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Evaluation of chronic toxicity study of *Nathaichoori Chooranam*- a Siddha polyherbal formulation

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ABSTRACT

Background: *Nathaichoori Chooranam* (NC), a Siddha formulation, is traditionally used in South India for hyperlipidemia, obesity and digestive disorders. NC, which is rich in phytochemicals such as alkaloids and flavonoids, shows promise in managing metabolic conditions. However, chronic toxicity studies are essential to confirm long-term safety, as long-term use of herbal medicines may lead to adverse effects. **Objective:** This study evaluates the safety profile of NC in a chronic toxicity model to support its therapeutic application. **Materials and Methods:** A 90-day chronic toxicity study was conducted using Wistar rats following OECD Guideline 452. Animals were randomized into 6 groups (n=10/sex/group): control (0.5% CMC vehicle) and dose groups (low, intermediate, high; 300, 600, 1000 mg/kg body weight, respectively) of *Nathaichoori* powder administered orally daily. The parameters assessed included body weight, food intake, hematological and biochemical markers (liver, kidney function), organ weights and histopathology of vital organs (liver, kidney, heart, spleen). Clinical signs and mortality were monitored daily. **Results:** No mortality or significant clinical abnormalities were observed in the groups. Body weight and food intake were comparable to controls. Hematological parameters (RBC, WBC, hemoglobin) and biochemical markers (ALT, creatinine, urea) did not show dose-related changes. Organ weights were unchanged, and histopathological analysis did not reveal treatment-related lesions in the liver, kidneys or other organs, indicating no chronic toxicity at the doses tested. **Conclusion:** *Nathaichoori Chooranam* did not exhibit chronic toxicity in rats at doses up to 1000 mg/kg for 90 days, indicating its safety for long-term use. These findings support the potential of NC as a safe herbal remedy, but human studies are needed to confirm safety and establish therapeutic doses.

Keywords: Biochemical paramters, Chronic toxicity study, Hematological parameters, *Nathaichoori Chooranam*.

INTRODUCTION

The Siddha system of medicine originating from Tamil Nadu, India, is one of the oldest traditional medical systems, emphasizing holistic healing through harmony between humans and nature [1]. This system uses polyherbal, herbo-mineral, and animal-derived formulations to balance the three humors – vata, pitta, and kabum – that regulate bodily functions [2-4]. Some polyhedral Siddha formulations are traditionally used for their therapeutic efficacy, particularly in managing symptoms such as Anti-inflammatory [5], Anti-microbial, Antioxidant [6], Anthelmintic [7,8], Antibacterial [9], Skin diseases [10], and Respiratory illness [11]. Long-term toxicity studies are important for assessing the safety of herbal medicines intended for long-term use, as they identify adverse effects in animal models after repeated exposure for 6 to 12 months [12,13]. These studies determine the no-observed-adverse-effect level (NOAEL). In Siddha medicine, formulations such as *Nathaichoori* Chooranam are prescribed for chronic conditions, and such evaluations are crucial to verify safety and support integration into modern healthcare. Previous studies have demonstrated that *Nathaichoori* Chooranam significantly reduces serum cholesterol, triglycerides, low-density lipoprotein (LDL), and very-low-density lipoprotein (VLDL), and increases high-density lipoprotein (HDL) in hyperlipidemic rats, with effects comparable to atorvastatin [14].

As recognized by the World Health Organization (WHO), the global growth in complementary and alternative medicine (CAM) underscores the need for standardized toxicological evaluations to ensure the safety, efficacy, and quality of herbal medicines [15]. Phytochemicals in *Nathaichuri* Chooranam contribute to its therapeutic effects but pose risks if not evaluated for long-term use. For example, prolonged exposure to some phytochemicals may result in hepatotoxicity or nephrotoxicity, as seen in some herbal formulations [16]. By evaluating clinical observations, body weight, hematological and biochemical parameters, and histopathological changes at multiple dose levels, this study aimed to establish the safety profile of *Nathaichuri* Chooranam for long-term use. These findings are expected to

contribute to the scientific validation of Siddha formulations, to address the global demand for safe natural remedies.

MATERIALS AND METHODS

Procurement and preparation of test item

The test compound *Nathaichoori chooranam* was collected from Pharmacy, Siddha Central Research Institute Chennai-106. It contains 50% of *Nathaichoori* vithai seed, 25% of chukka Rhizome, 25% of Avarampoo flowers, 12.5% of Koththumalli fruit, 12.5% of Kezhvaragu seed. Dose formulation was prepared a fresh daily. Required quantities of test item (*Nathaichoori chooranam*) was weighed separately and transferred into mortar. A small volume of 0.5% CMC was added to dissolve it. Triturate slowly and make it like paste. Remaining quantity of 0.5% CMC was added to make final required concentration.

Animal handling

The animals used in the present study (Wistar albino rats) were chosen as the test system because this species is commonly used for repeated oral toxicity testing and it meets the regulatory requirements of most regulatory agencies. These animals were collected from the Animal Facility, Tamil Nadu Veterinary and Animal Sciences University, Chennai-600051. The animals were handled as per the recommendations of the CPCSEA Guidelines for Laboratory Animal Facility after obtaining approval from the Institutional Animal Ethics Committee (IAEC) of Siddha Central Research Institute, Central Council for Research in Siddha (Ministry of AYUSH), Arumbakkam, Chennai-106. Approval No: 193/SCRI/PHARMA/2018. Approximately 100 (50 male + 50 female) animals weighing Male (205 – 375) g, Female (162 – 230) gand aged between Male (10-12) weeks Female (8-12) weeks at the time of dosing were used. Animals were housed in polypropylene rat cages with stainless steel tops, provided with feed and polycarbonate water bottles with sippers, and provided with a constant supply of pelleted rat feed containing 18.63% protein and RO water ad libitum in autoclaved corn cob bedding cages.

The experimental environment was maintained cleanly at a temperature and relative humidity between 18 - 25 ºC and 30 - 65%, with a lighting condition of 12 hours' light and dark cycle. Before the start of the study, animals were maintained for 7 days to acclimatize to the experimental conditions. At the end of the study, the animals were sacrificed using a high dose of general anaesthesia (pentobarbital sodium solution) induced by the intraperitoneal route. The bodies of the sacrificed animals were pooled in a biohazard plastic bag; they were then cremated.

Treatment procedures

Chronic toxicity study

Chronic toxicity assessments of Siddha herbal formulation were conducted based on the protocol given in OECD guideline number 452, i.e. the use of both sexes (male and female rats) [17]. In this study, hundred animals (50 males and 50 females) were distributed into six groups (consisting of 10 males and 10 females per group) namely G1 - Control - 0 mg/kg b.w., G2 - Low dose - 300 mg/kg b.w., G3 - Intermediate dose-1 - 600 mg/kg b.w., G4 - Intermediate dose-2 -1000 mg/kg b.w., G5 – Control reversal - 0 mg/kg b.w. and G6 - High dose reversal - 1000 mg/kg b.w (G5&G6 contains 5 male and female rats).The test item was administered for treatment group (Low, intermediate, high and high dose reversal) by gavage using stainless steel ball tipped oral intubation needle at the desired dose level continuously for up to 90 days. Homogeneity of the test item in the vehicle was maintained during administration. The dose volume administered at 10 mL/kg b.w. A 0.5% CMC was used as vehicle. Control animals were administered with the CMC alone. Reversal group animals were observed for further 28 days after completion of the treatment to check any reversibility of treatment related change/delayed test item related toxicity.

Clinical observations

All animals were examined twice daily for mortality during the observation period. Detailed clinical observations were made once before treatment was initiated and once weekly during the treatment and recovery periods. Observations included changes in the skin, fur, eyes, mucous membranes, secretions and excretions, and autonomic activity (e.g., lacrimation, piloerection, iris size, and abnormal respiratory pattern), gait, posture, and responsiveness to handling, as well as clonic or tonic movements, stereotypes (e.g., excessive grooming, repetitive circling), or aberrant behavior (e.g., self-mutilation, walking backwards). Body weights were recorded on the day of receipt, before randomization, on the day of dosing, and weekly thereafter for the treatment and recovery groups, and food and water consumption were recorded daily and reported weekly.

Clinical laboratory investigations

Blood samples for hematology and clinical biochemistry were collected from all animals on day 91 (G1, G2, G3, G4, G5 & G6). Animals were housed in metabolic cages and fasted overnight before urine and blood sampling, but were allowed access to water ad libitum. Blood samples were taken from the retro-orbital plexus using a micro-hematocrit heparinized glass capillary tube. Potassium EDTA was used as anticoagulant for hematology tests. For clinical chemistry, blood samples were kept in serum tubes at room temperature for approximately 30 minutes to obtain serum aliquots. After clotting, the blood tubes were centrifuged at 3000 rpm for 15 minutes. The supernatants were decanted and stored at 70ºC for further analysis.

Hematology

Hematological measurements and calculations were performed by using a haematology analyser (Exigo H400) from Mallard SolutionsLaboratories, New Delhi, Indiaforthe haematological analysis. The mentioned machine uses the flow cytometry principle to count and differentiate cells. Whereby parameters including Haemoglobin (Hb), packed cell volume (PCV), Erythrocytecount (RBC), Total leukocyte count (WBC), Platelet count (PLT), Mean corpuscular volume (MCV), Mean corpuscular haemoglobin concentration (MCHC), Mean platelet volume (MPV), Neutrophil, Lymphocyte and Monocyte were analysed from the collected blood samples [18].

Biochemistry

Biochemical parameters including calcium (Ca), Glucose (GLU), Urea, Creatinine (CRE), Uric acid, Alkaline phosphatise (ALP), Total bilirubin (TBIL), Serum Glutamic Pyruvic Transaminase (SGPT), Total protein (TP), Albumin (ALB) Total Cholesterol, Triglycerides (TGL) were analysed by using RA-50 auto analyser (Bayer) [19].

Necropsy and organ weights

Animals were sacrificed humanely by exsanguination under carbon dioxide after completion of treatment and reversal period. all tissues and organs samples were collected from all the animals at necropsy and fixed in 10% buffered neutral formalin solution.

Histopathology

The organs (liver, lung, kidney, heart etc., in both sex) excised from all the experimental rats were fixed in 10% formalin in labelled bottles, and processed for histological examination. Tissues embedded in paraffin wax were sectioned 5 mm thick and stained with haematoxylin and eosin, mounted on glass slides and examined under a standard light microscope [20].

Statistical analysis

The following statistical methods were used to analyze the body weight, organ weights as well as clinical pathology data. Data was summarized in tabular form. Statistical analysis was performed using graph pad prism software 8.3 version. Data for each group of animals was subjected to analysis of variance (ANOVA) followed by Post-hoc comparisons were made using Dunnett's test and unpaired t-test. Values were given as Mean ± Standard Deviation (SD).

RESULTS

Mortality and Clinical Observations

No treatment related mortality and clinical signs of toxicity were observed during entire study period in any of the animals from all groups. No significant difference was observed in feed consumption of treated animals when compared with control group animals in both sexes**.**

Body Weight and % Body Weight Change

There was no statistically significant difference in body weight and % body weight change throughout the experiment when compare to control animals as shown in Figure 1.

Hematology

The analysed hematology parameters of males and females were observed to be comparable with vehicle control throughout the experimental period. The observed statistically significant parameters were considered to be incidental and were considered not related to treatment. In the absence of dose response relationship, the observed statistical differences were observed to be very minimal as shown in Table 1.

Clinical Biochemistry

The analyzed clinical biochemistry of males and females treated groups were observed to be compared to concurrent control group on day 91 (Table 2). The observed statistically significant parameters were considered to be incidental and were considered not related to treatment. In the absence of dose response relationship, the observed statistical differences were observed to be very minimal and no correlating microscopic findings were observed.

Organ weights

The observed statistically significant parameters were considered to be incidental and were considered not related to treatment. In the absence of dose response relationship, the observed statistical differences were observed to be very minimal and no correlating microscopic findings was observed and it is confined to only one sex. No statistically significant changes were observed in both absolute organ weights and relative organ weights of treatment group and recovery group animals when compared to control group animals (Table 3).

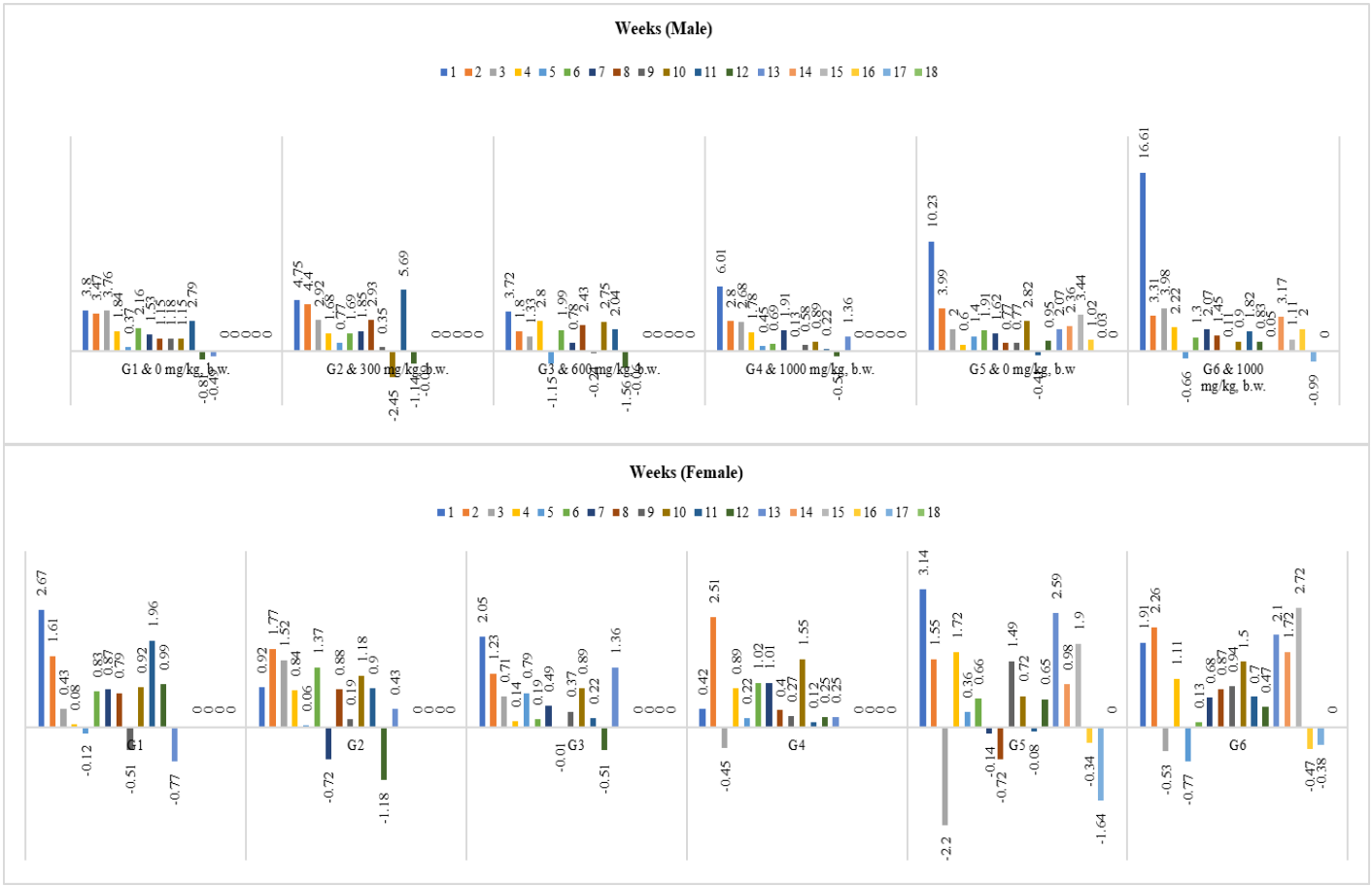

Figure 1: Summary of Weekly Body Weight Change (%)– Males & Females

Values are expressed as Mean, n=10
Values are not statistically significant (P value > 0.05)

Table 1: Summary of Biochemical Parameters- Male & Female

Parameters	G1 & 0 mg/kg, b.w.	G2 & 300 mg/kg, b.w	G3 & 600 mg/kg, b.w.	G4 & 1000 mg/kg, b.w.	G5 & 0 mg/kg, b.w.	G6 & 1000 g/kg, b.w.
Day 91 Male (n=10)						
GLU mg/dl	116.38±23.62	121.63±14.38	128.00±12.91	147.50±20.18	120.20±11.97	130.00±16.60
T. CHOL mg/dl	63.88±8.36	67.38±9.26	67.13±10.86	69.38±14.52	74.20±15.09	67.20±7.66
TGL mg/dl	141.88±34.36	132.50±28.83	142.75±44.20	182.38±42.07	146.40±26.97	153.20±29.98
HDL mg/dl	23.25±3.85	28.25±8.61	24.63±5.93	24.88±4.76	27.40±3.36	25.00±3.00
LDL mg/dl	12.38±7.44	12.38±10.35	14.00±7.03	9.00±8.18	17.00±11.27	12.00±10.25
UREA mg/dl	30.00±3.42	33.38±4.21	30.63±3.02	28.75±3.49	31.40±2.88	29.00±5.24
CK U/L	0.33±0.03	5.04±13.32	0.35±0.04	0.32±0.02	0.42±0.22	0.34±0.05
T. BILI mg/dl	0.12±0.04	0.11±0.05	0.10±0.01	0.10±0.00	0.10±0.00	0.10±0.00
SGOT U/L	195.75±29.75	193.88±27.00	175.63±19.03	176.50±42.68	198.80±43.97	206.20±62.96
SGPT U/L	72.00±11.49	84.00±18.02	71.63±5.18	72.50±19.15	80.80±7.98	73.80±12.93
ALP U/L	209.50±52.30	266.38±74.82	233.13±50.39	222.00±43.98	233.40±42.77	186.20±27.67
T.P g/dl	6.86±0.14	7.11±0.34	7.29±0.34	7.20±0.15	7.12±0.19	7.02±0.40
ALB g/dl	2.69±0.15	2.78±0.21	2.79±0.39	2.86±0.26	2.80±0.28	2.94±0.05
CALCIUM mg/dl	9.56±0.34	9.73±0.32	9.86±0.33	10.01±0.27	9.94±0.37	10.22±0.40
LDH U/L	1983.88±840.71	2362.63±503.98	2469.50±326.04	2513.00±214.67	2724.80±161.80	2378.60±518.33
CK MB U/L	240.63±92.44	166.13±36.08	159.38±37.69	174.13±59.74	193.80±26.40	194.60±71.10
PHOS mg/dl	5.25±0.30	5.09±0.38	4.91±0.45	5.39±0.37	5.12±0.37	5.06±0.36
Day 91 Female (n=10)						
GLU mg/dl	117.00±29.56	136.00±21.67	126.57±41.81	105.86±19.43	130.80±6.61	131.60±33.44
T. CHOL mg/dl	72.10±11.43	68.88±10.13	66.86±20.24	70.14±11.77	73.60±9.53	77.00±4.30
TGL mg/dl	124.40±36.61	156.00±66.16	136.57±51.57	97.57±34.84	196.80±58.00	196.80±37.52
HDL mg/dl	30.00±3.71	29.50±4.81	26.86±10.40	29.43±3.36	31.80±4.15	35.20±4.60
LDL mg/dl	17.10±11.56	11.88±14.13	14.14±12.68	22.14±11.55	7.00±8.94	4.60±4.93
UREA mg/dl	26.90±3.84	28.50±4.75	33.00±4.40	29.14±6.04	27.80±4.82	27.40±5.86
CK U/L	0.35±0.04	0.33±0.05	0.33±0.04	0.34±0.05	0.32±0.08	0.34±0.05
T. BILI mg/dl	0.12±0.02	0.13±0.04	0.12±0.03	0.18±0.04	0.10±0.00	0.10±0.00
SGOT U/L	173.50±31.05	175.88±34.03	168.43±20.35	175.57±28.10	171.40±20.86	153.40±19.62
SGPT U/L	73.40±10.38	76.38±16.39	72.43±14.28	69.71±10.83	73.60±7.89	69.40±5.86
ALP U/L	203.60±44.32	195.00±54.22	327.00±127.58	209.57±65.98	205.00±14.16	184.80±54.36
T.P g/dl	7.16±0.30	7.01±0.29	7.20±0.73	7.31±0.30	7.44±0.27	7.50±0.19
ALB g/dl	3.18±0.25	3.01±0.24	3.24±0.34	3.31±0.25	3.52±0.22	3.58±0.15
CALCIUM mg/dl	9.62±0.43	9.84±0.47	10.41±1.36	9.87±0.35	10.26±0.36	10.26±0.23
LDH U/L	2281.70±343.89	2133.88±542.23	2527.57±289.31	2197.57±429.94	2325.40±364.25	1743.60±523.97

CK MB U/L	138.20±39.00	129.38±48.23	145.71±35.83	105.14±38.69	147.20±37.99	105.20±25.90
PHOS mg/dl	4.08±0.64	4.33±0.43	4.67±0.90	4.73±0.91	4.32±0.48	4.36±0.50

*Values are expressed as Mean ± SD, n=10
Values are not statistically significant (P value > 0.05)

KEY: Glu - Glucose, T. chol. - Total Cholesterol, TGL - Triglycerides, HDL- High Density Lipoprotein, LDL- Low Density Lipoprotein, CK-Creatinine Kinase, TBilI - Total Bilirubin, SGOT - Serum glutamic oxaloacetic transaminase, SGPT - serum glutamate-pyruvate transaminase, ALP- Alkaline Phosphatase, T.P- Total Protein, ALB - Albumin, LDH - Lactate Dehydrogenase, Creatinine Kinase MB, PHOS – Phosphorus

Table 2: Summary of Haematology Parameters- Male & Female

Parameters	**G1 & 0 mg/kg, b.w.**	**G2 & 300 mg/kg, b.w**	**G3 & 600 mg/kg, b.w.**	**G4 & 1000 mg/kg, b.w.**	**G5 & 0 mg/kg, b.w.**	**G6 & 1000 mg/kg, b.w.**
Day 91 Male (n=10)						
WBC 10^9/l	10.91±2.33	9.87±2032	7.55±2.62	10.68±3.33	10.06±1.54	9.12±1.58
LYM %	69.31±2.70	66.45±5.97	63.42±10.21	64.20±5.53	64.84±8.69	64.12±4.31
MON %	4.66±1.79	5.90±2.19	6.25±2.98	7.83±0.62	6.40±3.69	5.32±2.48
NEU %	21.58±3.94	22.22±4.82	24.26±8.20	24.17±5.23	25.18±7.65	24.54±4.18
HGB g/dL	12.25±1.40	12.01±1.65	10.93±3.50	12.83±0.74	12.48±1.57	12.18±0.73
MCH pg	17.41±0.44	17.15±0.23	17.18±0.44	17.10±0.42	16.92±0.22	17.32±0.21
MCHC g/dL	37.36±1.65	36.46±1.67	35.76±1.96	36.41±0.90	36.00±1.13	35.14±0.5
RBC 10^{12}/l	7.03±0.71	7.01±0.95	6.33±1.98	7.50±0.46	7.36±0.84	7.03±0.42
MCV fl	46.65±1.28	47.13±2.19	48.16±2.24	47.03±1.44	47.02±0.87	49.38±1.29
HCT %	32.80±3.05	32.86±3.19	30.25±9.22	35.27±1.54	34.60±3.57	34.72±1.55
PLT 10^9/l	630.25±116.43	611.75±152.66	661.75±276.56	718.62±80.91	566.60±170.94	668.60±61.12
MPV fl	5.10±0.37	4.76±0.34	4.87±0.46	4.82±0.15	5.02±0.31	4.94±0.21
Day 91 Female (n=10)						
WBC 10^9/l	6.01±3.05	5.45±2.77	9.22±11.41	5.17±1.40	5.50±3.02	5.72±2.06
LYM %	69.27±6.02	68.57±6.63	67.27±7.87	68.62±8.69	70.22±3.10	70.96±8.15
MON %	6.24±2.37	4.87±2.91	5.62±2.05	4.31±3.69	5.62±2.54	4.60±2.55
NEU %	20.10±3.76	23.26±3.91	21.67±6.43	21.54±7.65	17.12±3.63	17.92±6.85
HGB g/dL	11.63±1.15	10.61±3.03	11.05±1.79	11.34±1.57	11.52±1.57	11.78±1.22
MCH pg	18.67±0.24	18.45±0.24	18.92±0.43	18.52±0.22	18.82±0.55	18.46±0.36
MCHC g/dL	36.21±1.03	35.76±1.21	35.67±1.04	35.61±1.13	35.04±0.99	35.12±0.63
RBC 10^{12}/l	6.23±0.57	5.75±1.62	5.85±1.01	6.13±0.84	6.12±0.74	6.39±0.66
MCV fl	51.60±1.39	51.65±1.16	53.12±1.88	52.10±1.68	53.66±1.27	52.60±1.24
HCT %	32.16±2.71	29.55±8.06	30.96±4.75	31.87±1.21	32.84±3.74	33.62±2.97
PLT 10^9/l	599.00±117.11	563.75±117.86	526.25±199.48	634.14±82.54	493.80±252.28	634.20±67.28
MPV fl	4.72±0.28	4.76±0.40	4.9±0.59	4.61±0.15	5.12±0.50	4.74±0.26

*Values are expressed as Mean ± SD, n=10
Values are not statistically significant (P value > 0.05)

KEY: WBC - Total leukocyte count, LYM-Lymphocyte, MON-Monocyte, NEU- Neutrophil, HGB-Hemoglobin, MCH-Mean corpuscular hemoglobin, MCHC-Mean corpuscular hemoglobin concentration, RBC- Erythrocyte count, MCV-Mean corpuscular volume, Hematocrit, PLT- Platelets, MPV- Mean Platelet Volume

Table 3: Summary of Relative Organ Weights (%)– Male & Female

Parameters	G1 & 0 mg/kg, b.w.	G2 & 300 mg/kg, b.w	G3 & 600 mg/kg, b.w.	G4 & 1000 mg/kg, b.w.	G5 & 0 mg/kg, b.w.	G6 & 1000 mg/kg, b.w.
Day 91 Male (n=10)						
BRAIN	0.45±0.04	0.49±0.07	0.50±0.05	0.48±0.07	0.38±0.03	0.43±0.05
STOMACH	0.56±0.03	0.57±0.03	0.62±0.05	0.60±0.04	0.56±0.02	0.55±0.02
LIVER	3.42±0.58	3.28±0.47	3.41±0.53	3.27±0.47	3.02±0.30	2.92±0.25
KIDNEYS	0.71±0.58	0.73±0.05	0.77±0.09	0.73±0.04	0.65±0.30	0.73±0.04
ADRENALS	5.26±0.05	10.19±4.33	20.40±33.04	0.01±0.00	0.01±0.04	0.01±0.01
SPLEEN	0.20±5.47	0.23±0.05	0.21±0.05	0.21±0.05	0.19±0.01	0.18±0.03
HEART	0.30±0.04	0.33±0.04	0.33±0.02	0.33±0.04	0.30±0.03	0.33±0.03
THYMUS	0.08±0.02	0.23±0.42	4.18±10.83	0.16±0.22	0.09±0.02	0.05±0.01
Lungs	0.68±0.04	0.69±0.14	0.66±0.08	0.72±0.07	0.62±0.08	0.61±0.07
Prostate	0.46±0.17	0.58±0.12	0.61±0.19	0.56±0.25	0.63±0.05	0.67±0.06
Testies	0.91±0.09	0.86±0.14	0.94±0.11	0.97±0.07	0.82±0.08	0.86±0.06
Epididymis	0.45±0.07	0.38±0.17	0.48±0.07	0.50±0.12	0.45±0.05	0.53±0.09
Day 91 Female (n=10)						
BRAIN	0.80±0.10	0.76±0.08	0.83±0.08	0.76±0.08	0.65±0.04	0.41±0.04
STOMACH	0.78±0.08	0.75±0.09	0.76±0.08	0.78±0.12	0.79±0.07	0.44±0.02
LIVER	3.32±0.47	3.31±0.49	3.94±1.12	3.63±0.41	3.05±0.36	1.68±0.73
KIDNEYS	0.87±0.15	0.84±0.09	0.90±0.05	0.85±0.07	0.78±0.07	0.49±0.02
ADRENALS	0.09±0.08	14.14±11.65	3.37±8.86	0.03±0.01	0.02±0.01	0.02±0.01
SPLEEN	0.27±0.05	0.27±0.04	0.47±0.52	0.25±0.04	0.23±0.01	0.14±0.01
HEART	0.39±0.05	0.39±0.02	0.42±0.06	0.41±0.06	0.35±0.03	0.20±0.01
THYMUS	0.08±0.04	6.07±15.74	0.43±0.81	0.09±0.02	0.05±0.03	0.06±0.01
Lungs	0.88±0.11	0.86±0.12	0.93±0.24	0.82±0.13	0.91±0.15	0.46±0.06
Urinary bladder	0.12±0.14	0.23±0.15	0.08±0.10	0.04±0.01	0.21±0.30	0±0
Ovaries & Uterus	0.44±0.11	0.45±0.08	0.41±0.12	0.45±0.17	0.56±0.15	0.27±0.15

*Values are expressed as Mean ± SD, n=10
Values are not statistically significant (P value > 0.05)

Histopathology

The histopathology of the lung showed pulmonary congestion and mild perivascular and interstitial mononuclear cell infiltration in G1, G2, and G3 males and G2, G3, and G4 females. The histopathology of the uterus showed mild neutrophilic infiltration in G3, G5, and G6 females. The histopathology of the liver showed multifocal mild mononuclear cell infiltration, mild hepatocellular degeneration, multifocal mild hydrophobic degeneration of hepatocytes, congestion in G1, G2, and G3 male and female, mild hepatocellular degeneration in G5 male, mild bile duct hyperplasia in G6 male, and mild periportal mononuclear cell infiltration in G6 female. The histopathology of the kidney showed congestion and mild tubular epithelial cell degeneration in G5 and G6 males and G5 females. The histopathology of the spleen showed congestion in G6 female, G5 male, G6 male, G4 male and female, G2 male and female, and G3 male. The histopathology of the heart showed congestion and mild mononuclear cell infiltration in the G1e G1 female. The histopathology of the uterus showed mild neutrophilic infiltration in G1, G2, G3, G5, and G6 females. The histopathology of ovaries showed congestion in G2 and G5 females, as shown in Figure 2.

DISCUSSION

A chronic toxicity study of *Nathaichoori Chooranam* conducted in Wistar albino rats according to OECD guideline 452 evaluated doses of 300 mg/kg, 600 mg/kg and 1000 mg/kg to assess its safety for long-term use. The absence of mortality, significant clinical signs or major histopathological changes in all dose groups supports the traditional use of this formulation for chronic conditions such as hyperlipidemia. These findings are aligned with acute and sub-acute toxicity studies, which reported no adverse effects at doses up to 2000 mg/kg, indicating a wide safety margin [21]. No significant changes in behavior, physical appearance or clinical signs (e.g., tremors, seizures) were observed in the treated groups. Body weight, a key indicator of systemic toxicity, was comparable to controls, indicating that *Nathaichoori Chooranam* did not impair growth or metabolic function. These results are consistent with studies on other siddha formulations such as *Karuveppilai Chooranam*, which did not show toxicity at high doses [22]. Hematological parameters such as red blood cell (RBC) count, white blood cell (WBC) count and haemoglobin (Hb) did not show any significant changes, indicating bone marrow suppression or immune dysfunction. Biochemical markers such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea and creatinine were within normal ranges, indicating no hepatotoxicity or nephrotoxicity. Stable lipid profiles further confirm the safety of the formulation. These findings are consistent with studies on *Seenthil Chooranam*, which reported normal hematological and biochemical parameters in 90-day studies [23]. Histopathological examination of vital organs - liver, kidneys, heart and spleen did not reveal any significant changes in the low and medium-dose groups.

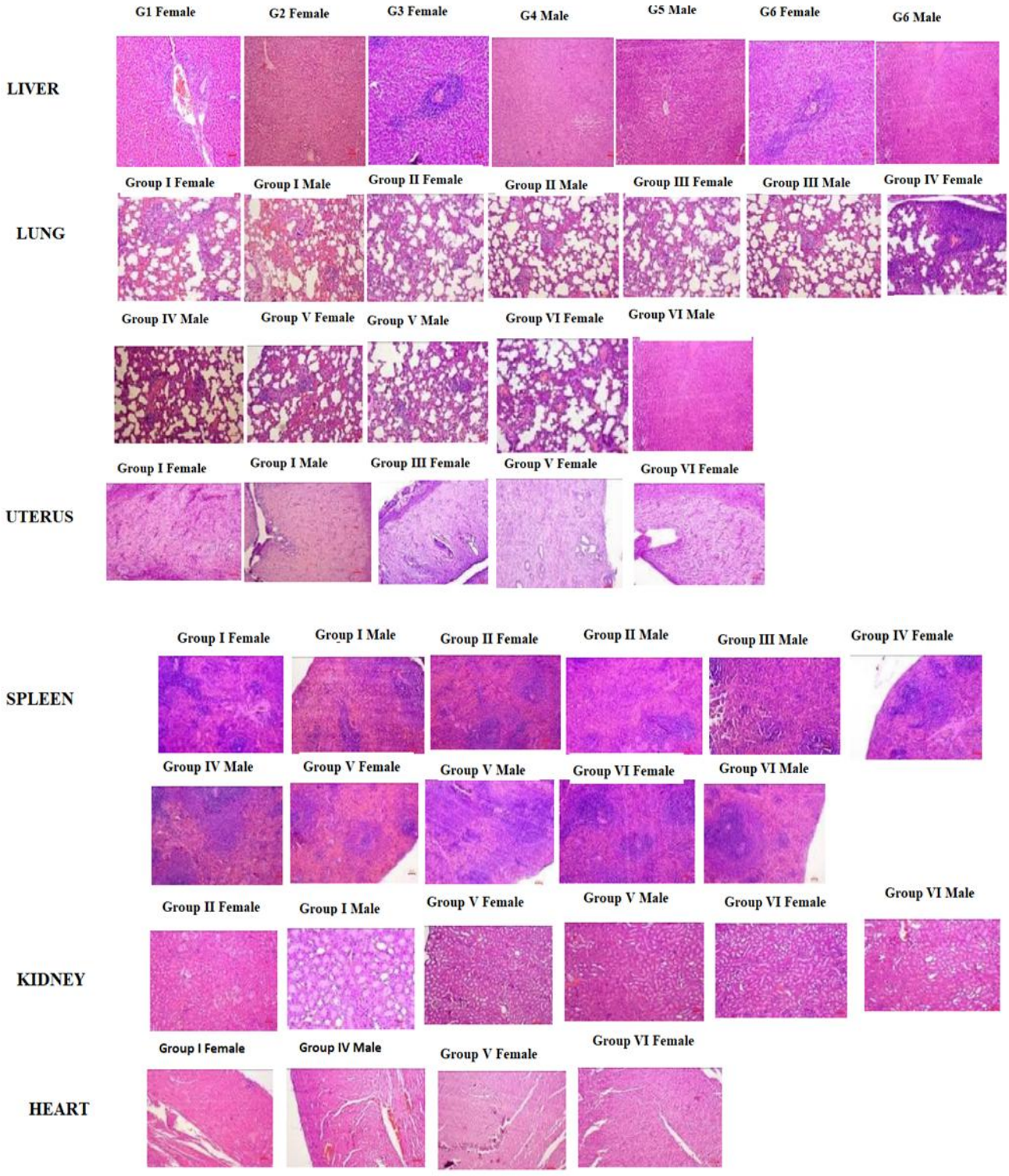

Figure 2: Effect of *Nathaichoori chooranam* on histopathology of Liver, Lung, Kidney, Uterus, Heart and Spleen– Male & Female

In the high-dose group (1000 mg/kg), mild sinusoidal dilatation in the liver and focal interstitial edema in the kidney were observed, but these were not associated with necrosis or severe inflammation. Similar mild changes have been reported in chronic toxicity studies o f*Vilangathi Chooranam*, which is considered non-toxic [24]. These findings suggest that *Nathaichoori Chooranam* produces minimal histopathological effects, which may not be clinically significant. The safety profile of *Nathaichoori Chooranam* is comparable to other siddha formulations. For example, *Karuveppilai Chooranam* did not show toxicity at 500 mg/kg in sub-acute studies [22], and *Seenthil Chooranam* was non-toxic up to 2000 mg/kg in repeated dose studies [23]. These studies highlight the safety of standardised formulations when prepared according to traditional protocols.

The 90-day period may not fully capture the effects of long-term human exposure, and interspecies differences limit direct extrapolation. Future studies should include non-rodent models and clinical trials to confirm safety and efficacy. Advanced techniques such as metabolomics can elucidate the mechanisms behind mild histopathological changes. Standardisation and phytochemical profiling are crucial for batch consistency.

CONCLUSION

On the basis of the results obtained in the chronic toxicity study of *Nathaichoori Chooranam* demonstrates its safety for long-term use, with no significant adverse effects on clinical, hematological,

biochemical, or histopathological parameters. it can be concluded that the No Observed Adverse Effect Level (NOAEL) of *Nathaichoori chooranam* in the present study was considered as 1000 mg/kg b.w. for both males and females of wistar rats. These findings validate its traditional use in Siddha medicine and support its integration into modern healthcare. Further research, including clinical trials and extended preclinical studies, is needed to confirm these results.

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Conflict of interest

The authors declared no conflict of interest.

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