Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 6, Issue 5, 2014

Original Article

PREPARATION, CHEMICAL ANALYSIS AND SUB-ACUTE TOXICITY EVALUATION OF LINGA PATHANGAM (A MERCURY BASED SIDDHA HERBO-METALLIC DRUG) IN RATS

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Received: 16 Apr 2014 Revised and Accepted: 22 May 2014

ABSRACT

Objective: *Lingapathangam* (LP) is a mercury based herbo-mineral drug used in Siddha Medicine for the management of chronic autoimmune arthritis. The toxicity profile of the drug has not been reported. This study was conducted to prepare LP as per traditional literature, evaluate chemical composition and to study the sub-acute toxicity of the LP in rats.

Methods: LP was prepared as per the traditional literature *Anupoga Vaithiya Navaneetham* Part-IV in our lab. The physico-chemical analysis was done to find out the presence of organic and inorganic compounds. Acute toxicity study was conducted in rats by administering single oral dose of three strengths. Abnormal behavior and death were observed for 14 days. Sub-acute toxicity study was done in rats by administering three different doses (2, 10 and 20 mg/kg) orally for 21 days. Body weight, food intake, water intake were monitored every day. At the end of the study, blood was collected from rats to estimate renal function parameters, liver function parameters, hematology parameters and lipid profile. Organs such as brain, lung, stomach and liver were collected for histopathology assessment.

Results: Quantitative analysis has revealed that 100gm of LP contains 70.43% mercury, 14.10% chloride, 1.80% silica and 0.98% sulphate and the absence of organic matter. Acute toxicity study suggests that LP fall under the category 2 (LD50 is > 5 – 50 mg/kg), which is considered as highly toxic category. Sub-acute toxicity revealed that LP is safe up to 10mg/kg. There is no renal impairment even at high dose (20mg/kg). LP at high dose produced toxicity in blood parameters, liver functions and lipid profile, and showed mild histological changes.

Conclusion: *Linga pathangam* is safe up to 10mg/kg in rats, which corresponds to human dose of 112mg/70kg in man. The currently practiced clinical dose (50mg/day) given with palm jiggery only up to 7 days is considered as the safe and non-toxic dose.

Keywords: Siddha, Ayurveda, Mercury, Traditional medicine, Lingapathangam, Cinnabar.

INTRODUCTION

Nature has given many remedies for human's ailment which have diverged around the earth. Siddha system of medicine, one of the oldest Asian traditional medical systems, derives drugs from plants, animal products, minerals and metals. Siddhars were ancient spiritual scientists, considered as superhuman who defined the age and other laws of nature, to which all human being are subjected to. They expounded their medical wisdom to this world, which is named after them as "Siddha system of medicine"[1].Currently, the health consumers started searching indigenous system of medicines for the management of chronic diseases in order to minimize unwanted side effects of modern drug. In Siddha Medicine, mercury and its four salt forms are together classified as *panjasootham* and reserved for the management of certain specific clinical conditions such as cancer, autoimmune disorders and infectious diseases etc. As Siddha literatures consider lingam (cinnabar or mercury sulfide) as the safest among mercurial drugs, lingam based preparations are frequently used by siddha physicians. The *Linga pathangam* (LP) is one such preparation, prescribed especially for management of chronic arthritis including autoimmune etiology. The drug is given at the dose of 50mg/day with palm jaggery for 3 to 7 days depend on the severity of the disease with strict dietary regimen. There are a set of herbals been used to alleviate mercury induced toxicity after the LP therapy[2]. The query about its toxicity has not been properly addressed. In the present study, we have analyzed the chemical composition of LP and also sub-acute toxicity was evaluated in rats.

MATERIALS AND METHOD

Preperation of lingapathangam.[2]

Ingredients: LP was prepared in Pharmaceutical lab of National Institute of Siddha, Chennai, India, following standard manufacturing procedures. *Lingam* (cinnabar or mercury II sulfide), *valaiyaluppu* (bentonite) and *kariuppu* (Sodium chloride) are the three mineral ingredients of LP. All the mineral raw materials were purchased from Rajendra raw herbal shop, Thakalay, Tamil Nadu. *Acalypa indica, Citrus limon, Thespesia populnea* and cow milk are the other ingredients used to prepare the drug. These three herbals were collected locally and authenticated by a Taxonomist from National Institute of Siddha, Chennai. LP was prepared using traditional clay-made sublimation apparatus. Since the drug is prepared by sublimation process (*pathangam* in Siddha), it acquired the name as *lingapathangam*.

Purification of cinnabar*:* Before the actual preparation process, as per the procedure, cinnabar is supposed to be processed with certain juices to detoxify (purify). Cinnabar was purified by dipping into honey and cow milk each for one day. After cleaning and drying, cinnabar was again subjected to heat on mud pot for three hours. While heating, the purifying liquid (prepared by mixing juices of *Acalypa indica, Citrus limon* and cow milk) was added drop by drop on cinnabar. This is considered as purified lingam and was used to prepare *lingapathangam*[2]. Since LP was the mercury based drug and cinnabar was the only source of mercury, the amount of mercury was estimated in cinnabar before its purification, after purification process and in the LP (finished product) using Perkin Elmer model 400/HGA900/AS800 (USA) coupled with Mercury Hydride System-15.

Siddha Sublimation apparatus (fig.1): The traditional sublimation apparatus was set as per the literature procedure. Shortly; two fresh mud pots were purchased and inner side was given coating with the leaf juice of *Thespesia populnea*. After getting dried, the coating procedure was repeated using the same leaf juice for seven times to seal the minute holes in the mud pot and also to enhance the sublimation yield of the drug.

Sublimation process: Equal quantity of cinnabar, *valaiyaluppu* (bentonite) and sodium chloride were mixed together and placed in

the lower pot of Siddha sublimation apparatus. Lower pot with the mineral ingredients was closed by placing the mouth of upper pot on its mouth. Gap between the facing areas of both pots were sealed with a cotton cloth coated by clay plaster. Lower pot was subjected to heat by medium flame using gas stove for four hours. The vapor formed inside the sublimation apparatus spread within the closed mud pots and got sublimated on the inner side of the upper pot (Fig.1). Adequate cooling to collect sublimated drug was done by spreading cold water wet cotton-cloth upon upper pot. The apparatus was allowed to cool after four hours of heat and the sublimated ash colored LP powder was carefully collected from inner side of upper pot by using a brush.

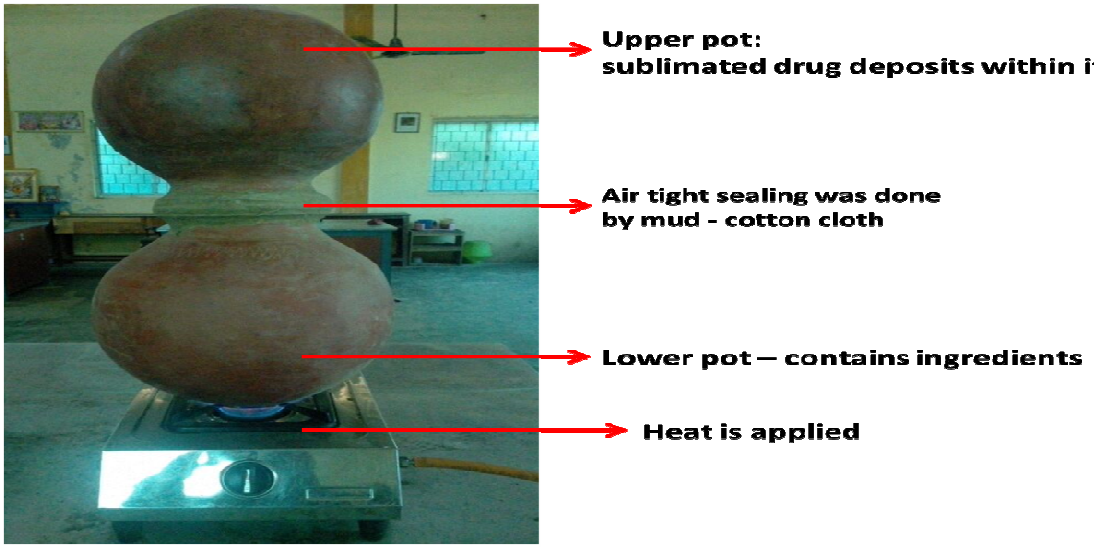

Fig. 1: Siddha Sublimation apparatus made by clay used to prepare *linga pathangam*

Physico-chemical analysis

The LP was analyzed for its physical properties such as color, odor, loss of weight on drying, pH, water solubility and acid solubility by using standard protocol. A preliminary qualitative chemical analysis was done in LP for the following chemicals; silicate, nitrate, nitrite, copper, sodium, sulphate, chloride, phosphate, carbonate, fluoride, oxalate, borate, lead, aluminum, iron, zinc, calcium, magnesium, ammonium, potassium, mercury, starch, reducing sugar, alkaloid, tannic acid, unsaturated compound, amino acid, aliphatic amino acids, oxyquinolone epinephrine, pyro catechol, antipyrine, meconic acid, apomorphine salicylate, resorcinol, morphine, phenol, cresol and hydroquinone. LP was subjected to quantitative elemental analysis using Atomic absorption spectrometer for which it showed positive in preliminary qualitative analysis.

Experimental animals

The study was done after getting ethical committee approval (PGC/84/290/CPCSEA-2000/IAEC-10) from Institutional Animal Ethics Committee. Inbred adult female wistar albino rats (150-230 gm) were obtained from the animal housing facility of King Institute, Guindy, Chennai. The animals were maintained in a well – ventilated room with 12:12 hour light/dark cycle in polypropylene cages. Standard pellet feed and tap water were provided *ad libitum* throughout the experimental period. Animals were acclimatized to laboratory conditions one week prior to initiation of experiments.

Acute Toxicity study

Acute toxicity study of linga pathangam was evaluated in rats as per the Organization of Economic Co-operation and Development (OECD) guideline 423[3]. Rats were fasted overnight with water *ad libitum.* All animals received a single oral dose of LP mixed with 2% palm jaggery solution as this is how it is being used in patients. According to OECD, acute toxicity study is based on stepwise procedure with the use of the minimum number of animal per step. Three animals were used for each step. Depending on the mortality and/or morbidity status of the animals, on and average 2-4 steps may be necessary to allow judgment on the acute toxicity of the test substance. A single oral dose (0.1 ml/ 10 gm body weight) of 5, 50, 300 and 2000 mg/kg body weight was administered stepwise according to the guideline. The general behaviors of the rats were continuously monitored for first one hour after dosing, followed by every 4th hour periodically till first 24 hours and then daily thereafter for a total of 14 days. Changes in normal psychomotor activity, external morphology and mortality of rats were observed and recorded.

Sub-acute toxicity study

Sub-acute toxicity studies was carried out according to OECD guideline 407[4]. As the LP is administered 3-7 days to patients, 21 days sub-acute toxicity was carried out. Three doses for sub-acute toxicity have been chosen based on the acute toxicity study as well as clinical dose. Based on acute toxicity study (LD50 dose range > 5 – 50mg/kg), 2mg/kg, 10mg/kg and 20mg/kg were chosen. Rats were divided into four groups of 10 animals each (5male and 5 female). Group 1 received vehicle (2% palm jaggery solution). Other three groups received LP at the doses of 2, 10 and 20mg/kg. Both the vehicle and drugs were administered orally once daily for 21days and the volume was kept constant as 0.1ml/10gm body weight to the individual rat. Toxic symptoms such as signs of toxicity, body weight changes, food and water intake and mortality were monitored daily. At the end of the study period, rats were anaesthetized by ketamine and blood was collected for hematological parameters. Then, all rates were sacrificed by injecting high dose of ketamine.

Blood analysis

Blood sample was used for determining hematological parameter, lipid profile, liver function parameter and renal function parameter. Hematological parameters estimated include total red blood cells, Hemoglobin (HB), packed cell volume (PCV), mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), total white blood corpuscles (TWBC) and differential count of white blood corpuscles (DC-WBC). Blood glucose level and lipid profiles (total cholesterol, high density lipoprotein (HDL) and triglycerides.) were estimated. Liver function parameters estimated were total bilirubin, direct bilirubin, indirect bilirubin, total protein, albumin, alkaline phosphatase (ALP), serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT). Renal parameters estimated include urea, uric acid and calcium.

Histopathological examination

Organs such as brain, lung, liver and stomach were isolated from the animals belong to control group and high dose LP treatment groups. All tissues were preserved in 10% formalin solution for histopathological examination. Tissues were fixed in 10% formalin, embedded in paraffin at 50°C and stained with eosin and haematoxylin.

Statistical analysis

Statistical analysis was carried out by using SPSS software. *P*< 0.05 was considered as significant. Values were expressed in mean ± SD and the values were analyzed by one way ANOVA followed by Tukey's test.

RESULT AND DISCUSSION

Physico-chemical properties

Mercury level in lingam before and after purification process were 78.4% and 82.7% respectively, which indicates the purification process involves in removing of other impurities or compounds, but not mercury, hence mercury level gets concentrated after purification. Drug pH was found to be 2.47, which is acidic in nature, hence the drug absorption in gastric acidic medium would be high than absorption occurs in small intestine alkaline environment. (Table1).

Preliminary qualitative analysis of LP has shown the presence of four elements such as mercury, sulphate, silica and chloride. It showed the absence of organic matters. Quantitative analysis has revealed that 100gm of LP contains 70.43% mercury, 14.10% chloride, 1.80% silica and 0.98% sulphate.

Acute toxicity study

At the dose of 2000 mg/kg and 300mg/kg, all three rats were died within 4 hours. At 50 mg/kg dose, 2 animals were died within 12hours. Rats given with 5 mg/kg dose did not produce any behavioral abnormality and were survived after 14 days of dug administration. Thus, the drug is considered as category 2 (LD50 is > 5 – 50 mg/kg), which falls under highly toxic category [5].

Sub-acute toxicity study

Three different doses of LP (2,10 and 20mg/kg) and vehicle were administered for 21 days for four groups of rats. There was no statistically significant alteration in body weight and food intake during the drug treatment. There was a dose dependent reduction in water intake. This was observed more in day 14 and day 21 (Table 2-4).

Table 1: Physical properties of *lingapathangam*

Parameter	Results
Colour	Dull white
Odour	Odourless
Loss on drying @ 105°C (%)	0.10
pH @ 25°C (1:10 Ratio)	2.47
Ash value @ 550° C (%)	4.33
Water soluble (%)	44.50
Acid insoluble ash (%)	2.40
Alkalinity as $CaCO_3$ in water soluble ash (%)	NIL

Table 2: Effect of *lingapathangam* (LP) on changes in rat body weight:

Groups	Body weight in gram (mean ± S.D)			
(Dose) (n=10)	Baseline	7th day	14th day	21st day
Control (Vehicle)	148.02 ± 10.1	151.66 ± 12.9	156.66 ± 12.1	160.33 ± 12.6
Low dose (LP 2mg/kg)	117.0 ± 11.3	120.0 ± 16.4	124.16 ± 18.7	132.5 ± 14.3
Medium dose (LP 10mg/kg)	142.5 ± 11.7	147.5 ± 16.9	154.83 ± 17.9	160.83 ± 23.9
High dose (LP 20mg/kg)	115.30 ± 10.1	118.33 ± 13.29	120.0 ± 14.44	116.66 ± 16.33

* P < 0.05 vs. control

Table 3: Effect of *lingapathangam* (LP) on changes in food intake:

Groups (n=10)	Food weight in gram/day (mean ± S.D)			
(Dose)	Baseline	7th day	14th day	21st day
Control (Vehicle)	43.94 ± 1.63	44.83 ± 2.63	45.16 ± 1.94	45.16 ± 2.56
Low dose (LP 2mg/kg)	30.01 ± 2.30	30.33 ± 1.03	30.16 ± 1.32	30.16 ± 1.94
Intermediate dose (LP 10mg/kg)	39.10 ± 2.82	40.16 ± 1.94	42.16 ± 2.13	40.33 ± 2.87
High dose (LP 20mg/kg)	38.98 ± 1.14	40.16 ± 1.2 4	40.33 ± 2.87	40.16 ± 2.18

* P < 0.05 vs. control

Renal parameters and hematology

The 21 days treatment with LP did not cause abnormality in renal function even at higher dose when compared to control group (Table 5). There was no significantly change in hematology parameters in low dose and medium dose treatment. Only high dose treatment with LP caused reduction in total RBC, Hb, MCHC and WBC. These clearly say that this drug is safe in low and medium dose (up to 10mg/kg) and toxic at higher doses. When we convert human therapeutic dose (50mg/70kg man/day) to corresponding rat dose based on body surface area, it is 4.5mg/kg, which clearly indicate that the human therapeutic dose is in the safe range (Table 6).

Table 4: Effect of *lingapathangam* (LP) on changes in water intake (ml/day)

Groups (n=10) (Dose)	Water intake in ml/day (mean ± S.D)			
	Baseline	7th day	14th day	21st day
Control (Vehicle)	44.23 ± 3.11	44.83 ± 2.63	45.16 ± 1.94	45.16 ± 2.56
Low dose (LP 2mg/kg)	45.4 ± 2.01	45.5 ± 1.71	35 ± 2.58*	32.5 ± 1.12*
Intermediate dose (LP 10mg/kg)	46.23 ± 2.43	46.66 ± 1.05	31.66 ± 3.58*	27.5 ± 1.12*
High dose (LP 20mg/kg)	48.72 ± 1.23	48.33 ± 1.05	35 ± 3.42*	26.66 ± 1.05*

* P < 0.05 vs. control

Table 5: Effect of *lingapathangam* (LP) on renal function test

Renal function parameter	Control (Vehicle)	Low dose (LP 2mg/kg)	Intermediate dose (LP 10mg/kg)	High dose (LP 20mg/kg)
Urea (mg/dl)	57.5 ± 11.9	54.5 ± 10.7	46.8 ± 9.8	46.8 ± 10.8
Uric acid (mg/dl)	0.6 ± 0.3	0.4 ± 0.2	0.5 ± 0.3	0.4 ± 0.2
Calcium (mg/dl)	8.9 ± 2.1	10.1 ± 1.8	8.5 ± 1.7	10.1 ± 4.4

Values are expressed in mean ± S.D (n=10)

Table 6: Effect of *lingapathangam* (LP) on hematological parameter

Hematological parameter	Control (Vehicle)	Low dose (LP 2mg/kg)	Intermediate dose (LP 10mg/kg)	High dose (LP 20mg/kg)
Red blood cells(cells/cu.mm)	5.68 ± 0.06	5.22 ± 0.39	5.8 ± 0.16	2.34 ± 0.14*
Hemoglobin (mg %)	20.16 ± 0.74	15.00 ± 1.14	16.9 ± 0.81	10.4 ± 0.81*
Packed cell volume	34.16 ±1.01	38.8 ±1.43	39.2 ± 1.24	36.4 ± 1.03
Mean corpuscular hemoglobin (pg)	3.16 ± 0.60	2.45 ± 0.65	2.66 ± 0.09	4.54 ± 0.10
Mean corpuscular volume (fL)	88.66 ± 1.38	85.07 ± 0.13	84.4 ± 0.17	81.49 ± 0.06
Mean corpuscular hemoglobin concentration (g/dl)	33.0 ± 0.78	48.86 ± 1.89	58.74 ± 1.75	28.1 ± 1.69*
Total white blood corpuscles (thousands/cu.mm)	8.53 ± 0.16	7.22 ± 0.92	7.25 ± 0.74	6.78 ± 0.87
Neutrophil	31.8 ± 6.3	30.3 ± 4.5	28.3 ± 4.9	35.4 ± 5.2
Lymphocyte	67.16 ± 7.7	67.83 ± 4.8	70.0 ± 6.3	63.8 ± 5.9
Eosinophil	0.83 ± 1.3	0.83 ± 0.7	0.60 ± 1.3	0.8 ± 0.01
Monocyte	1.16 ± 1.2	1.00 ± 0.8	1.80 ± 1.2	1.8 ± 0.01

Values are expressed in mean ± S.D (n=10), * P < 0.01 vs. control

Table 7: Effect of *lingapathangam* (LP) on biochemical parameter and lipid profile

Biochemical parameter	Control (Vehicle)	Low dose (LP 2mg/kg)	Intermediate dose (LP 10mg/kg)	High dose (LP 20mg/kg)
Total Bilirubin (mg/dl)	3.24 ± 0.01	3.26 ± 0.05	3.26 ± 0.04	4.48 ± 0.04*
Direct Bilirubin (mg/dl)	2.7 ± 0.06	2.4 ± 0.03	2.42 ± 0.04	4.28 ± 0.04*
Indirect Bilirubin (mg/dl)	0.50 ± 0.01	0.76 ± 0.03	0.78 ± 0.02	0.13 ± 0.01*
ALP (U/L)	829.33 ± 166.2	791.58 ± 101.1	724.5 ± 150.01	613.2 ± 183.9*
SGOT(U/L)	150.33 ± 31.9	172.33 ± 6.4	149.66 ± 18.45	160.4 ± 15.12
SGPT(U/L)	48.5 ± 21.3	41.83 ± 13.7	50.83 ± 14.8	68.6 ± 10.9*
Total protein(g/dl)	10.6 ± 0.4	10.85 ± 16.24	3.76 ± 0.2*	3.53 ± 0.5*
Albumin(g/dl)	3.85 ± 0.13	3.88 ± 0.4	3.68 ± 0.3	3.44 ± 0.3
Blood glucose (mg/dl)	122.83 ± 6.8	125.16 ± 18.2	133.66 ± 28.2	163 ± 9.2*
Total Cholesterol (mg/dl)	53.5 ± 7.4	44.6 ± 20.6	37.2 ± 8.9†	40.4 ± 14.7†
High density lipoprotein (mg/dl)	14.3 ± 1.5	16.5 ± 3.6	23.3 ± 6.5†	23.8 ± 8.7†
Triglycerides (mg/dl)	88 ± 11.6	75.5 ± 20.2	70.8 ± 11.2	72.4 ± 11.2

Values are expressed in mean ± S.D (n=10), *P<0.01 vs. control, †P < 0.05 vs. control

Liver function parameters and lipid profile

All the biochemical parameters are normal in the low and medium dose treated groups, whereas high dose LP treatment significantly altered bilirubin (total, direct, indirect), ALP, SGPT, total protein and blood glucose levels. Medium dose and high dose treatment also reduced the total cholesterol level and raised high density lipoprotein compared to low dose treatment (Table 7).

Histopathology of organs

Organs from control group showed normal histological appearance in brain, lung, stomach and liver. High dose of LP showed mild gliosis in brain, mild congestive changes in lung and hyperplastic changes with focal ulceration in stomach. Liver showed chronic venous congestion and presence of apoptotic bodies with binuclear hepatocytes. These findings support for the toxicity of high dose of LP in sub-acute toxicity study (Finger 2-5).

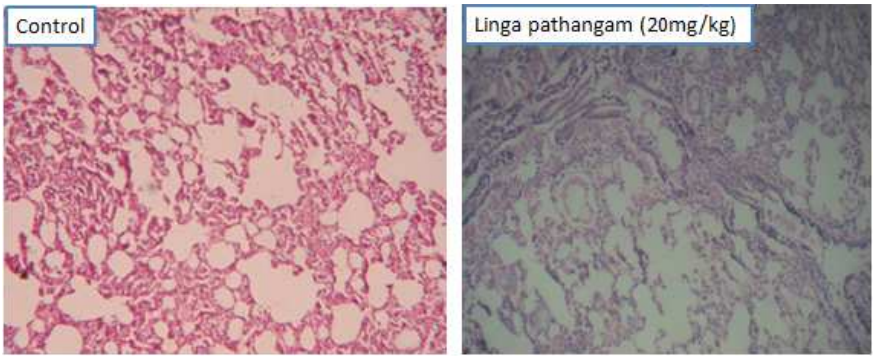

Fig. 2: Effect of linga pathangam in lung

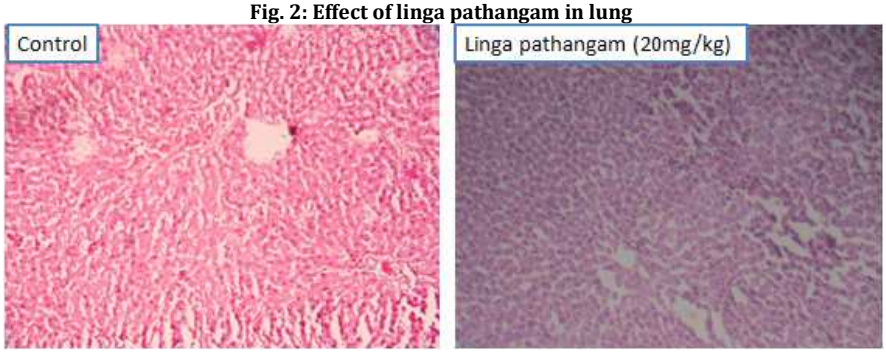

Fig. 3: Effect of linga pathangam in liver

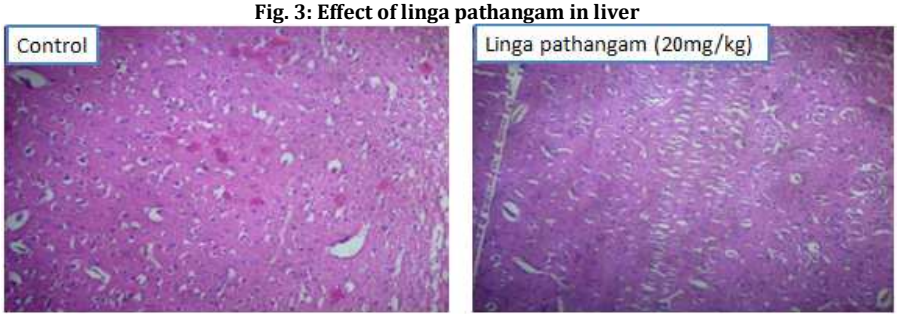

Fig. 4: Effect of linga pathangam in brain

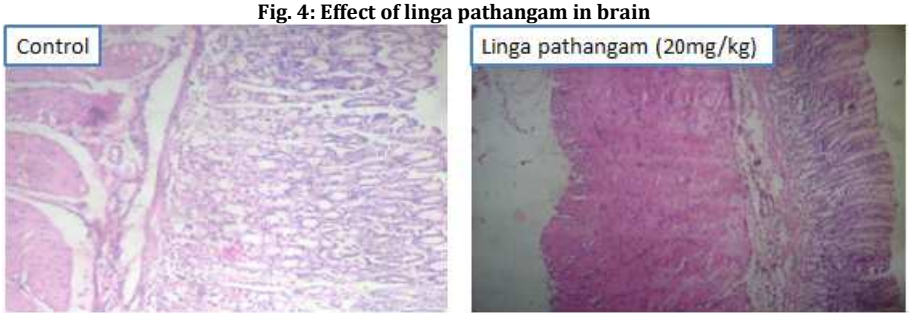

Fig. 5: Effect of linga pathangam in stomach

CONCLUSION

Linga pathangam is being used thousands of years by Siddha physicians for the management of chronic arthritis including autoimmune etiology. The drug is given at the dose of 50mg/day with palm jaggery for 3 to 7 days depend on the severity of the disease with strict dietary regimen. Acute toxicity study has revealed that this drug belongs to highly toxic category 2 and the corresponding human lethal dose would be 4g. In sub-acute toxicity, the drug is safe up to 10mg/kg in rats which corresponds to human dose of 112mg/70kg in man. But, the clinical dose is half of this safe dose (50mg/day) and given only up to 7 days. The drug is administered in the palm jiggery, which is believed to protect body from mercury toxicity. If at all, the drug showed mercury related toxicities, then it is counteracted by a set of herbals after *lingapathangam* therapy. Thus, the current practice of *lingapathangam* with low dose for short duration with other precautionary measures to counteract mercury toxicity could be considered as safe practice of mercury based medicines in Siddha. Further, there is a need to evaluate the role of herbals in reduction of mercury toxicity during metal drugs in Siddha Medicine.

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