PRELIMINARY ACUTE TOXICOLOGICAL SCREENING OF A NOVEL SIDDHA METALLO-MINERAL FORMULATION “KAALAMEGA NARAYANA CHENDHOORAM” AS MENTIONED IN “ATHMARAKSHA MIRTHAM ENNUM VAITHIYA SAARA SANGERAHAM” IN WISTAR ALBINO FEMALE RATS AS PER OECD GUIDELINES 423.

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ABSTRACT

Background: Kaalamega Narayana Chendhooram(KMNC) was a novel siddha formulation, had a indication for cancer as mentioned in the siddha classical literature Athmaraksha Mirtham Ennum Vaithiya Saara Sangeraham. Aim and Objective: The aim of the present study is to validate the the safety of a novel siddha metallo-mineral formulation Kaalamega Narayana Chendhooram as mentioned in Athmaraksha Mirtham Ennum Vaithiya Saara Sangeraham through a acute toxicological studies as per OECD guigelines 423. Methods: Administration of a natural medicines is used to cure the diseases of the human beings from the time immemorial. Every civilization had its own medicine and own tradition, they were considered as a traditional medicines. Siddha system of medicine was a one of the traditional system of medicine, which have been practiced by the south Dravidian people of the South India. This system of medicine contains flora, fauna, metals and minerals in their medicinal
preparations. The current scientific committee of the World insisting that the administration of metals and minerals to human usage must be undergo the toxicity studies. *Kaalamega Narayana Chendhooram (KMNC)* was the one of the higher order medicine in Siddha formulation contains heavy metals in their formulation. As per the safety regulations drug should be proved its short and long term safety in rodents before extrapolating its clinical benefits in humans. Present investigation aimed at evaluating the safety profile of the test drug *KMNC* by acute oral toxicity in female wistar rats in accordance with OECD regulatory guidelines. In the acute study, a single dose of 2000 mg/kg was orally administered and animals were monitored for 14 days. Then the results of toxicity study was noted. In this paper an attempt was made to screen the acute toxicological studies of *KMNC* as per OECD 423 guidelines. **Results:** At the end of this preliminary acute toxicological research study, that the trail drug of a novel siddha metallo-mineral formulation *Kaalamega Narayana Chendhooram* as mentioned in *Athmaraksha Mirtham Ennum Vaiithiya Saara Sangeraham* in acute toxicity study in Wistar rats showed that there were no significant toxicity changes seen during the 14 days of study. The body weight, food, water intake, behavioral, changes were slightly changes but they are within the non significant in compared with the control group. There was no mortality rate seen at 14 days of observation. **Conclusion:** The present preliminary acute toxicological research study of a potent siddha metallo-mineral formulation *Kaalamega Narayana Chendhooram* as mentioned in *Athmaraksha Mirtham Ennum Vaiithiya Saara Sangeraham* showed some changes in the report because of its anticancerous effect, but the changes are non significant in compared with control no mortality rate was observed during the course of this study.

**KEYWORDS:** *Kaalamega Narayana Chendhooram, KMNC, Chendhooram, Siddha, metallo-mineral formulation, OECD guidelines, Acute toxicity 423.*

**INTRODUCTION**

Siddha system of medicine is a unique system among Indian medical systems, a boon of Siddhars offered to dravidan people of South India. It contains flora, fauna, metals and minerals in their medicinal preparation. It plays a major role in treating acute and chronic ailments.\(^1\)

The unique formulations in Siddha include Parpam (mineral/metallic oxides), Chendhooram (mineral/metallic sulphides), Chunnam (caustic or major oxides) and Pathangam (sublimation). *KMNC* is a *chendhooram* that belongs to category of metallic sulphides.\(^2\)
Toxicology is the study of the adverse effects of chemical or physical agents on living organisms. Our society’s dependence on chemicals and the need to assess potential hazards have made toxicologists an increasingly important part of the decision-making processes. Regulatory requirements have strongly recommending the need of toxicological profiling of medicinal preparations before intended to use in humans for clinical efficacy. Hence it becomes highly essential for a drug to prove its safety and efficacy before prescribing the same for clinical application. KMNC is one of the higher order Siddha formulation had a wide variety of metals and minerals in their formulation had a indication for Cancer. The main aim of the present research work is to evaluate the safety of the drug KMNC in Acute toxicological study in Wistar albino female rat for 14 days of observation as per OECD guidelines.

Hence, the traditionally practised medicine has to be validated by the modern techniques to prove the safety of the drug. So, in this study, an effort was made to evaluate toxicity of the metallo-mineral formulation KMNC.

MATERIALS AND METHODS

SELECTION OF THE DRUG

For this present study, the metallo-mineral formulation “KAALAMEGA NARAYANA CHENDHOORAM” was taken as the compound drug preparation for oral cancer mentioned in the classical Siddha literature “Athmarakshamirthham Ennum Vaithiya Saara Sangeraham” written by Kandhasamy Mudhaliyaar, pg no:493, First Edition 1931.[3]

Ingredients of the drug
1. Purified Vediuppu [Potassium nitrate] – 840 gm
2. Purified Thurusu [Cupric sulphate] – 210 gm
3. Purified Padikaaram [Aluminium potassium sulphate (Alum)] – 840 gm
4. Purified Vengaram [Sodium bicarbonate (Borax)] – 210 gm
5. Purified Navacharam [Ammonium Chloride]-210gm
6. Purified Pooneeru [Impure Sodium Carbonate (Fullers Earth)] – 105 gm
7. Purified Jaathilingam [Red sulphate of mercury]-525gm
9. Purified Kalluppu [Sodium chloride]- 210 gm
10. Purified Rasam [Hydragyrum] – 1050 gm
11. Purified Aritharam [Tri sulphate of Arsenic (Yellow Orpiment)]- 350 gm
12. Purified Manosilai [Di sulphate of Mercury (Red Orpiment)]- 140gm

**Collection of the raw materials**

All the raw materials were purchased from R.N. Rajan country drug store, Parrys corner, Chennai.

**Identification and Authentication of the drug**

The raw materials were identified and authenticated by the experts of Gunapadam, Government Siddha Medical College, Arumbakkam, Chennai- 106.

The specimen sample of each raw material has been kept in the PG Gunapadam department individually for future reference.

**Purification of the drugs**

Purification process was done as per the classical Siddha literature.

1. **Purification of Pottasium Nitrate (Vediuppu)**

**Materials Required**

<table>
<thead>
<tr>
<th>Material</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salt</td>
<td>100 gm</td>
</tr>
<tr>
<td>Water</td>
<td>400 gm</td>
</tr>
<tr>
<td>Fermented butter milk</td>
<td>100 gm</td>
</tr>
<tr>
<td>Lime juice</td>
<td>100 gm</td>
</tr>
</tbody>
</table>

**Procedure**

Water was added to the pottasium nitrate and boiled on a hearth with mild flames. The white yolk of eggs (4 nos) were added to every 1400gm of salt and the bubbles thus appeared with impure substances were removed with wooden spoon.

The ingredients were then transferred to another pot, sealed with mud pasted cloth, filtered and transferred to another pot, sealed with mud pasted cloth, filtered and kept in places without aeration. Next day the water was filtered and salt was sun shade. This process was repeated for seven times to get it purified.

2. **Purification of Padikaaram (Aluminium potassium sulphate (Alum))**

The alum was dissolved in water and it was filtered, boiled. Then it was cooled to get purified form.
3. Purification of Thurusu (Copper sulphate)
The copper sulphate was fried, till it turns to whitish.

4. Purification of Vengaram (Sodium biborate)
Borax was bundled and hanged in the buffalo’s dung solution and boiled. The bundle was cleaned with fresh water and insolated to get it in purified form.

5. Purification of Navacharam (Ammonium chloride)
Navacharam (Ammonium chloride) was dissolved in hot water and filtered. After it was cooled, it was poured in a broad mouthed vessel and insolated; the salt was formed in a purified form. It was preserved with small quantity of the root of jequirity in a bottle.

6. Purification of Kalluppu (Sodium chloride)
Kalluppu was dissolved in vinegar and clean with a cloth, dried in a sunshade.

7. Purification of Pooneeru (Impure Sodium Carbonate)
Fuller’s earth 1.3 litre was soaked in dew’s water 5.2 litres and allowed to settle. Next morning it was churned well and the outer cream layer was removed. The remaining mixture was in procelin plates and insolated to obtain purified form. This process was repeated for ten times and stored in a bottle.

8. Purification of Rasam (Mercury) Materials Required:

<table>
<thead>
<tr>
<th>Material</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mercury</td>
<td>- 35 gm</td>
</tr>
<tr>
<td>Brick powder</td>
<td>- 100 gm</td>
</tr>
<tr>
<td>Turmeric powder</td>
<td>- 100 gm</td>
</tr>
<tr>
<td>Acalypha juice (Acalypha indica)</td>
<td>- 1.3 litre</td>
</tr>
</tbody>
</table>

Procedure
Mercury was triturated with brick powder and turmeric powder for one hour respectively and washed with water. Then the Mercury was boiled with the juice of Indian Acalypha till the juice completely evaporates. And thus mercury was purified.

9. Purification of Lingam (Cinnabar)
Lime juice, cow’s milk and the Acalypha indica juice were mixed together in equal proportion and allowed to fuse Cinnabar so as to get it in a purified potent form.
10. Purification of Thaalagam (Yelow Orpiment): Materials required

<table>
<thead>
<tr>
<th>Material</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arsenic trisulphide</td>
<td>– 35 gm</td>
</tr>
<tr>
<td>Cow’s urine</td>
<td>– 1 litre</td>
</tr>
<tr>
<td>Indian acalypha juice</td>
<td>– 300 ml</td>
</tr>
<tr>
<td>Lime stone</td>
<td>– 300 gm</td>
</tr>
</tbody>
</table>

Procedure
Arsenic trisulphide was bundled and kept immersed in the mixture of limestone, *Acalypha indica* juice and cow’s urine and heated to get purified.

11. Purification of Gandhagam (sulfur)

Materials Required:

<table>
<thead>
<tr>
<th>Material</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sulphur</td>
<td>35 gm</td>
</tr>
<tr>
<td>Butter</td>
<td>35 gm</td>
</tr>
<tr>
<td>Cow’s milk</td>
<td>150 ml</td>
</tr>
</tbody>
</table>

Procedure
Sulphur was placed in an iron spoon. Butter was added and the spoon was heated till the butter melts, this mixture was immersed in inclined position in cow’s milk. The procedure was repeated for about 7 times and thus sulphur was purified. Fresh milk was used each time.

12. Purification of Manosilai (Red orpiment) Materials required

<table>
<thead>
<tr>
<th>Material</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red orpiment</td>
<td>35 gm</td>
</tr>
<tr>
<td>Cow’s butter milk</td>
<td>125 ml</td>
</tr>
</tbody>
</table>

Procedure
Red orpiment was triturated with cow’s butter milk for 3 hours. It was dried to get purified form.\(^4\)

Preparation of the trial drug – “KAALAMEGA NARAYANA CHENDHOORAM”

1. Purified Vediuppu [Potassium nitrate] – 840 gm
2. Purified Thurusu [Cupric sulphate] – 210 gm
3. Purified Padigaram [Aluminium potassium sulphate (Alum)] – 840 gm
4. Purified Vengaram [Sodium bicarbonate (Borax)] – 210 gm
5. Purified Navacharam [Ammonium Chloride]-210gm
6. Purified Pooneeru [Impure Sodium Carbonate (Fullers Earth)] – 105 gm
7. Purified Jaathilingam [Red sulphate of mercury]-525gm
9. Purified Kalluppu [Sodium chloride]- 210 gm
10. Purified Rasam [Hydragyrum(Mercury)] – 1050 gm
11. Purified Aritharam [Tri sulphate of Arsenic (Yellow Orpiment)]- 350 gm
12. Purified Manosilai [Di sulphate of Mercury (Red Orpiment)]- 140gm.

Procedure
- 840 gm of 8th solution of Vediuppu [Potassium nitrate] and Padigaram [Aluminium potassium sulphate (Alum)] were taken.
- Along with that, 210 gm of Thurusu [Cupric sulphate], Vengaram [Sodium bicarbonate (Borax)], Navacharam [Ammonium Chloride], Kalluppu [Sodium chloride Impura] were taken and then mixed with 105 gm of Pooneeru [(Impure Sodium Carbonate (Fullers Earth)].
- Above ingredients were ground into fine powder and divided into 3 parts.
- First part of the powder was underwent distillation process, the end product was mixed with 2nd part of powder and dried.
- Second part of the powder was underwent distillation process, the end product was mixed with 3rd part of powder and dried.
- Third part of the powder was undergoes distillation process, the final end product was taken and kept in a sealed bottle.
- The Jaathilingam [Red sulphate of mercury]-525 gm, Rasam (Mercury)-1050gm, Aritharam [Tri sulphate of Arsenic (Yellow orpiment)]-350 gm, Gandhagam [Sulphur] 420 gm, Manosilai [Di sulphate of mercury (Red Orpiment)] 140 gm wereground, along with the end product of distillation for 12 hours (4 saamam) and made into fine powder and dried.
- Dried powder was kept in a mud pot which was sealed with 7 mud pasted plaster.
- Another mud pot with small quantity of sand was taken and above preparation was kept into it and sealed the lid with mud pasted plaster.
- The mud pot was ignited by using Aavarai stick for 30 hours (10 saamam), after 30 hours “Chendhooram” was obtained.
Drug profile

<table>
<thead>
<tr>
<th>Drug name</th>
<th>Kaalamega Narayana Chendhooram</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dosage</td>
<td>244 mg of Chendhooram [1/2 Panavedai]</td>
</tr>
<tr>
<td>Route</td>
<td>Enteral (oral)</td>
</tr>
<tr>
<td>Adjuvant</td>
<td>Thipili chooranam with honey (bd for 48 days – 1 mandalam)</td>
</tr>
<tr>
<td>Indications</td>
<td>Kannaputru [ORAL CANCER], Elaippu [Tuberculosis], Kuttam 18 [Hansen’s Disease]</td>
</tr>
<tr>
<td>Reference</td>
<td>“AthmarakshaMirutham Ennum Vaithiya Saara Sangeeraham”,[13]</td>
</tr>
</tbody>
</table>

Purified Vediuppu [Potassium nitrate]

Purified Thurusu [Cupric sulphate]
Purified Padigaram [Aluminium potassium sulphate (Alum)]

Purified Vengaram [Sodium bicarbonate (Borax)]

Purified Navacharam [Ammonium Chloride]
Purified Kalluppu [Sodium chloride Impura]

Purified Pooneeru [Impure Sodium Carbonate (Fullers Earth)]

Purified Rasam [Hydragyrum]
Purified Jaathilingam [Red sulphate of mercury]

Purified Aritharam [Tri sulphate of Arsenic (Yellow orpiment)]

Purified Gandhagam [Sulphur]
Purified *Manosilai [Red Orphiment]*

Fig no 1: Ingredients of Kaalamega Narayana Chendhooram.

Process 1.

Preparing for *Thravagam*
Process 2.

Divided into 3 parts

Process 3.

1\textsuperscript{st} part undergoes distillation process

Collection of Thravagam

Process 4.

The obtained *Thravagam* was used grind the second part

Again the second part underwent distillation to process
Process 5.

The obtained *Thravagam* is used to grind the third part

Again the third part underwent distillation process

Process 6.

The end product of distillation was sealed in a bottle.

Process 7.
Grinding of prepared medicine

Process 8.

Final product was sealed with mud pasted cloth.

Process 9.

Ignition of final *Chendhooram*
TOXICOLOGICAL STUDY
ACUTE TOXIICY STUDY 423

Principle of the Test
It is the principle of the test that based on a stepwise procedure with the use of a minimum number of animals per step, sufficient information is obtained on the acute toxicity of the test substance to enable its classification. The substance is administered orally to a group of experimental animals at one of the defined doses. The substance is tested using a stepwise procedure, each step using three animals of a single sex. Absence or presence of compound-related mortality of the animals dosed at one step will determine the next step, i.e.
− no further testing is needed
− dosing of three additional animals, with the same dose
− dosing of three additional animals at the next higher or the next lower dose level. The method will enable a judgment with respect to classifying the test substance to one of a series of toxicity classes.

Animal
The experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC) under CPCSEA (IAEC approved Number: IAEC/XLVIII/13CLBMCP/2016) at C.L. Baid Metha College of pharmacy, Thuraipakkam, Chennai.

The Healthy adult Wistar albino rats were used for the study. The animals were housed in poly propylene cages and were kept in well ventilated with 100% fresh air. A 12 light / dark
cycle were maintained. Room temperature was maintained between 22°C (+3°C) and relative humidity 50–65%. They were provided with food (Sai feeds, Bangalore, India) and water *ad libitum*. Females should be nulliparous and non-pregnant. Animals may be grouped and tagged by dose, but the number of animals per cage must not interfere with clear observations of each animal. The animals are randomly selected, marked to permit individual identification, and kept in their cages for at least 7 days prior to dosing to allow for acclimatization to the laboratory conditions. The studies were conducted in the animal house of C.L. Baid Metha College of pharmacy, Thuraipakkam, Chennai.

**Acute oral toxicity – OECD Guidelines – 423**

Acute toxicity study was carried out as per OECD guideline (Organization for Economic Co-operation and Development) Guideline-423.

The animals were randomly divided into control group and treatment groups of 6 female wistar albino rats of 3 in each group. The animals were fasted overnight (12-16 hrs) with free access to water. Animals were fasted prior to dosing. Following the period of fasting, the animals were weighed and then the test substance was administered. After the substance has been administered, food was withheld for a further 3-4 hours. *Kaalamega Narayana Chendhooram* was prepared as per the classical Siddha literature was suspended in 2% CMC with uniform mixing and was administered to the groups of Wistar albino rats. Group I served as control and the study was conducted with single oral administration of study drug *Kaalamega Narayana Chendhooram* (*KMNC*) 2000mg/kg (p.o) by gavages using a feeding needle to the group II rats. The animals were observed continuously for first 72 hours and then 14 days for emerging signs of behavioral changes, body weight changes and for mortality. Occurrence of toxicity in animals were observed continuously for the first 4 to 24 hr and observed. Periodically for the next 14 days. Observation includes the change in skin, fur, eyes and mucus membrane. Appearance of C.N.S, C.V.S and A.N.S related toxicity such as tremors, convulsions, sedation, steric behavior, respiratory distress, cardiovascular collapse, response to sensory stimuli, salivation, diarrhea, lethargy, sleep, coma and mortality were observed with special attention for 14 days.

Finally, the number of survivors was noted after 24 hours and these animals were then maintained for a further 14 days and observations made daily. The toxicological effect was assessed on the basis of mortality.
Justification for choice of vehicle

The vehicle selected as per the standard guideline was pharmacologically inert and easy to employ for new drug development and evaluation technique.

Test Substance: *Kaalamega Narayana Chendhooram (KMNC)*

Animal Source: TANUVAS, Madhavaram, Chennai.

Animals: Wister Albino Rats (Female-3+3)

Age: 6-8 weeks

Body Weight on Day 0: 150-200gm.

Acclimatization: Seven days prior to dosing.

Veterinary examination: Prior and at the end of the acclimatization period.

Identification of animals: By cage number, animal number and individual marking by using Picric acid.

Number of animals: 3 Female/group,

Route of administration: Oral

Diet: Pellet feed supplied by Sai meera foods Pvt Ltd, Bangalore

Water: Aqua guard portable water in polypropylene bottles.

Housing & Environment: The animals were housed in Polypropylene cages provided with bedding of husk.

Housing temperature: between 22ºC + 3ºC.

Relative humidity: between 30% and 70%.

Air changes: 10 to 15 per hour and

Dark and light cycle: 12:12 hours.

Duration of the study: 14 Days

Body weight was recorded periodically. At the end of the experiment all animals were subjected for gross necropsy and observed for pathological changes.[5]

Statistical analysis

The statistical analysis will be 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 Dunnet’s test for the control and treatment group. P-values less than 0.05 were set as the level of significance.[6]
RESULTS AND DISCUSSIONS

ACUTE ORAL TOXICITY

Dose finding experiment and its behavioral Signs of Toxicity for Kaalamega Narayana Chendooram

Observation done

Effect of KMNC on clinical signs of rats in Acute Oral Toxicity Study

The dose of KMNC 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 a long period (14 days). The result of acute toxicity of Kaalamega Narayana Chendooram has been tabulated below Table 1.

Table no 1.

<table>
<thead>
<tr>
<th>SL</th>
<th>Group CONTROL</th>
<th>Observation</th>
<th>SL</th>
<th>Group TEST</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Body weight</td>
<td>Normal</td>
<td>1</td>
<td>Body weight</td>
<td>Normal</td>
</tr>
<tr>
<td>2</td>
<td>Assessments of posture</td>
<td>Normal</td>
<td>2</td>
<td>Assessments of posture</td>
<td>Normal</td>
</tr>
<tr>
<td>3</td>
<td>Signs of Convulsion, Limb paralysis</td>
<td>Absence</td>
<td>3</td>
<td>Signs of Convulsion Limb</td>
<td>Absence of sign (-)</td>
</tr>
<tr>
<td>4</td>
<td>Body tone</td>
<td>Normal</td>
<td>4</td>
<td>Body tone</td>
<td>Normal</td>
</tr>
<tr>
<td>5</td>
<td>Lacrimation</td>
<td>Normal</td>
<td>5</td>
<td>Lacrimation</td>
<td>Absent</td>
</tr>
<tr>
<td>6</td>
<td>Salivation</td>
<td>Normal</td>
<td>6</td>
<td>Salivation</td>
<td>Normal</td>
</tr>
<tr>
<td>7</td>
<td>Change in skin color</td>
<td>No significant color change</td>
<td>7</td>
<td>Change in skin color</td>
<td>No significant color change</td>
</tr>
<tr>
<td>8</td>
<td>Piloerection</td>
<td>Normal</td>
<td>8</td>
<td>Piloerection</td>
<td>Normal</td>
</tr>
<tr>
<td>9</td>
<td>Defecation</td>
<td>Normal</td>
<td>9</td>
<td>Defecation</td>
<td>Normal</td>
</tr>
<tr>
<td>10</td>
<td>Sensitivity response</td>
<td>Normal</td>
<td>10</td>
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<tr>
<td>11</td>
<td>Locomotion</td>
<td>Normal</td>
<td>11</td>
<td>Locomotion</td>
<td>Normal</td>
</tr>
<tr>
<td>12</td>
<td>Muscle gripness</td>
<td>Normal</td>
<td>12</td>
<td>Muscle gripness</td>
<td>Normal</td>
</tr>
<tr>
<td>13</td>
<td>Rearing</td>
<td>Mild</td>
<td>13</td>
<td>Rearing</td>
<td>Mild</td>
</tr>
<tr>
<td>14</td>
<td>Urination</td>
<td>Normal</td>
<td>14</td>
<td>Urination</td>
<td>Normal</td>
</tr>
</tbody>
</table>

Table no 2: Behavioural Signs of Toxicity for KMNC.

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

(+ Present, - Absent)

Table no: 3: (Body weight Observation).

<table>
<thead>
<tr>
<th>DOSE</th>
<th>DAYS 1</th>
<th>DAYS 7</th>
<th>DAYS 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL</td>
<td>280.2±42.30</td>
<td>281.4 ± 64.12</td>
<td>282.6 ±26.18</td>
</tr>
<tr>
<td>HIGH DOSE</td>
<td>279.4± 21.24</td>
<td>279± 3.64</td>
<td>279.4 ±2</td>
</tr>
<tr>
<td>P value (p)*</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS- Significant, **(p < 0.01), *(p <0.05), values are mean ± S.D (One way ANOVA followed by Dunnett’s test)

Effect of KMNC on Body weight of rats in acute toxicity study
No significant change was observed in body weight of female rats treated with KMNC at the dose of 2000mg/ kg. The results were tabulated in Table 3.

Table no 4: (Water intake (ml/day) of Wistar albino rats group exposed to Kaalamega Narayana Chendooram.

<table>
<thead>
<tr>
<th>DOSE</th>
<th>DAYS 1</th>
<th>DAYS 7</th>
<th>DAYS 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL</td>
<td>61± 1.12</td>
<td>62±2.22</td>
<td>62.9±1.14</td>
</tr>
<tr>
<td>HIGH DOSE</td>
<td>61.2±1.1</td>
<td>61±1.14</td>
<td>61.2±24</td>
</tr>
<tr>
<td>P value (p)*</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

N.S- Not Significant, **(p < 0.01), *(p <0.05), values are mean ± S.D (One way ANOVA followed by Dunnett’s test)

Effect of KMNC on Water intake of rats in acute toxicity study
No significant change was observed in Water intake of female rats treated with KMNC at the dose of 2000mg/ kg. The results were tabulated in Table 4.

Table no 5: Food intake (gm/day) of Wistar albino rats group exposed to Kaalamega Narayana Chendooram.

<table>
<thead>
<tr>
<th>DOSE</th>
<th>DAYS 1</th>
<th>DAYS 7</th>
<th>DAYS 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL</td>
<td>56.24±2.22</td>
<td>56.2±7.42</td>
<td>57.4±3.46</td>
</tr>
<tr>
<td>High dose</td>
<td>56.6±1.63</td>
<td>55.6±2.62</td>
<td>55.1±5.38</td>
</tr>
<tr>
<td>P value (p)*</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

N.S- Not Significant, **(p < 0.01), *(p <0.05), values are mean ± S.D (One way ANOVA followed by Dunnett’s test)
Effect of KMNC on Food intake of rats in acute toxicity study

No significant change was observed in Food intake of female rats treated with KMNC at the dose of 2000mg/kg. The results were tabulated in Table 5.

Acute toxicity Discussion

Observations

Animals are observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours, and daily thereafter, for a total of 14 days, except where they need to be removed from the study and humanely killed for animal welfare reasons or are found dead. It should be determined by the toxic reactions, time of onset and length of recovery period, and may thus be extended when considered necessary. The times at which signs of toxicity appear and disappear are important, especially if there is a tendency for toxic signs to be delayed. All observations are systematically recorded with individual records being maintained for each animal.

Mortality

Animals will be observed intensively at 0.5, 2.0, 4.0, 6.0, 12.0, 24.0 and 48.0 hours following drug administration on day 1 of the experiment and daily twice thereafter for 14 days. No mortality rate was observed for 14 days.

Body weight

Individual weight of animals was determined before the test substance was administered and weights will be recorded at day 1, 7, and 14 of the study. Weight changes were calculated and recorded. At the end of the test, surviving animals were weighed and humanly killed. The changes observed were non significant.

Cage-side observation

These include changes in skin and fur, eyes and mucous membranes and also respiratory, circulatory, autonomic and central nervous systems, somatomotor activity and behavioral patterns. Attention should be directed to observations of tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma. No obvious observation were seen.

Data and reporting

All data were summarized in tabular form, showing for each test group the number of animals used, the number of animals displaying signs of toxicity, the number of animals found dead
during the test, description of toxic symptoms, weight changes, food and water intake. The changes observed during the studies were non significant.

In the acute toxicity study, the rats were treated with the concentration of *Kaalamega Narayana Chendooram* in the range of 2000mg/kg as per OECD guidelines.

This dose level did not produce the signs of toxicity, behavioral changes, and mortality in the test groups as compared to the controls when observed during 14 days of the acute toxicity experimental period. These results showed that a single oral dose of the extract showed no mortality of these rats even under higher dosage levels indicating the high margin of safety of this drug.

However the behavior changes, Body weight, Water intake, food intake does not produce much significant, they may be the slight changes have been noted in the Body weight, water intake, food intake these changes are due to the anticancer effect of the trail drug but they are non significant in compared with the control group. Thus the results are in non-significant and explored that the safety of the drug due to lack of moratlity during the course of this study of about 14 days. This Acute toxicological report of *KMNC* shows the safety of the drug. In acute toxicity test the *Kaalamega Narayana Chendooram* was found to be nontoxic at the dose level of 2000mg/ kg body weight.

**CONCLUSION**

The preliminary acute toxicological study as per OECD guidelines 423 showed that the *KMNC* was a safer drug for clinical dose in observing 14 days of experiments. No mortality rate was seen during the study. The drug *KMNC* had a variety of metals in their preparation, these metals loses their toxicity during the purification process. The purification process of metals and minerals takes a variety of steps to attain the toxic form of the substances to the non-toxic form. The drug before and after purification process was described in materials and methods of this paper. These purification process was a boon offered by the Siddhars. Thus this paper provoked the safety of metallo-mineral formulation *Kaalamega Narayana Chendooram* as mentioned in *Athmaraksha Mirtham Ennum Vaithiya Saara Sangeraham* through a acute toxicological studies as per OECD guidelines 423 and recommended for further clinical trail.
ACKNOWLEDGEMENT

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REFERENCES