Dose finding experiment and its behavioral Signs of Toxicity**

| S.No | Dose mg/kg | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 2000 | + | - | - | - | + | + | + | - | - | - | - | - | - | - | + | - | + | + | - | - |

1. Alertness 2. Aggressiveness 3. Pile erection 4. Grooming 5. Gripping 6. Touch Response 7. Decreased Motor Activity 8. Tremors 9. Convulsions 10. Muscle Spasm 11. Catatonia 12. Muscle relaxant 13. Hypnosis 14. Analgesia 15.Lacrimation 16. Exophthalmos 17. Diarrhoea 18. Writhing 19. Respiration 20. Mortality

**Table No.2: Body weight (g) changes of rats exposed to APE**

| S.No | Dose (mg/kg/day) | Days | | | | |
|---|---|---|---|---|---|---|
| | | 1 | 7 | 14 | 21 | 28 |
| 1 | Control | 122.37 ± 3.21 | 124.14 ± 4.09 | 128.21 ± 2.17 | 129.21 ± 5.11 | 133.32 ± 1.89 |
| 2 | 315 | 136.4±6.2 | 133.62±6.8 | 137.24±3.4 | 138.46±1.2 | 13.46±5. |
| 3 | 1575 | 128.2±3.4 | 130.46±2.4 | 133.4±4.6 | 134.6±2.3 | 135.72±1.6 |
| 4 | 3150 | 126.3±4.2 | 130.28±6.2 | 131.6±2.6 | 135.8±3.4 | 137.82±8.6 |

Values are expressed as mean ± S.E.M. N=10

**Table No.3: Effect of APE on Haematological parameters in rats**

| S.No | Parameter | Control | 315 mg/kg | 1575 mg/kg | 3150 mg/kg |
|---|---|---|---|---|---|
| 1 | RBC (x $10^6$/$mm^3$) | 7.51 ± 0.16 | 6.46±0.26 | 6.697±0.46 | 7.2±0.43 |
| 2 | PCV (%) | 48.2 ± 1.3 | 42.4±3.2 | 46.46±4.2 | 48.6±2.6 |
| 3 | Hb (%) | 15.6 ± 0.19 | 14.3±0.8 | 14.6±0.4 | 15.2±1.2 |
| 4 | WBC (x $10^3$/$mm^3$) | 10.12 ± 1.2 | 10.6±1.2 | 11.2±2.3 | 9.6±0.89 |
| 5 | Neutrophils (%) | 22 ± 4 | 27.3±1.6 | 18.3±0.67 | 24.6±1.34 |
| 6 | Mononuclear cells (%) | 76 ± 2 | 69.4±2.2 | 78.6±1.8 | 72.14±3.2 |
| 7 | Eosinophils(%) | 2.4 ± 0.6 | 3.4±0.12 | 3.12±0.16 | 2.4±0.46 |
| 8 | Platelets(x $10^3$/$mm^3$) | 423.2 ± 48.8 | 463.4±34.3 | 472.6±23.46 | 522.68±48.2 |

Values are expressed as mean ± S.E.M (Dunnett's test).*P<0.05,**P<0.01,***P<0.001 *vs* control; N=10

**Table No.4: Effect of APE on biochemical parameters in rats**

| S.No | Parameters | Control | 315 mg/kg | 1575 mg/kg | 3150 mg/kg |
|---|---|---|---|---|---|
| 1 | Protein (g/dl) | 8.62 ± 1.3 | 7.62±1.3 | 8.46±0.85 | 7.2±1.16 |
| 2 | Albumin (g/dl) | 4.8 ± 0.6 | 3.4±0.48 | 3.42±0.6 | 4.12±0.8 |
| 3 | BUN (mg/dl) | 19.2 ± 1.2 | 21.6±1.4 | 19.2±2.1 | 17.4±0.78 |
| 4 | Urea (mg/dl) | 64.24 ± 3.11 | 54.3±2.6 | 52.6±4. | 49.6±3.14 |
| 5 | Creatinine (mg/dl) | 0.82 ± 0.16 | 0.64±0.02 | 0.72±0.04 | 0.63±0.24 |
| 6 | Total Cholesterol mg/dl) | 91.24 ± 1.35 | 111.34±1.62 | 91.56±2.46 | 96.2±5.8 |
| 7 | Triglycerides ( mg/dl) | 50.15 ± 3.21 | 54.6±2.8 | 56.3±3.4 | 57±4.6 |
| 8 | Glucose (mg/dl) | 110.16 ± 8.62 | 104.6±3.4 | 95.6±11.2 | 87.2±8.2 |
| 9 | Total Bilirubin (mg/dl) | 0.205 ± 0.04 | 0.314±0.06 | 0.21±0.04 | 0.46±0.04 |
| 10 | SGOT (U/L) | 73 ± 2.4 | 86±2.4 | 79.2±5.6 | 72.6±3.2 |
| 11 | SGPT(U/L) | 28.4 ± 1.2 | 37±4.2 | 25.2±4.3 | 29.3±4.2 |
| 12 | Alkaline phosphatase(U/L) | 102.4 ± 3.6 | 97.4±4.3 | 98.6±3.2 | 101.4±11.2 |
| 13 | Sodium (mEq/L) | 138.12 ± 3.14 | 128.2±4.3 | 114.2±4.6 | 132.6±2.3 |
| 14 | Potassium (mEq/L) | 7.2 ± 1.34 | 6.4±0.8 | 5.6±0.4 | 5.7±0.6 |

Values are expressed as mean ± S.E.M (Dunnett's test). *P<0.05, **P<0.01, ***P<0.001 *vs* control; N=10

**Table No.5: Effect of APE on Urine parameters in rats**

| S.No | Parameters | Control | 315 mg/kg | 1575 mg/kg | 3150 mg/kg |
|---|---|---|---|---|---|
| 1 | Colour | Yellow | Yellow | Yellow | Yellow |
| 2 | Transparency | Clear | Clear | Clear | Clear |
| 3 | Specific gravity | 1.010 | 1.01 | 1.02 | 1.03 |
| 4 | PH | 7.2 | 7.4 | 6.8 | 6.9 |
| 5 | Protein | Nil | Nil | Nil | Nil |
| 6 | Glucose | Nil | Nil | Nil | Nil |
| 7 | Bilirubin | -ve | -ve | -ve | -ve |
| 8 | Ketones | -ve | -ve | -ve | -ve |
| 9 | Blood | Absent | Absent | Absent | Absent |
| 10 | RBCs | Nil | Nil | Nil | Nil |
| 11 | Epithelial cells | Nil | Nil | Nil | Nil |
| 12 | Casts | Nil | Nil | Nil | Nil |

**Table No.6: Effect of APE on Organ weight in rats**

| S.No | Dose (mg/kg) | Control | 315 mg/kg | 1575 mg/kg | 3150 mg/kg |
|---|---|---|---|---|---|
| 1 | Liver (g) | 5.24±0.14 | 4.34±0.26 | 5.62±1.02 | 4.26±0.46 |
| 2 | Heart (g) | 0.70±0.05 | 0.60±0.04 | 0.58±0.03 | 0.62±0.07 |
| 3 | Lung (g) | 1.78±0.25 | 1.46±0.32 | 1.62±0.46 | 1.70±0.21 |
| 4 | Spleen (g) | 0.74 ±0.07 | 0.62±0.08 | 0.56±0.07 | 0.82±0.04 |
| 5 | Brain (g) | 1.43±0.18 | 1.37±0.21 | 1.46±0.26 | 1.52±0.46 |
| 6 | Kidney (g) | 0.70±0.05 | 0.63±0.04 | 0.75±0.04 | 0.82±0.06 |

Values are expressed as mean ± S.E.M (Dunnett's test). *P<0.05, **P<0.01, ***P<0.001 *vs* control; N=10

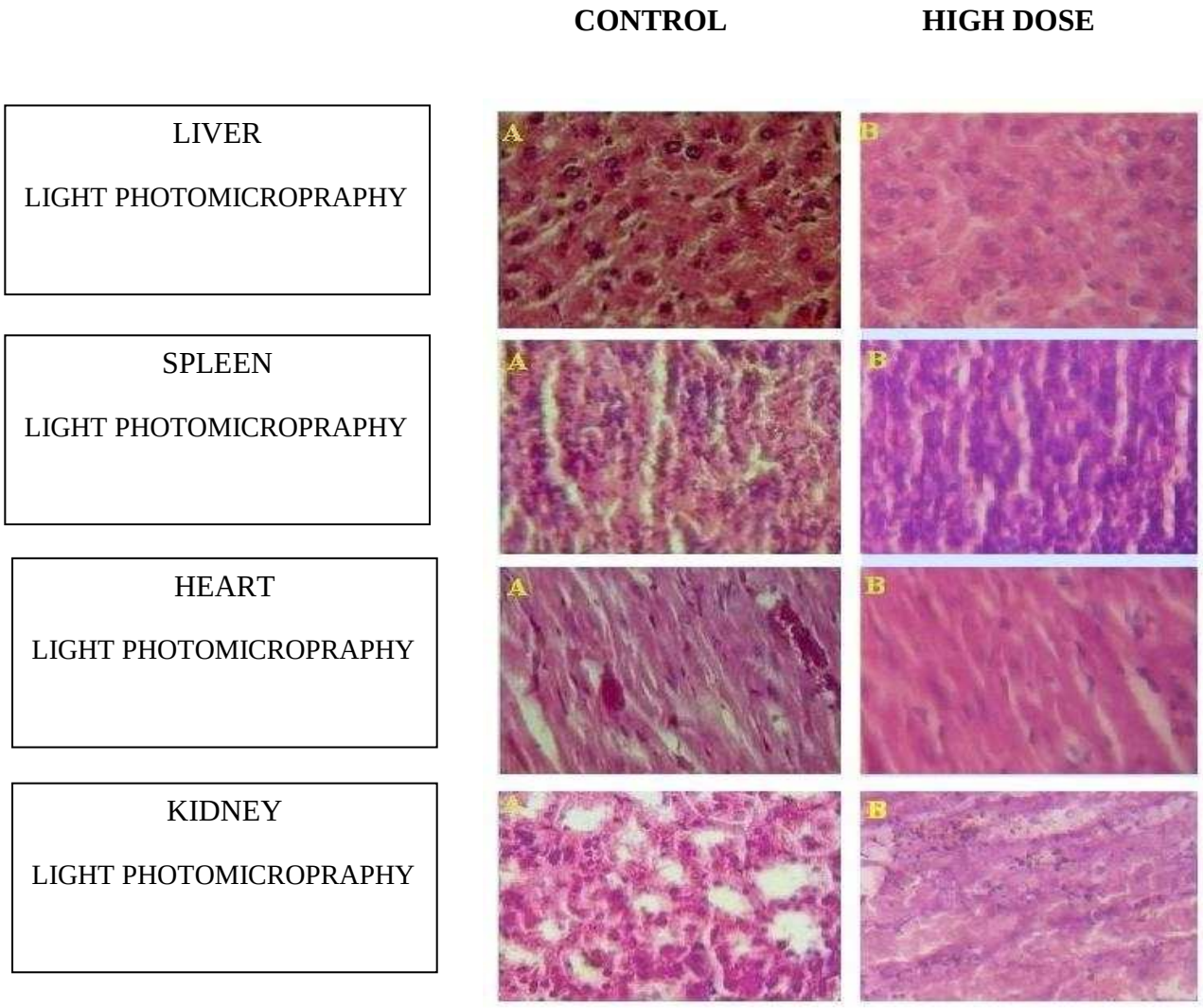


**Figure No.1: Histo Pathological Analysis of Sub –Acute Toxicity Study**

No Abnormality is seen in hepatocytes, sinusoids.
Spleen shows no abnormality is seen in trabeculae, capsule.
Heart shows no abnormality is seen in nuclei of myocyte, myocardium.
Kidney shows no abnormality is seen in glomeruli, bowman's capsule, capillaries.

## CONCLUSION

Based on above findings, no toxic effects were observed upto 3150 mg/kg of body weight of APE treated via oral route over a period of 28 days. So this study concluded that the APE is suitable for therapeutic use in human with the dosage recommendations of upto 3150 mg/kg of body weight per oral.

## ACKNOWLEDGEMENT

The Grateful Author HOD. Department of Maruthuvam, Government Siddha Medical College, Chennai, Tamil Nadu, India.

## CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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**Please cite this article in press as:** Vadivelan and Parthibhan. Acute and sub-acute toxicity study on siddha drug atthi pinchuennai, *International Journal of Research in Pharmaceutical and Nano Sciences*, 3(2), 2014, 139-146.