Toxicity Study of Thalaga Parpam in Rats

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ABSTRACT

Introduction: Thalaga Parpam is a Siddha Herbo-mineral formulation useful in wide range of diseases and disorders. Efficacy of formulation depends on their genuineness of herbs and mineral used. Authentication of herbs by experts is first and fundamental step for standardization of herbo-mineral formulation. In this paper acute and sub acute toxicity study of Thalaga Parpam were studied. Methods: The standard methods recommended in toxicity study guidelines Methods for chemicals by OECD was followed. Results: No any abnormal behavioural observed in acute and sub acute toxicity studies of Thalaga Parpam. Conclusion: Findings of the study is helpful in standardization of toxicity studies showed in LD50 value 2000mg/kg body weight and NOAEL in Sub acute toxicity study of Siddha formulation Thalaga Parpam, which will promote global acceptance of the formulation and reputation of the Siddha medical system.

KEYWORDS: Acute and sub acute toxicity study, Thalaga Parpam, Siddha Medicine

I. INTRODUCTION

Siddha system is profuse with remedies for many classified and unclassified diseases. Siddhar’s are the pioneers in using metal and minerals as medicine recipes based on metals, minerals are formulated for long shelf life, mainly metals like Mercury, Arsenic, Lead, Copper, Iron, Zinc, Tin etc. are used. These drugs are effective in smaller doses and long acting and also the potency of the drug can be maintained for a long period. Although the clinical efficacy is apparent the toxicological profile is incomplete.

Toxicology studies are essential in order to establish the safety and efficacy of new drugs or natural substances which will be used later in human as a health supplement or medicine. A toxicology study covers pharmacological aspects which deal with the adverse effects of bioactive substance on living organisms and acts as a guide for the researchers to make evaluation on the suitability of a new drug to be adopted or applied for clinical use (Anadón, 2016; Gossau, 2016; Parasuraman, 2011). By identifying the safe range of dosage to consume, Thalaga Parpam could provide maximum advantages to human health with minimum side effects. Thalaga parpam is an Arsenic preparation. So, the present study is focussed to evaluate the toxicity of Thalaga parpam. Acute and sub acute toxicity studies were done.

II. MATERIALS AND METHOD

Method of preparation of Thalaga parpam:

Thalagam is purified by panankal (palm toddy) and processed into parpam by Agasakarudan kizhangu juice (Corallocarpus epigae). This method is taken from textbook of gunapadam thathu jeeva vaguppu – R. Thiagarajan. P. 251.

Animal study:

Wistar strain, adult healthy male rats were procured from King Institute, Guindy. They weighed on an average of 150 gms. They were maintained in a clean environment.

Animal selection:

Thirty (30) clinically healthy Wistar strain male rats weighing between 120-300 g (age between 5-7 weeks) were randomly selected for the sub-acute oral toxicity study. All animals were housed inside a standard environmental conditions at a temperature of 25 ± 1°C with 12 hours light and 12 hours dark cycle. The animals were acclimatized to hygienic laboratory conditions for at least 7 days prior to the experiment. Animals were fed a standard commercial pellet diet and tap water ad libitum. This study was approved by the King Institute, Guindy, Chennai of Research following the university’s ethical standards with reference number IAEC/KIC/2002/11 dated 24 January 2002.

Acute toxicity study:
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The applied method was modified from Dollah et al. (2013). The experiment was divided into Phase I and Phase II. Fixed dose procedure was followed as described in OECD guidelines 420 (2001) for oral toxicity study in the aim to determine the Lethal Dose (LD₅₀). In Phase I, thirty Wistar albino rats (Takrif Bistari Enterprise, Seri Kembangan) were divided into five groups which consisted of six animals per group (n=6). Group 1 served as negative control (untreated group), Group 2 (orally treated with diluted thalaga parpam at 05 mg/kg/day), Group 3 (50 mg/kg/day), Group 4 (300 mg/kg/day), and Group 5 (with the highest tested dose at 2000 mg/kg/day). Treatment rats were treated once daily with diluted THALAGA PARPAM using a sterile size 14 ball-tipped oral gavage needle (Harvard Apparatus, US) for 14 days. Close observation was conducted for the first four hours to examine any toxic symptoms such as abnormal behaviour, abnormal posture, evidence of diarrhoea, blood in urine, and increase of heart beat potentially caused by the thalaga parpam. Body weight was recorded on days 0, 4, 7, 11 and 14 during the experimental period. Blood was withdrawn from the tail vein using a sterile needle on day 7 and 14 respectively and subject to differential white blood cells counting via Wright’s staining. Whole blood samples were collected on day 14 and key hepatic enzyme assays were performed by using Spotchem EZ SP-4430 analyser. Half of the survived rats (n=3) from each group were euthanized on day 14 of the sub-acute oral toxicity study to obtain Liver, Kidney, Spleen, Lung and Heart for organ relative weight measurements. Toxicity effects in the Liver and Kidney were further analysed by histopathological analysis for any abnormalities.

Fourteen days recovery period

In Phase II of toxicity, the method modified from Takahashi et al. (2012) and as described in OECD (2001) was followed. Half of the survived rats (n=3) for each group were returned to their own cage and kept for another 14 days of observation period.

During the recovery period, all groups of the rats were left untreated (without treatment of any thalaga parpam) and had access to food pellets and water ad libitum. The occurrence of delayed toxicity symptoms was observed twice daily, including abnormal behaviour, increase of heart beat and abnormal posture. Body weight changes were recorded on days 0, 4, 7, 11 and 14. All rats were euthanized at the end of day 14 of the recovery period to obtain blood and organ samples for differential white blood cell counting, hepatic enzyme assays and organ weight measurements. Liver and kidney samples were subjected to histopathological analysis.

Sub-acute oral toxicity study (Repeated dose 14 days):

The drug (thalaga parpam) was continued further for 14 days to the different set of animals at doses of 14mg, 70mg,140mg for body weight of animal. After the study period the blood samples were collected and tested for Liver and Kidney function test and haematological parameters.

Blood: RBC Count, WBC Count, Hb.
Serological Test: Liver function test – SGPT,SGOT,SAP
Kidney function test – Urea, Creatinine

Group 1 – Control
Group 2 - Low Dose – 14mg/kg/b.wt.
Group 3 - Mid Dose –70mg/kg/b.wt.
Group 4 - High Dose –140mg/kg/b.wt.

Histopathological analysis

All liver and kidney samples were fixed in 10% buffered formalin for 48 hours and subjected to tissue processor (LEICATM, Germany). Processed tissue samples were embedded in paraffin wax, sectioned at approximately 5 μm using Rotary Microtome Machine (LEICATM, Germany), and stained with haematoxylin and eosin (H&E) (Sigma, US). All stained tissues were examined under light microscope to observe any abnormalities in the liver and kidney tissue samples (modified from Takahashi et al., 2012).

Statistical analysis

All data were analysed using IBM Statistical Package for the Social Sciences (SPSS) Statistics software (Version 20). The differences of all toxicological parameters between treatment and control (untreated) groups were compared using one-way ANOVA followed by multiple comparison Tukey post-hoc tests. All data are presented as mean ± S.D. In all analysis, p < 0.05 was taken to indicate significant difference.
III. RESULTS

In acute toxicity study there is no cross changes in behaviour, there is no mortality food and water intake does not alter. No alteration in faecal and urine output. There is no appearance of palpable masses.

As regard the toxicity analysis the animals experienced no deviation from that of control parameters.

Results indicated that the lethal dose (LD₉₀) of Thalaga Parmam could not be determined in this study, as no lethality observed in any animal during the 28 days of the experiment (Phase I: 14 days of sub-acute toxicity followed by Phase II: 14 days recovery period). The LD₉₀ of Thalaga Parmamis thus more than 2000 mg/kg body weight and may be ranked to Globally Harmonised System (GHS) Category 5 (LD₉₀ = 2000-5000 mg/kg body weight) (OECD, 2001a). Gross observations revealed that the oral administration of Thalaga Parmam all dosages tested did not produce any sign of distress and significant change in behaviour, breathing and nervous responses in tested male rats upon the first 4 hours of the treatment. This indicates that the oral feeding of Thalaga Parmamid not cause any acute toxicity effect (Lippmann et al., 2007; Pinault, 2008). Furthermore, no significant bodyweight increment of all animals compared to untreated group during the 28 days of experiments indicating normal body metabolism and no occurrence of toxic effect even after administration has been stopped. This result is in alignment with a study by Dollah et al. (2013), which found that oral administration of grounded Thalaga Parmam for 28 days continuously shown insignificant change in bodyweight.

Assessment of organ weight and ratios to its body weight is important as alteration in organ-to-body weight ratio may be an indicator as a result of organ damage and precede morphological changes (Olaniyan et al., 2016). The present findings indicated no significant difference (p > 0.05) in the relative organ weights of all treatment groups as compared to normal untreated group which demonstrated that the consumption of Thalaga Parmam may not elicit any deleterious effects to the host and was not toxic to the organs in both 14 days of sub-acute toxicity study and 14 days of recovery period.

Differential white blood cells count

Blood plays an important role in regulating normal body physiological functions and homeostasis (Doctor & Spinella, 2012). Differential white blood cells count of circulating peripheral blood was performed in order to investigate if continuous consumption of Thalaga Parmam for 14 days during sub-acute toxicity study could cause any inflammation or any delayed allergic reactions for the next 14 days. Results demonstrated no significant alteration in percentage of leukocytes subtypes between treatment groups and normal untreated group on day 7 and 14 for both Phase I and Phase II toxicity studies. However, there was a significant increase (p < 0.05) in basophil counts in dosage group of 140 mg/kg compared to normal untreated group at day 14 of Phase I, indicating the possible occurrence of inflammation reactions (Miyake & Karasuyama, 2017). However, this result can be neglected as the basophil counts is in the normal proportion as proposed by Voehringer (2016), that basophil contribute 1-2%, eosinophil contribute around 5% and monocyte contribute for 2-8% to the circulating blood. This indicates that no inflammation or delayed allergic reactions had occurred upon administration of Thalaga Parmams all the leukocytes subtypes in normal proportion as compared to normal untreated group. This contradicts the results of studies by Abel-salam (2012) and Kamil (2013), which reported inflammation due to an increase of granulocytes upon administration of Thalaga Parmam. The difference of outcomes as compared to the current study might be due to higher dosage given, form of administration and administration duration. Moreover, this study investigated on mixture of Thalaga Parmam.

Table 1: Serum clinical chemistry parameters in different groups of animals:

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I (control)</th>
<th>Group III (High Dose given)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC count</td>
<td>6.4±0.9</td>
<td>5.6±0.7</td>
</tr>
<tr>
<td>WBC count</td>
<td>7±0.4</td>
<td>8±0.3</td>
</tr>
<tr>
<td>Hb (%)</td>
<td>13±0.8</td>
<td>11±0.6</td>
</tr>
<tr>
<td>Urea mg/dl</td>
<td>38±2.6</td>
<td>36±2.5</td>
</tr>
<tr>
<td>Serum Glutamate Pyruvate Transaminase (IU/L)</td>
<td>62±2.7</td>
<td>74±1.5</td>
</tr>
<tr>
<td>Serum Glutamate Oxaloacetate Transaminase (IU/L)</td>
<td>96±1.4</td>
<td>89±1.8</td>
</tr>
<tr>
<td>Serum Alkaline Phosphatase KA units</td>
<td>17±1.2</td>
<td>16.2±1.4</td>
</tr>
<tr>
<td>Creatinine mg/dl</td>
<td>0.3±0.006</td>
<td>0.3±0.005</td>
</tr>
</tbody>
</table>

Histopathological analysis

Histopathological analysis on the livers obtained from sub-acute study and recovery period confirmed that the consumption of Thalaga Parmamid did not give any toxicity effect at any administered dosage. No abnormalities were detected in the central vein, hepatocytes, sinusoids and no fatty change occurred indicating that no lesion has been developed in all the tested groups when compared to control rat’s liver. In addition,
observationson kidneys obtained from sub-acute study and recovery period revealed non-toxic effects upon the consumption of Thalaga Parpam. Kidney samples from both studies show that the glomerulus encircled with Bowman’s capsule in all tested dose did not experience any inflammatory reaction when compared to normal untreated rat kidneys.

IV. CONCLUSION

From the toxicity study of Thalaga Parpam the author came to the conclusion that in short term therapy by Thalaga Parpam there is no toxicity. This study confirms that daily administration of 140 mg/kg body weight (equivalent to 140 mg/day for 60 kg human) of Thalaga Parpam can be concluded as safe and did not cause any adverse or delayed toxicity effects. All toxicity parameters which were evaluated, such as animal behaviour, body weight, relative organ weight, differential white blood cells count, hepatic enzyme assays and histopathological analysis were unaffected following this administration. It is suggested that a chronic toxicity study should be conducted to increase the duration of administration. Furthermore, blood glucose level, lipid profile and renal function test could be conducted to further examine the effects of THALAGA PARPAM consumption on other biochemical measurement.

Conflict of interest
There is no any conflict of interest

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REFERENCES

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