*ejpmr, 2021,8(4), 415-426*

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

# SAFETY EVALUATION OF SIDDHA FORMULATION THEER ANDHASKHAN MATHIRAI BY ACUTE AND SUB-ACUTE TOXICITY STUDIES IN WISTAR RATS

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**Article Received on 24/01/2021** **Article Revised on 12/02/2021** **Article Accepted on 05/03/2021**

## ABSTRACT

In *Siddha* system of medicine, thousands of raw drugs are used to treat various kinds of diseases. These drugs are categorized into four groups namely Metals, Minerals, Herbals and Animal products. But in certain life-threatening diseases and especially in many chronic diseases, the herbal medicines alone have not been much effective. Herbal and herbo mineral preparations are being traditionally used in Indian system of Medicine especially in *Siddha* system. According to the Shelf life Herbo mineral preparations have more actions then any other drugs. Here ***THEER ANDHASKHAN MATHIRAI(TAM)*** is prepared as per *Siddha* classical text book Anuboga vaithiya Navaneetham, part 4, page no:105 – 106 to treat RA. Before clinical study there should be a need to undergone Preclinical trial as per WHO guidelines. The Clinical study was approved by Institutional Ethics Committee (IEC) and the approval number is **IEC No: GSMC-CH-ME-5/004/2018**. It was registered in Clinical Trials Registry-India (CTRI) and the registered number is CTRI/2019/08/020797.The present preclinical study aims to carry out safety and toxicity of the experimental protocol *Theer Andhaskhan Mathirai* was approved by Institutional Animal Ethics Committee **(IAEC)** of Sathyabama University, Chennai, Tamil Nadu, India. The approval number is **SU/CLATR/IAEC/XIII/125/2019.** Acute & Sub acute toxicity studies were carried out as per OECD guidelines 423 and 407. Hematological Parameters, biochemical parameters histopathological studies were performed for all animals. The study concludes that on oral administration of 100mg/kg of bodyweight of TAM to Wistar albino rats, there was no change in behaviour movements and no characteristic clinical sign of toxicity or mortality observed. From the result it was concluded that the Siddha preparation TAM offers wide margin of safety in tested rodents and further long term usage of drugs will be considered as safe for the ailment of various disease.7

**KEYWORD:** Siddha, OECD guidelines, Acute, Sub-acute, TAM, IAEC.

## INTRODUCTION

Siddha system of medicine is one of the ancient indigenous medical system compiled by Siddhars, who lived a spiritual life in the southern region of India.[1] In Siddha system, metals and minerals are widely used, when compared to other traditional medical systems. In such conditions, Siddhars enumerated some Herbo – metal and Herbo – mineral formulations. Some of the mineral drugs and also plant drugs produces some toxic effects to human. So before entering into the clinical trial there is a huge need for preclinical studies. Toxicity is defined as any harmful effect of chemical or a drug on a target organism. Acute toxicity has been defined by various experts. The Organization for Economic Co-operation and Development panel of experts (OECD Guidelines) defines acute toxicity as "the adverse effects occurring within a short time of administration of a single dose of a substance or multiple dose given within 24 hours.[2] Toxicity profiling of Siddha preparations are highly essential to prove the safety and efficacy of this Herbo mineral formulation before administration in humans. It provides a base for fixation of dose to the pharmacological study. The main purpose of toxicity studies reveals the information regarding LD50. The OECD Guidelines for the testing of chemicals are a collection of the most relevant internationally agreed testing methods used by government, industry, and independent laboratories to characterize potential hazards of new and existing chemical substances and chemical preparations, mixtures.[3] The main aim of the present investigation is to evaluate the safety of the Siddha formulation *Theer Andhaskhan Mathirai* in rodents at fixed dose level by acute and sub-acute toxicity studies in accordance with OECD guidelines.

## MATERIALS AND METHODS STUDY DRUG

**Trial drug name:** Theer Andhaskhan Mathirai.

**Reference:** Anuboga vaithiya Navaneetham, part 4, page no:105 –106.[4]

**Required ingredients:** Rasa Karpooram (*Hydragyrum subchloride*- 5 varagan (21gms) Milagu (*Piper nigrum*) powder-5 varagan (21gms) Lavangam (*Syzygium aromaticum*) powder-5 varagan (21gms) Omam (*Carum copticum*) powder-5 varagan (21gms) Poolan Kizhangu (*Curcuma zedoaria*) powder -5 varagan (21gms) Aatruthumati Kai (*Citrullus colcocynthis*)-125 Nos (extracted juice).

## STANDARD OPERATIVE PROCEDURE

***Source of Raw Drugs***

The required raw drugs were procured from a well reputed indigenous raw drug shop. The raw drugs taken for study was authenticated by the Botanist, Dept. of Medicinal botany and mineral drug was authenticated by HOD, Gunapadam department, Government Siddha medical college, Chennai - 106. Certificate was enclosed.

***Purification of Raw Drugs*[5]**

The raw drugs were purified as mentioned in "Sikitcha Rathna Deepam Ennum Vaithiya Nool".

**1. Rasa Karpooram (*Hydragyrum subchloride*)**

- Hydrargyrum Sub Chloride - 35 gm
- Betel leaf *(Piper betel*) - 8.75 gm
- Pepper (*Piper nigrum*) - 8.75 gm
- Water - 1.3 litre

**Procedure**

The Poultice made of betel leaf and pepper was taken and dissolved in water. Rasa Karpooram (Calomel) was tied in a cloth and immersed in the liquid hung from the cross bar and heated. After the water was reduced to ¾ of its volume, the Rasa Karpooram (Calomel) was taken out washed with water and dried to get purified form.

**2. Milagu (*Piper nigrum*)**

Pepper was soaked into sour butter milk for 3 hours and dried to get purified form of
pepper.

**3. Lavangam (*Syzygium aromaticum*)**

Clean without any debris and keep it dry at night & get purified form.

**4. Omam (*Carum copticum*)**

Take omam and soaked into lime water then dried to get purified form.

**5. Poolan Kizhangu (*Curcuma zedoaria*)**

Clean without any debris in upper surface and keep it dry at night then powdered it.

**6. Aatruthumati Kai (*Citrullus colcocynthis*) juice**

Take Aatruthumati kai and shoot at straw fires until that upper skin colour turned into charcoal colour then leave it few minutes, now remove the that upper skin part and take that inner pulp part then crushed it well to get a juice.

## METHOD OF PREPARATION

Take the Purified Pooram in a kalvam and make it into fine powder. Add all the powders and grind them altogether with Aatruthumati kai juice by adding little by little. After the entire quantity of the juice has been totally utilized, make into pills of Kundri size (130mg).

## DRUG STORAGE

Dried well in shade and stored in an air-tight porcelain jar.

## PRE-CLINICAL STUDY

**In Vivo studies**

The experimental protocol *Theer Andhaskhan Mathirai* was approved by Institutional Animal Ethics Committee (**IAEC**) of Sathyabama University, Chennai, Tamil Nadu, India. The approval number is **SU/CLATR/IAEC/XIII/125/2019.**

**Toxicological study**

Acute oral toxicity study was carried out as Per OECD guideline 423.

Sub acute toxicity study 28 days repeated dose oral toxicity study was carried out as Per OECD guideline 407.

**Study center**

Sathyabama Institute of science and technology, Chennai – 119, Tamilnadu, India.

Medicine name : Theer Andhaskhan Mathirai
Dosage : 2 Tablets /BD
Adjuvant : Jaggery
Duration **:** 48 days

## ACUTE TOXICITY STUDY

Acute toxicity study of the study drug Theer Andhaskhan Mathirai (TAM) was carried out as per OECD guideline (Organization for Economic Co-operation and Development) Guideline-423.

**Animal**

Healthy adult Wistar albino rat weighing between 180-200 g were used for the study. The animals were housed in poly propylene cages and were kept in well ventilated with 100% fresh air by air handling unit (AHU). A 12 light / dark cycle were maintained. Room temperature was maintained between 22 $\pm$ $2^0$ C and relative humidity 50–65%. They were provided with food (Amruth, Kvat, Hyderabad, India) and water *ad libitum*. All the animals were acclimatized to the laboratory for 7 days prior to the start of the study. The experimental protocol was

approved by The Institutional Animal Ethics Committee of Sathyabama Institute of science and technology, Chennai, Tamil Nadu, India.

**Acute toxicity Study**
Acute toxicity study will be carried out in accordance with OECD guideline 423. The animals were fasted overnight with free access to water. The study was conducted with single oral dose administration of TAM.

| IAEC | SU/CLATR/IAEC/XIII/125/2019 |
|---|---|

**Animal Grouping**
One group consist of 6 female rats were used for this study. The dose utilized for evaluation of acute toxicity study is about 2000 mg/kg higher than that of the therapeutic dose.

**Study Description**
The animals were fasted overnight (12-16 hrs) with free access to water. The study was conducted with single oral administration of study drug TAM at the dose of 2000mg/kg (p.o) prepared in respective recommended adjuvant. The animals were observed continuously for first 72 h and then 14 days for emerging signs of behavioral changes, body weight changes and for mortality.

**Clinical signs Observation**
Lacrimation, Salivation, Animal appearance, open field behavior, cage behavior, Convulsion, Laxative action, sensory responses, Mobility /balancing, skin tone, CNS abnormalities, CVS abnormalities, respiratory distress, Muscle strength/ Coordination, Urine analysis, fecal pellet consistency and Mortality.

**Report**
The dose of TAM used for acute toxicity study is 2000mg/kg is higher than the normal therapeutic dose. No mortality observed at this dose level, further no significant change with respect to clinical signs on acute toxicity observed for (24-48 h) and for a period of 14 days. There is no significant change in the body weight, organ weight and gross observational changes of the treated animals which confirms the wide margin of safety of the study drug.

**SUB-ACUTE TOXICITY STUDY**
**Guideline:** Sub-acute toxicity study was carried out as per OECD guidelines Guideline-407.[21]

**Animal**
Healthy adult Wistar albino rat weighing between 180-200 g were used for the study. The animals were housed in poly propylene cages and were kept in well ventilated with 100% fresh air by air handling unit (AHU). A 12 light / dark cycle were maintained. Room temperature was maintained between 22 ± 2$^{o}$ C and relative humidity 50–65%. They were provided with food (Amruth, Kvat, Hyderabad, India) and water *ad libitum*. All the animals were acclimatized to the laboratory for 7 days prior to the start of the study. The experimental protocol was approved by The Institutional Animal Ethics Committee of Sathyabama Institute of Science and technology, Chennai, Tamil Nadu, India.

**Animal Grouping**
Animals were divided into three groups of 06 animals each consist of 3 male and 3 female rats.
**GROUP I :** Animals received saline 5 ml/kg b.w (p.o)
**GROUP II :** Animals received low dose of test drug 200 mg/kg (p.o)
**GROUP III :** Animals received high dose of test drug 400 mg/kg (p.o)

The animals were randomly divided into control group and drug treated groups for two different doses viz. low dose (200 mg/kg b.w) and high dose (400 mg/kg b.w) prepared in respective recommended adjuvant.

The animals were administrated with the study drug once daily for 28 days. The animals in group I (control group) received normal saline 5 ml/kg b.w. The animals in group II received low dose of *TAM* 200 mg/kg b.w (p.o) and group III received high dose of *TAM* 400 mg/kg b.w (p.o).

The rats were weighed periodically and observed for signs of toxicity pertains to C.N.S, C.V.S, A.N.S including behavioral changes, food - water intake and morphological changes. At the end of 28$^{th}$ day, the animals were fasted for overnight with free access to water. On 29$^{th}$ day the animals were sacrificed with excess anesthesia. Blood samples were collected from aorta and stored in EDTA (ethylenediamine–tetra acetate) for Hematological analysis and for serum generation for biochemical analysis(6). The vital organs including heart, brain, lungs, spleen, kidneys, liver, stomach, testes, and ovary were harvested and carefully examined for gross lesions. The organs were preserved in 10% formalin for histopathological assessment and interpretation.

**Hematological analysis[7]**
Blood samples were analyzed using established procedures and automated Bayer Hematology analyzer. Parameters evaluated include Packed Cell Volume (PCV), Red Blood Cells (RBC) count, White blood cell count (WBC), Platelet Count, Hemoglobin (Hb), Mean cell Hemoglobin Concentration (MCHC), Mean Red Cell Volume (MCV), Mean Cell Hemoglobin (MCH), Mean platelet volume (MPV), Neutrophils, Eosinophil's, Basophils, Lymphocytes and Monocytes.

**Biochemical analysis[8]**
Serum samples were analyzed for High Density Lipoprotein (HDL), Low density Lipoprotein (LDL), Very low density Lipoprotein (VLDL), Triglycerides (TGL), Total Cholesterol , Blood urea nitrogen (BUN), Creatinine, Albumin, Total Protein, Glucose, Uric acid, Aspartate Transaminase (AST), Alanine amino

Transaminase (ALT) and Alkaline Phosphatase (ALP) using Mind ray auto analyzer model BS 120.

**Histopathological evaluation**[9]

Organs included of heart, brain, lungs, spleen, kidneys, liver, stomach, testes and ovary. Histological slides of organs were made and observed under the microscope. The pathological observations of cross section of these organs were performed on gross and microscopic bases. Histological examinations were performed on the preserved tissues with particular emphasis on those which showed gross pathological changes.

**Statistical analysis**

The statistical analysis was carried by one-way ANOVA (GRAPH PAD PRISM 5 computer program). Results were expressed as mean ± standard error. A statistical comparison was carried out using the Dunnett's test for the control and treatment group.

***ADMINISTRATION OF TRIAL DRUG***

**METHODOLOGY**

***A. Fecal Pellet Analysis:*** Rats of control and treatment group were allowed to explore to open field on clean and sterile Stainless-steel tray. The collected pellets were analyzed for consistency, color, Shape, Presence of blood cells etc.

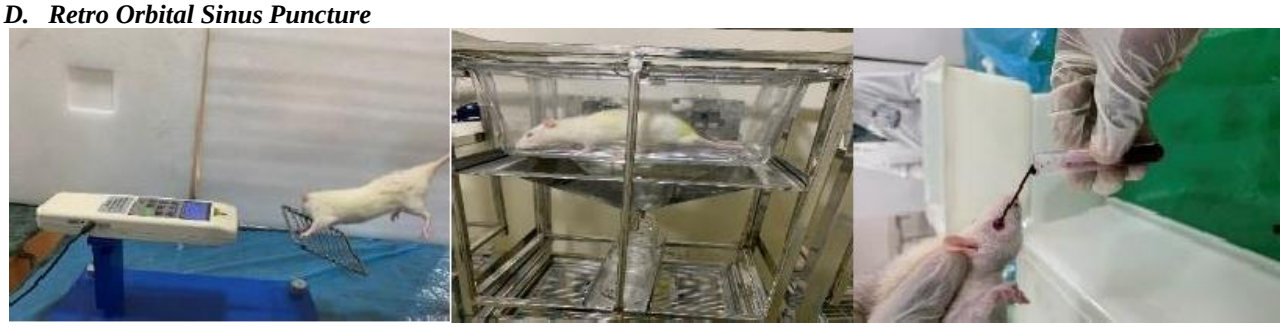
| Acute Toxicity Study | |
|---|---|
| **Analysis** | **TAM** |
| **Consistency** | Soft |
| **Shape** | Irregular |
| **Colour** | Pale greenish |
| **Mucous Shedding** | Absent |
| **Blood Cells** | Absent |
| **Signs of Infection** | None Observed |

| Sub-Acute Toxicity Study | | | |
|---|---|---|---|
| **Analysis** | **Control** | **Low Dose** | **High Dose** |
| **Consistency** | Rigid | Soft | Soft |
| **Shape** | Oblong | Irregular | Irregular |
| **Colour** | Greenish | Pale greenish | Pale greenish |
| **Mucous Shedding** | Absence | Absence | Absence |
| **Blood Cells** | Absent | Absent | Absent |
| **Signs of Infection** | None Observed | None Observed | None Observed |

***B. Muscle Grip Strength Analysis:*** The grip strength test is a simple non-invasive method designed to evaluate rat muscle force in vivo. Rats of control and drug treated group was allowed to hold the pull bar with both the hind limbs firmly then the animal was gently pulled back with the tail until the animal lost the grip toward the bar. The procedure was repeated to get the average value. Muscle grip ness of the drug treated group was compared to that of the control rat to ensure the change in coordination.

***C. Metabolic Cage Observation:*** Rat of control and treatment group was placed individually in metabolic cage with free access to feed and water. Urine dropping from the animal was collected using specialized wire mesh system fixed at the base of the cage having provision to trap the fecal pellet mixed with urine sample. The collected urine sample was subjected to analysis with respect to colour, pH, glucose, ketone bodies, pus and blood cells.

***D. Retro Orbital Sinus Puncture***



**B** **C** **D**

**RESULTS**

**Assessment of clinical signs in rats treated with *(TAM)* on Acute toxicity study.**

| Parameter | TAM | Parameter | TAM |
|---|---|---|---|
| **Lacrimation** | Absence | **Response to Sound** | Normal |
| **Salivation** | Absence | **Response to Light** | Normal |
| **Animal appearance** | Normal | **Mobility** | Normal |
| **Tonic Movement** | Absence | **Respiratory Distress** | Nil |
| **Clonic Movement** | Absence | **Skin Color** | Normal |
| **Laxative action** | Absence | **Stereotype behavior** | Absence |
| **Touch Response** | Normal | **Piloerection** | Absence |
| **Limb Paralysis** | Absence | **Open field behavior** | Normal |
| **Posture** | Normal | **Gait Balancing** | Normal |
| **Freezing Behaviour** | Absent | **Sings of Stress and Anxiety** | None observed |
| **Muscular coordination** | Normal | **Muscle grip** | Normal |
| **Sedation** | Absence | **Social Behavior** | Normal |
| **Urine Analysis** | No abnormality | **Urine Colour** | Yellowish |
| **Urine Ph** | 6 | **Urine - Glucose** | Absence |
| **Urine - Ketones** | Absence | **Urine- Bilirubin** | Absence |
| **Urine-Blood Cells** | Negative | **Urine - Pus cells** | Negative |
| **Mortality** | Nil | | |

**Quantitative data on the body weight of rats treated with *(TAM)* in Acute toxicity study**

| TAM | Body wt in gms | |
|---|---|---|
| | Initial | Final |
| **Mean** | **180.3** | **187.2** |
| **Std. Deviation** | **2.944** | **3.189** |
| **Std. Error** | **1.202** | **1.302** |

Values are mean ± S.D (n = 6 per group). Statistical significance carried out using one-way ANOVA followed by Dunnett's test.

**Assessment of clinical signs in rats treated with *(TAM) on* Sub-Acute toxicity study**

| | SUB ACUTE TOXICITY STUDY | | |
|---|---|---|---|
| **Parameter** | **CONTROL** | **LOW DOSE** | **HIGH DOSE** |
| **Clinical Signs Parameters for the duration of 28 days** | Normal Saline | **TAM 200 mg/kg** | **TAM 400 mg/kg** |
| **Lacrimation** | Absence | Absence | Absence |
| **Salivation** | Absence | Absence | Absence |
| **Animal appearance** | Normal | Normal | Normal |
| **Tonic Movement** | Absence | Absence | Absence |
| **Clonic Movement** | Absence | Absence | Absence |
| **Laxative action** | Absence | Absence | Absence |
| **Touch Response** | Normal | Normal | Normal |
| **Response to Sound** | Normal Response | Normal Response | Normal Response |
| **Response to Light** | Normal Response | Normal Response | Normal Response |
| **Mobility** | Normal Response | Normal Response | Normal Response |
| **Resp.Distress** | Nil | Nil | Nil |
| **Skin Color** | Normal | Normal | Normal |

| | | | |
|---|---|---|---|
| **Stereotype behavior** | Absence | Absence | Absence |
| **Piloerection** | Absence | Absence | Absence |
| **Limb Paralysis** | Absence | Absence | Absence |
| **Posture** | Normal | Normal | Normal |
| **Open field behavior** | Normal | Normal | Normal |
| **Gait Balancing** | Normal | Normal | Normal |
| **Freezing Behaviour** | Absent | Absent | Absent |
| **Sings of Stress and Anxiety** | None Observed | None Observed | None Observed |
| **Muscular coordination** | Normal | Normal | Normal |
| **Muscle grip** | Normal | Normal | Normal |
| **Sedation** | Absence | Absence | Absence |
| **Social Behavior** | Normal | Normal | Normal |
| **Urine Analysis** | No Abnormality | No Abnormality | No Abnormality |
| **Urine Colour** | Yellowish | Yellowish | Yellowish |
| **Urine pH** | 7 | 6 | 6 |
| **Urine -Glucose** | Absence | Absence | Absence |
| **Urine -Ketones** | Absence | Absence | Absence |
| **Urine- Bilirubin** | Absence | Absence | Absence |
| **Urine-Blood Cells** | Negative | Negative | Negative |
| **Urine - Pus cells** | Negative | Negative | Negative |
| **Mortality** | Nil | Nil | Nil |

**Effect of *(TAM) on* Body weight of Rats in Sub-acute toxicity study.**

| **Group** | **TAM** | |
|---|---|---|
| **Control** | **Body wt in gms** | |
| | **Initial** | **Final** |
| **Mean** | 184.5 | 218.8 |
| **Std. Deviation** | 1.871 | 5.636 |
| **Std. Error** | 0.7638 | 2.301 |
| | **Body wt in gms** | |
| **TAM Low Dose** | **Initial** | **Final** |
| **Mean** | 184.8 | 219.7 |
| **Std. Deviation** | 3.92 | 8.548 |
| **Std. Error** | 1.6 | 3.49 |
| | **Body wt in gms** | |
| **TAM High Dose** | **Initial** | **Final** |
| **Mean** | 183.8 | 216.8 |
| **Std. Deviation** | 2.787 | 4.622 |
| **Std. Error** | 1.138 | 1.887 |

Values are mean ± S.D (n = 6 per group of which 3 males and 3 females). Control and treatment groups were compared statistically using one-way ANOVA followed by Dunnett's test.

**Quantitative data on food intake of rats treated with *TAM)* for 28 days in Sub-acute toxicity study.**

| **Average Feed Intake in gms** | **Control** |
|---|---|
| 18.17 | **Mean** |
| 0.7528 | **Std. Deviation** |
| 0.3073 | **Std. Error** |
| **Average Feed Intake in gms** | **TAM Low Dose** |
| 15.83 | **Mean** |
| 1.472 | **Std. Deviation** |
| 0.6009 | **Std. Error** |
| **Average Feed Intake in gms** | **TAM High Dose** |
| 16.67 | **Mean** |
| 1.862 | **Std. Deviation** |
| 0.7601 | **Std. Error** |

Values are mean ± S.D (n = 6 per group of which 3 males and 3 females). Control and treatment groups were compared statistically using one-way ANOVA followed by Dunnett's test.

**Quantitative data on water intake of rats treated with *(TAM)* for 28 days in Sub-acute toxicity study.**

| **Control** | **Average Water Intake in ml** |
|---|---|
| **Mean** | 23.67 |
| **Std. Deviation** | 3.882 |
| **Std. Error** | 1.585 |
| **TAM Low Dose** | Average Water Intake in ml |
| **Mean** | 24.67 |
| **Std. Deviation** | 2.944 |
| **Std. Error** | 1.202 |
| **TAM High Dose** | Average Water Intake in ml |
| **Mean** | 24.83 |
| **Std. Deviation** | 3.971 |
| **Std. Error** | 1.621 |

Values are mean ± S.D (n = 6 per group of which 3 males and 3 females). Control and treatment groups were compared statistically using one-way ANOVA followed by Dunnett's test.

**Quantitative data on absolute Organ weight of control group rats.**

| **Con male** | **Brain** | **Heart** | **Lung** | **Stomach** | **Liver** | **Spleen** | **Kidney** | **Testes** | **Uterus** | **Ovary** |
|---|---|---|---|---|---|---|---|---|---|---|
| Mean | 1.757 | 0.57 | 1.378 | 1.458 | 4.641 | 0.6254 | 1.102 | 1.366 | | |
| Std.Dev | 0.01541 | 0.1716 | 0.1599 | 0.1754 | 0.3403 | 0.3935 | 0.2501 | 0.4483 | | |
| Std. Error | 0.008895 | 0.09907 | 0.09229 | 0.1013 | 0.1965 | 0.2272 | 0.1444 | 0.2588 | | |
| **Con female** | **Brain** | **Heart** | **Lung** | **Stomach** | **Liver** | **Spleen** | **Kidney** | **Testes** | **Uterus** | **Ovary** |
| Mean | 1.398 | 0.4593 | 1.394 | 1.286 | 4.387 | 0.602 | 0.8334 | | 0.2232 | 0.05983 |
| Std. Dev | 0.1652 | 0.08049 | 0.1775 | 0.2363 | 1.012 | 0.1188 | 0.2198 | | 0.09344 | 0.00948 |
| Std. Error | 0.09537 | 0.04647 | 0.1025 | 0.1364 | 0.5845 | 0.06861 | 0.1269 | | 0.05395 | 0.00547 |

Values are mean ± S.D (n = 3 per group of which 3 males and 3 females). Control and treatment groups were compared statistically using one-way ANOVA followed by Dunnett's test.

**Quantitative data on absolute Organ weight of treatment group rat.**

| **L.D.M** | **Brain** | **Heart** | **Lung** | **Stomach** | **Liver** | **Spleen** | **Kidney** | **Testes** | **Uterus** | **Ovary** |
|---|---|---|---|---|---|---|---|---|---|---|
| Mean | 1.612 | 0.5821 | 1.282 | 1.268 | 4.06 | 0.3286 | 1.212 | 1.738 | | |
| Std. Dev | 0.1276 | 0.1031 | 0.08642 | 0.2988 | 0.6671 | 0.02945 | 0.1127 | 0.3389 | | |
| Std. Error | 0.07366 | 0.05952 | 0.04989 | 0.1725 | 0.3851 | 0.017 | 0.06508 | 0.1957 | | |
| L.D.F | Brain | Heart | Lung | Stomach | Liver | Spleen | Kidney | Testes | Uterus | Ovary |
| Mean | 1.582 | 0.587 | 1.856 | 1.547 | 4.075 | 0.3853 | 1.011 | | 0.1487 | 0.09447 |
| Std. Dev | 0.04367 | 0.134 | 0.9227 | 0.2947 | 0.6306 | 0.143 | 0.1353 | | 0.04568 | 0.01417 |
| Std. Error | 0.02521 | 0.07735 | 0.5327 | 0.1702 | 0.3641 | 0.08259 | 0.07809 | | 0.02637 | 0.00818 |
| **H.D.M** | **BRAIN** | **HEART** | **LUNG** | **STOMACH** | **LIVER** | **SPLEEN** | **KIDNEY** | **TESTES** | **UTERUS** | **OVARY** |
| Mean | 1.646 | 0.5324 | 0.9496 | 1.456 | 3.945 | 0.3042 | 1.183 | 1.089 | | |
| Std. Dev | 0.1004 | 0.08777 | 0.1087 | 0.1809 | 0.1769 | 0.05838 | 0.239 | 0.4323 | | |
| Std. Error | 0.05795 | 0.05067 | 0.06277 | 0.1045 | 0.1022 | 0.03371 | 0.138 | 0.2496 | | |
| H.D.F | Brain | Heart | Lung | Stomach | Liver | Spleen | Kidney | Testes | Uterus | Ovary |
| Mean | 1.51 | 0.6078 | 1.741 | 1.591 | 5.556 | 0.8579 | 1.141 | | 0.3075 | 0.4794 |
| Std. Dev | 0.2122 | 0.06278 | 0.2962 | 0.3681 | 0.9132 | 0.5153 | 0.1853 | | 0.08281 | 0.7056 |
| Std. Error | 0.1225 | 0.03625 | 0.171 | 0.2125 | 0.5272 | 0.2975 | 0.107 | | 0.04781 | 0.4074 |

L.D.M - Low Dose Male, L.D.F- Low Dose Female, H.D.M - High Dose Male, H.D.F- High Dose Female Values are mean ± S.D (n = 3 per group of which 3 males and 3 females). Control and treatment groups were compared statistically using one-way ANOVA followed by Dunnett's test.

**Effect of Siddha formulation TAM on Haematology profile of rats in sub-acute toxicity study.**

| Control | RBC ($\times 10^6$ µl) | WBC1 ($\times 10^3$ µl) | PLT ($\times 10^3$ µl) | HGB (g/dl) | MCH (pg) | MCV (fl) |
|---|---|---|---|---|---|---|
| Mean | 5.867 | 7.55 | 720.7 | 13.25 | 18.88 | 56.12 |
| Std. Deviation | 1.226 | 1.696 | 71 | 0.8044 | 3.056 | 3.294 |
| Std. Error | 0.5004 | 0.6922 | 28.99 | 0.3284 | 1.248 | 1.345 |
| Low dose | Rbc ($\times 10^6$ µl) | Wbc ($\times 10^3$ µl) | Plt ($\times 10^3$ µl) | Hgb (g/dl) | Mch (pg) | Mcv (fl) |
| Mean | 6.733 | 10.78 | 813.5 | 12.25 | 18.6 | 56.13 |
| Std. Deviation | 1.082 | 2.456 | 230.7 | 1.95 | 1.364 | 2.887 |
| Std. Error | 0.4417 | 1.002 | 94.17 | 0.796 | 0.556 | 1.129 |
| High dose | Rbc ($\times 10^6$ µl) | Wbc ($\times 10^3$ µl) | Plt ($\times 10^3$ µl) | Hgb (g/dl) | Mch (pg) | Mcv (fl) |
| Mean | 7.15 | 8.6 | 756.8 | 12.9 | 19.92 | 59.7 |
| Std. Deviation | 1.228 | 2.661 | 63.23 | 1.765 | 2.477 | 5.279 |
| Std. Error | 0.501 | 1.086 | 25.81 | 0.7206 | 1.011 | 2.155 |

Values are mean ± S.D (n = 6 per group of which 3 males and 3 females). Control and treatment groups were compared statistically using one-way ANOVA followed by Dunnett's test.

**Effect of Siddha formulation TAM on Haematology profile of rats in sub-acute toxicity study.**

| Control | Neutrophils $10^3$/mm $^3$ | Eosinophils (%) | Basophils (%) | Lymph (%) | Mon(%) |
|---|---|---|---|---|---|
| Mean | 2.917 | 1.383 | 0.1667 | 78.32 | 4.4 |
| Std. Deviation | 0.5269 | 0.1722 | 0 .4082 | 8.401 | 0.613 |
| Std. Error | 0.2151 | 0.0703 | 0 .1667 | 3.43 | 0.250 |
| Low Dose | Neutrophils $10^3$/mm $^3$ | Eosinophils (%) | Basophils (%) | Lymph (%) | Mon (%) |
| Mean | 2.4 | 1.733 | 0 | 77.62 | 3.267 |
| Std. Deviation | 0.3347 | 0.1366 | 0 | 11.57 | 1.418 |
| Std. Error | 0.1366 | 0.0557 | 0 | 4.725 | 0.578 |
| High Dose | Neutrophils $10^3$/mm $^3$ | Eosinophils (%) | Basophils (%) | Lymph (%) | Mon (%) |
| Mean | 2.017 | 1.517 | 0 | 83.72 | 3.55 |
| Std. Deviation | 0.2483 | 0.3312 | 0 | 5.324 | 1.898 |
| Std. Error | 0.1014 | 0.1352 | 0 | 2.173 | 0.7749 |

**ABSTRACT**

In *Siddha* system of medicine, thousands of raw drugs are used to treat various kinds of diseases. These drugs are categorized into four groups namely Metals, Minerals, Herbals and Animal products. But in certain life-threatening diseases and especially in many chronic diseases, the herbal medicines alone have not been much effective. Herbal and herbo mineral preparations are being traditionally used in Indian system of Medicine especially in *Siddha* system. According to the Shelf life Herbo mineral preparations have more actions then any other drugs. Here ***THEER ANDHASKHAN MATHIRAI(TAM)*** is prepared as per *Siddha* classical text book Anuboga vaithiya Navaneetham, part 4, page no:105 – 106 to treat RA. Before clinical study there should be a need to undergone Preclinical trial as per WHO guidelines . The Clinical study was approved by Institutional Ethics Committee (IEC) and the approval number is **IEC No: GSMC-CH-ME-5/004/2018**. It was registered in Clinical Trials Registry-India (CTRI) and the registered number is CTRI/2019/08/020797.The present preclinical study aims to carry out safety and toxicity of the experimental protocol *Theer Andhaskhan Mathirai* was approved by Institutional Animal Ethics Committee **(IAEC)** of Sathyabama University, Chennai, Tamil Nadu, India. The approval number is **SU/CLATR/IAEC/XIII/125/2019.** Acute & Sub acute toxicity studies were carried out as per OECD guidelines 423 and 407. Hematological Parameters, biochemical parameters histopathological studies were performed for all animals. The study concludes that on oral administration of 100mg/kg of bodyweight of TAM to Wistar albino rats, there was no change in behaviour movements and no characteristic clinical sign of toxicity or mortality observed. From the result it was concluded that the Siddha preparation TAM offers wide margin of safety in tested rodents and further long term usage of drugs will be considered as safe for the ailment of various disease.

**KEYWORD:** Siddha, OECD guidelines, Acute, Sub-acute, TAM, IAEC.

Values are mean ± S.D (n = 6 per group of which 3 males and 3 females). Control and treatment groups were compared statistically using one-way ANOVA followed by Dunnett's test.

**Effect of Siddha formulation TAM on Serum Bio-chemistry profile of rats in sub-acute toxicity study.**

| Control | BUN (mg/dl) | Serum Creatinine (mg/dl) | Total Bilirubin (mg/dl) | SGOT (IU/ml) | SGPT (IU/ml) |
|---|---|---|---|---|---|
| Mean | 15.33 | 0.666 | 0.45 | 87 | 26.83 |
| Std. Deviation | 3.502 | 0.163 | 0.1975 | 17.5 | 12.06 |
| Std. Error | 1.43 | 0.066 | 0.080 | 7.146 | 4.922 |
| Low Dose | BUN (mg/dl) | Serum Creatinine (mg/dl) | Total Bilirubin (mg/dl) | SGOT (IU/ml) | SGPT (IU/ml) |
| Mean | 13.83 | 0.6 | 0.3833 | 92.5 | 24.83 |
| Std. Deviation | 3.061 | 0.063 | 0.1472 | 10.54 | 4.792 |
| Std. Error | 1.249 | 0.025 | 0.060 | 4.303 | 1.956 |
| High Dose | BUN (mg/dl) | Serum Creatinine (mg/dl) | Total Bilirubin (mg/dl) | SGOT (IU/ml) | SGPT (IU/ml) |
| Mean | 15.67 | 0.816 | 0.333 | 81.83 | 21.5 |
| Std. Deviation | 3.386 | 0.040 | 0.121 | 6.555 | 3.886 |
| Std. Error | 1.382 | 0.016 | 0.049 | 2.676 | 1.586 |

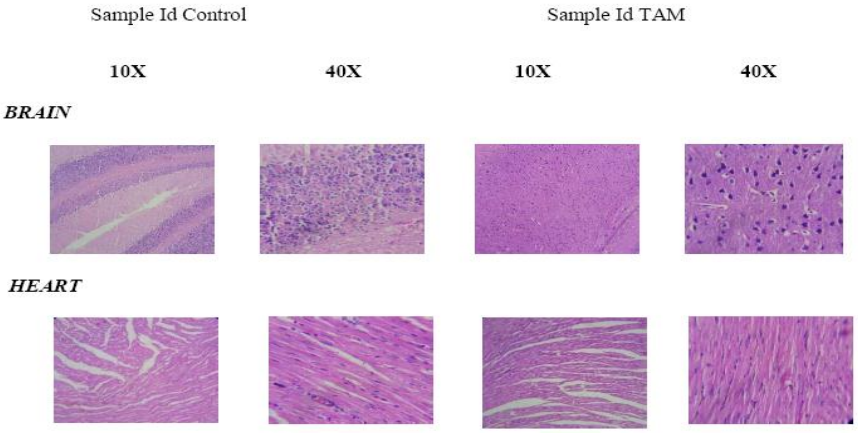
Values are mean ± S.D (n = 6 per group of which 3 males and 3 females). Control and treatment groups were compared statistically using one-way ANOVA followed by Dunnett's test.

**Effect of Siddha formulation TAM on Serum Bio-chemistry profile of rats in sub-acute toxicity study.**

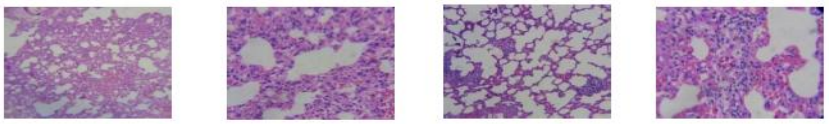
| Control | Total cholesterol (mg/dl) | HDL (mg/dl) | LDL (mg/dl) | VLDL (mg/dl) | TG (mg/dl) |
|---|---|---|---|---|---|
| Mean | 119.4 | 52.83 | 50 | 16.58 | 32.67 |
| Std. Deviation | 6.972 | 4.021 | 5.292 | 1.329 | 7.394 |
| Std. Error | 2.846 | 1.641 | 2.16 | 0.5425 | 3.018 |
| Low Dose | Total cholesterol (mg/dl) | HDL (mg/dl) | LDL (mg/dl) | VLDL (mg/dl) | TG (mg/dl) |
| Mean | 121.2 | 57 | 47.5 | 16.67 | 29.83 |
| Std. Deviation | 8.802 | 5.404 | 6.221 | 3.33 | 9.152 |
| Std. Error | 3.596 | 2.206 | 2.54 | 1.359 | 3.736 |
| High Dose | Total cholesterol (mg/dl) | HDL (mg/dl) | LDL (mg/dl) | VLDL (mg/dl) | TG (mg/dl) |
| Mean | 126.7 | 63 | 49.5 | 16.15 | 28.17 |
| Std. Deviation | 10.22 | 6.663 | 11.74 | 3.289 | 6.338 |
| Std. Error | 4.172 | 2.72 | 4.794 | 1.343 | 2.587 |

Values are mean ± S.D (n = 6 per group of which 3 males and 3 females). Control and treatment groups were compared statistically using one-way ANOVA followed by Dunnett's test.

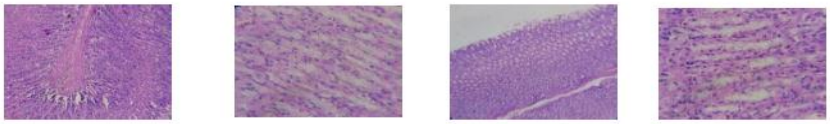
***Histopathology of Male*** **{Low Power Magnification 10X, High Power Magnification 40X}**



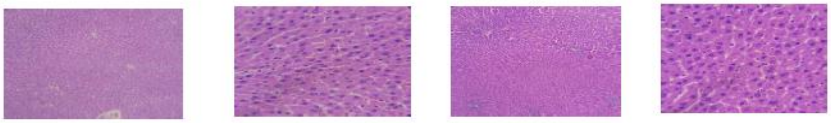
*LUNG*



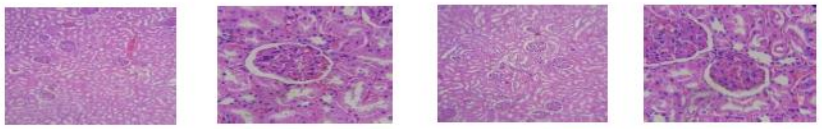
*STOMACH*



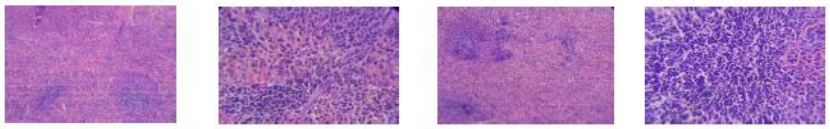
*LIVER*



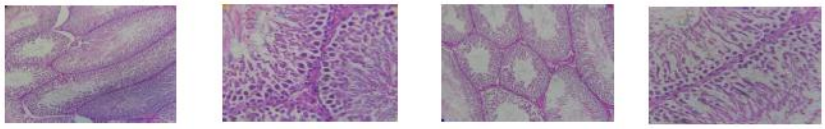
*KIDNEY*



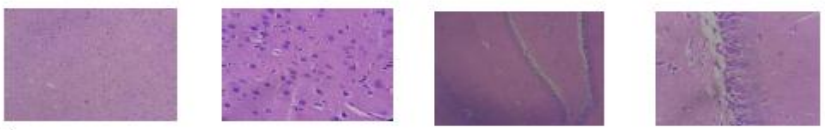
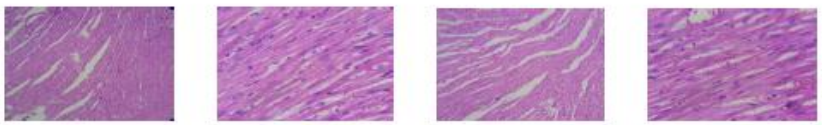
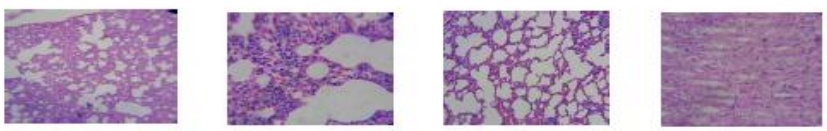
*SPLEEN*

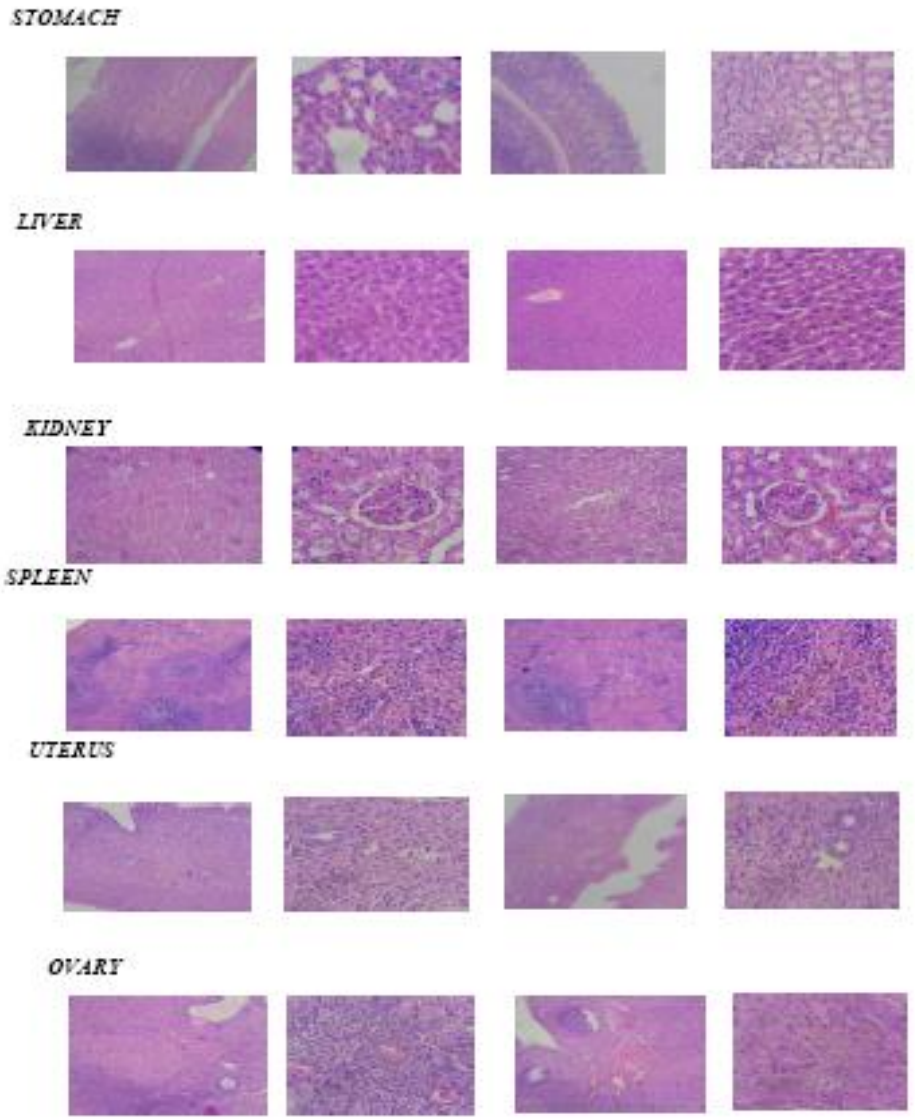


*TESTES*



***Histopathology of Female*** **{Low Power Magnification 10X High Power Magnification 40X}**

| | Sample Id: Control | | Sample Id: TAM | |
|---|---|---|---|---|
| | 10X | 40X | 10X | 40X |
| *BRAIN* |  | | | |
| *HEART* |  | | | |
| *LUNG* |  | | | |




## DISCUSSION

Toxicological evaluation of Siddha formulation *Theer Andhaskhan Mathirai* (TAM) has provided an evidence-based data with respect to C.N.S, A.N.S and C.V.S system on the tested animals. In acute toxicity study Siddha formulation TAM administered at the dose of 2000 mg/kg had no adverse effect on the treated rats in up to 14 days of observation. No significant change in the body weight, behavioral and sensory parameters were observed in acute toxicity study.

In acute toxicity study, there was no mortality up to a maximum dose of 2000 mg/kg body weight of TAM after per oral administration. The changes in bodyweight and other Clinical signs like skin color change, fecal consistency, gait analysis, urine analysis, sensory responses, animal behavior abnormalities, neuro muscular coordination have been used as an indicator of adverse effect. Since no remarkable changes were observed in animal behavior, body weight and organ weight at dose in treated rats as compared to control group, it can be inferred that Siddha formulation TAM is nontoxic at the administered dose of 2000mg/kg.

In sub-acute toxicity study treatment with TAM at 200 and 400 mg/kg reveals no significant change in the body weight of the treated rats. The low and high dose selected for toxicity covers the average therapeutic dosage of the test drug TAM in humans. Results of the study reveals that 28-day daily dose treatment with the TAM elicited no clinical signs of toxicity, morbidity, or mortality across all the treatment groups hence it may have concluded that the formulation TAM is safe at the tested doses over the observation period.

Results of the present investigation showed that there was no sign of toxicity and no mortality after single and repeated administration *of the test drug TAM at varying doses in tested rats.* There was no significant difference in mean body weight, food/water intake, behavioral, C.N.S, C.V.S, A.N.S vitals in control and test group rats. Further no changes in the gross observation of all the vital organs in both male and female rats. Single and repeated oral administration of the Siddha drug TAM

may be safe and considered as relatively non-toxic at both the doses levels of 200 and 4000 mg/kg dose level.

## CONCLUSION

In **acute toxicity study,** the dose of Theer Andhaskhan Mathirai (TAM) used for acute toxicity study is 2000mg/kg is higher than the normal therapeutic dose. No mortality observed at this dose level, further no significant change with respect to clinical signs on acute toxicity observed for (24-48 h) and for a period of 14 days. There is no significant change in the body weight, organ weight and gross observational changes of the treated animals which confirms the wide margin of safety of the study drug.

In **sub-acute toxicity study,** treatment with TAM at 200 and 400 mg/kg reveals no significant change in the body weight of the treated rats. The low and high dose selected for toxicity covers the average therapeutic dosage of the test drug TAM in humans. Results of the study reveals that 28-day daily dose treatment with the TAM elicited no clinical signs of toxicity, morbidity, or mortality across all the treatment groups hence it may have concluded that the formulation TAM is safe at the tested doses over the observation period.

## ACKNOWLEDGEMENT

I wish to acknowledge my thanks to The Tamil Nadu Dr. M.G.R. Medical University,Chennai, Tamil Nadu, India and ***Sathyabama Institute of Science and Technology, Chennai 600 119, Tamil Nadu, India.***

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