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Toxicity Study of Thalaga Parpam in Rats

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ABSTRACT
Introduction*:** Thalaga Parpamis aSiddhaHerbo-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 Parpamwere 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 ofThalaga Parpam.Conclusion*:** Findings of the study is helpful in standardization of toxicity studies showed in LD_{50} 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 coverspharmacological 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; Gosslau, 2016; Parasuraman, 2011).By identifying the safe range of dosage to consume, Thalaga Parpamcould 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 text book 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 between5-7 weeks) were randomly selected for the sub-acuteoral toxicity study. All animals were housed instandard environmental conditions at a temperatureof 25 ± 1°C with 12 hours light and 12 hours darkcycle. The animals were acclimatized to hygieniclaboratory conditions for at least 7 days prior to theexperiment. Animals were fed a standard commercialpellet diet and tap water *ad libitum*. This study wasapproved by the King Institute, Guindy, Chennai of Research following the university'sethical standards with reference number IAEC/KIC/2002/11 dated 24 January 2002.

Acute toxicity study:

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 (LD_{50}). 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 wererecorded 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 relative organ weight. Liver and kidney samples were subjected to histopathological analysis.

during the experimental period they were observed for;

1. Any cross change in behaviour
2. Mortality if any
3. Changes in food and water
4. Faecal and urine output
5. Palpable masses if any

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 palbable masses.

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

Results indicated that the lethal dose (LD_{50}) of Thalaga Parpamcould not be determined in this study, as nolethality observed in any animal during the 28 daysof the experiment (Phase I: 14 days of sub-acutestudy followed by Phase II: 14 days recovery period).The LD_{50} of Thalaga Parpamis thus more than 2000 mg/kgbody weight and may be ranked to GloballyHarmonised System (GHS) Category 5 (LD_{50} = 2000-5000 mg/kg body weight) (OECD, 2001a). Grossobservations revealed that the oral administration ofThalaga Parpamat all dosages tested did not produce any signof distress and significant change in behaviour,breathing and nervous responses in tested male ratsupon the first 4 hours of the treatment. Thisindicates that the oral feeding of Thalaga Parpamdid not causeany acute toxicity effect (Lippmann *et al*., 2007;Pinault, 2008). Furthermore, no significant bodyweight increment of all animals compared tountreated group during the 28 days of experimentindicating normal body metabolism and nooccurrence of toxic effect even after administrationhas been stopped. This result is in alignment with astudy by Dollah *et al.* (2013), which found that oraladministration of grounded Thalaga Parpamfor 28 dayscontinuously shown insignificant change in bodyweight.

Assessment of organ weight and ratios to itsbody weight is important as alteration in organ-tobodyweight ratio may be an indicator as a result oforgan damage and precede morphological changes(Olaniyan *et al.*, 2016). The present findingsindicated no significant difference ($p > 0.05$) in therelative organ weights of all treatmentgroups ascompared to normal untreated group whichdemonstrated that the consumption of Thalaga Parpammaynot elicit any deleterious effects to the host and wasnot toxic to the organs in both 14 days of sub-acutetoxicity study and 14 days of recovery period.

Differential white blood cells count

Blood plays an important role in regulatingnormal body physiological functions andhomeostasis (Doctor & Spinella, 2012). Differentialwhite blood cells count of circulating peripheralblood was performed in order to investigate ifcontinuous consumption of Thalaga Parpamfor 14 daysduring sub-acute toxicity study could cause anyinflammation or any delayed allergic reactions forthe next 14 days. Results demonstrated nosignificant alteration in percentage of leukocytessubtypes between treatment groups and normaluntreated group on day 7 and 14 for both Phase Iand Phase II toxicity studies.However, there was a significant increase ($p < 0.05$)in basophil counts in dosage group of 140 mg/kgcompared to normal untreated group at day 14 ofPhase I, indicating the possible occurrence ofinflammation reactions (Miyake & Karasuyama,2017). However, this result can be neglected as thebasophil counts is in the normal proportion asproposed by Voehringer (2016), that basophilcontribute 1-2%, eosinophil contribute around 5%and monocyte contribute for 2-8% to the circulatingblood. This indicates that no inflammation ordelayed allergic reactions had occurred uponadministration of Thalaga Parpamas all the leukocytes subtypesis in normal proportion as compared to normaluntreated group. This contradicts the results ofstudies by Abel-salam (2012) and Kamil (2013),which reportedinflammation due to an increase ofgranulocytes upon administration of Thalaga Parpam. Thedifference of outcomes as compared to the currentstudy might be due to higher dosage given, form ofThalaga Parpamused andadministration duration.Moreover, this study investigated on mixture ofThalaga Parpam.

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

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

Histopathological analysis

Histopathological analysis on the liversobtained from sub-acute study and recovery periodconfirmed that the consumption of Thalaga Parpamdid not give any toxicity effect at any administereddosage. No abnormalities were detected in thecentral vein, hepatocytes, sinusoids and no fattychange occurred indicating that no lesion has beendeveloped in all the tested groups when comparedto control rat's liver. In addition,

observationson kidneys obtained from sub-acute study andrecovery period revealed non-toxic effects upon theconsumption of Thalaga Parpam. Kidney samples from bothstudies show that the glomerulus encircled withBowman's capsule in all tested dose did notexperience any inflammatory reaction whencompared 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 of140mg/kg body weight (equivalent to 140 mg/day for60 kg human) of Thalaga Parpamcan be concluded as safe and didnot cause any adverse or delayed toxicity effects.

All toxicity parameters which were evaluated, suchas animal behaviour, body weight, relative organweight, differential white blood cells count, hepaticenzyme assays and histopathological analysis areunaffected following this administration. It issuggested that a chronic toxicity study should beconducted to increase the duration of administration.

Furthermore, blood glucose level, lipidprofile and renal function test could be conductedto further examine the effects of THALAGA PARPAM consumptionon other biochemical measurement.

Conflict of interest

There is no any conflict of interest

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REFERENCES

[1]. ThiagarajanR., *Gunapadam thathu jeeva vaguppu*, indian medicine and homoeopathy department, chenai, edition, 1999. P. 251

[2]. Abel-salam, B.K.A. 2012. Immunomodulatory effectsof black seeds and garlic on alloxan-inducedDiabetes in albino rat. *Allergology Immunopathology*,**40(6)**: 336-340.

[3]. Afroz, R., Tanvir, E.M., Hossain, M.F., Gan, S.H.,Parvez, M., Aminul Islam, M. & Khalil, M.I.2014. Protective effect of Sundarban honeyagainst acetaminophen-induced acute hepatonephrotoxicityin rats.

[4]. Al-Waili, N.S. 2003a. Effects of daily consumptionof honey solution on hematological indices andblood levels of minerals and enzymes in normalindividuals. *Journal of Medicinal Food*, **6(2)**:135-140.

[5]. Al-Waili, N.S. 2003b. Intravenous and intrapulmonaryadministration of honey solution tohealthy sheep: effects on blood sugar, renal andliver function tests, bone marrow function, lipidprofile, and carbon tetrachloride-induced liverinjury. *Journal of Medicinal Food*, **6(3)**: 231-247.

[6]. Alimohammadi, S., Hobbenaghi, R., Javanbakht, J.,Kheradmand, D., Mortezaee, R., Tavakoli, M. &Akbari, H. 2013. Protective and antidiabeticeffects of extract from *Nigella sativa* on bloodglucose concentrations against streptozotocin(STZ)-induced diabetic in rats: An experimentalstudy with histopathological evaluation.*Diagnostic Pathology*, **8(137)**: 1-7.

[7]. Anadón, A. 2016. Perspectives in veterinarypharmacology and toxicology. *Frontiers inVeterinary Science*, **3(82)**: 1-12.

[8]. Assayed, M.E. 2010. Radioprotective effects ofblack seed (*Nigella sativa*) oil against hemopoieticdamage and immunosuppression ingamma-irradiated rats. *Immunopharmacologyand Immunotoxicology*, **32(2)**: 284-296.

[9]. Bhat, R. 2011. The disease-preventive potentialof some popular and underutilized seeds.*Functional Foods, Nutraceuticals, andDegenerative Disease Prevention*. John Wiley& Sons, Inc. pp. 171-192.

[10]. Chan, B.K., Haron, H., Talib, R.A. & Subramaniam,P. 2017. Physical properties, antioxidantcontent and anti-oxidative activities ofMalaysian stingless kelulut (*Trigona* spp.)Honey. *Journal of agricultural Science*, **9(13)**:32-40.

[11]. Chavez-santoscoy, R.A., Gutierrez-uribe, J.A.,Granados, O., Torre-villalvazo, I., Tovar, A.R.,Serna-saldivar, S.O. & Palacios-gonza, B. 2014.Flavonoids and saponins extracted from blackbean(*Phaseolus vulgaris L*.) seed coatsmodulate lipid metabolism and biliarycholesterol secretion in C57BL / 6 mice. *BritishJournal of Nutrition*, **112**: 886-899.

[12]. Chua, L.S. & Adnan, N.A. 2014. Biochemical andnutritional components of selected honeysamples. *Acta Scientiarum Polonorum TechnologiaAlimentaria*, **13(2)**: 169-179.

[13]. Doctor, A. & Spinella, P. 2012. Effect of processingand storage on red blood cell function *in vivo.*

[14]. *Seminars in Perinatology*, **36(4)**: 248-259.

[15]. Dollah, M.A., Parhizkar, S., Latiff, L.A. & Hassan,M.H. 2013. Toxicity effect of *Nigella sativa* onthe liver function of rats. *Advanced PharmaceuticalBulletin*, **3(1)**: 97-102.

[16]. Goharshenasan, P., Amini, S., Atria, A., Abtahi, H. &Khorasani, G. 2016. Topical application of honeyon surgical wounds: a randomized clinical trial.*Forschende Komplementärmedizin/ Research inComplementary Medicine*, **23**: 12-15.

[17]. Gosslau, A. 2016. Assessment of food toxicology.*Food Science and Human Wellness*, **5(3)**: 103-115.

[18]. Isopescu, R.D., Josceanu, A.N.A.M., Minca, I., Colta,T., Postelnicescu, P. & Mateescu, C. 2012.Characterization of Romanian honey based onphysico-chemical properties and multivariateanalysis. *Revista De Chimie*, **65(4)**: 381-385.

[19]. Kaatabi, H., Bamosa, A.O., Lebda, F.M., Elq, A.H.Al. & Al-sultan, A.I. 2012. Favorable impact of*Nigella sativa* seeds on lipid profile in type 2diabetic patients. *Journal of Family andCommunity Medicine*, **19(3)**: 155-162.

[20]. Kaleem, M., Kirmani, D., Asif, M., Ahmed, Q. &Bano, B. 2006. Biochemical effects of *Nigellasativa L* seeds in diabetic rats. *Indian Journalof Experimental Biology*, **44**: 745-748.

[21]. Kamil, Z.H. 2013. Effect of crude oil of black seeds(*Nigella sativa*) on white blood cell andhematocrit of male albino mice treated with lowtoxic dose of paracetamol. *Medical Journal ofBabylon*, **10(4)**: 1005-1012.

[22]. Kek, S.P., Chin, N.L., Tan, S.W. & Yusof, Y.A. 2016.Classification of honey from its bee origin viachemical profiles and mineral content. *FoodAnalytical Methods*, **10(1)**: 19-30.

[23]. Khalil, M.I., Sulaiman, S.A. & Gan, S. H. 2010. High5-hydroxymethylfurfural concentrations arefound in Malaysian honey samples stored formore than one year. *Food and ChemicalToxicology*, **48(8–9)**: 2388-2392.

[24]. Lippmann, M., Bress, A., Nemeroff, C.B., Plotsky,P.M. & Monteggia, L.M. 2007. Long-termbehavioural and molecular alterations associatedwith maternal separation in rats. *EuropeanJournal of Neuroscience*, **25**: 3091-3098.

[25]. Miyake, K. & Karasuyama, H. 2017. Emergingroles of basophils in allergic inflammation.*Allergology International*, **66**: 382-391.

[26]. Oddo, L.P., Heard, T.A., Rodríguez-malaver, A.,Pérez, R.A., Fernández-muiño, M., Sancho, M.T.& Vit, P. 2008. Composition and antioxidantactivity of *Trigona carbonaria* honey fromAustralia. *Journal of Medicinal Food*, **11(4)**:789-794.

[27]. OECD. 2001a. Acute Oral Toxicity – Acute toxicclass method. *Oecd Guideline for Testing ofChemicals*, (December), 1–14.

[28]. OECD. 2001b. Acute Oral Toxicity – Fixed doseprocedure (chptr). *Oecd Guideline for Testingof Chemicals*, (December), 1–14.

[29]. Olaniyan, J.M., Muhammad, H.L., Makun, H.A.,Busari, M.B. & Abdullah, A.S. 2016. Acute andsub-acute toxicity studies of aqueous andmethanol extracts of *Nelsonia campestris* inrats. *Journal of Acute Disease*, **5(1)**: 62-70.

[30]. Oroian, M. 2012. Physicochemical and rheologicalproperties of romanian honeys. *Food Biophysics*,**7**: 296-307.

[31]. Parasuraman, S. 2011. Toxicological screening.*Journal of Pharmacology and Pharmacotherapeutics*,**2(2)**: 74-79.

[32]. Pinault, D. 2008. N-Methyl d-Aspartate receptorantagonists ketamine and MK-801 inducewake-related aberrant oscillations in the ratneocortex. *Biology Psychiatry*, **63**: 730-735.

[33]. Sahebkar, A., Soranna, D., Liu, X., Thomopoulos, C.,Simental-Mendia, L.E., Derosa, G. & Parati, G.2016. A systematic review and meta-analysis ofrandomized controlled trials investigating theeffects of supplementation with *Nigella sativa*(black seed) on blood pressure. *Journal ofHypertension*, **34(11)**: 2127-2135.

[34]. Takahashi, M., Kato, H., Doi, Y., Hagiwara, A. &Hirata-koizumi, M. 2012. Sub-acute oraltoxicity study with fullerene C60 in rats. *TheJournal of Toxicological Sciences*, **37(2)**: 353-361.

[35]. Tan, M.K., Sharifah, D., Adli, H., Tumiran, M.A.,Abdulla, M.A. & Yusoff, K.M. 2012. Theefficacy of gelam honey dressing towardsexcisional wound healing. *Evidence-BasedComplementary and Alternative Medicine*,**2012**: 1-6.

[36]. Voehringer, D. 2016. Regulation and function ofbasophil, eosinophil, and mast cell responses.*The Type Immune Response in Health andDisease: From Host Defense and Allergy toMetabolic Homeostasis and Beyond*. W.C.

[37]. Gause & D. Artis (Eds.), New York, NY: SpringerNew York. pp. 1-12.Wilson, J.I., George, B.O. & Umukoro, G.E. 2011.Effects of honey on the histology of liver inadult Wistar rats. *Biology and Medicine*, **3(1)**:1-5.

[38]. Yusof, H.M., Manan, M.A. & Sarbon, N.M. & Ali,A. 2017. Gender differences on the effects ofhoney and black seed mixture supplementation.*Journal of Sustainability Science and Management*,**(3)**: 119-134.

[39]. Zaoui, A., Cherrah, Y., Mahassini, N., Alaoui, K.,Amarouch, H. & Hassar, M. 2002. Acute andchronic toxicity of *Nigella sativa* fixed oil.*Phytomedicine*, **9(1)**: 69-74.