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EVALUATION OF ACUTE AND SUB- ACUTE TOXICITY STUDIES OF *AYA POONAGA CHENDURAM, SEENTHIL CHOORNAM* AND THEIR COMBINATION IN WISTAR RATS

G. Dayanand Reddy[1], M. Kannan[1], R. Ganesan[1], P. Sathiya rajeswaran[1], K. Venkataraman[1], B. Rama Devi[*1], K. Dhanaraj[2], K. Naga Rani[3]

[1]Siddha Central Research Institute, Ministry of AYUSH, Chennai, Tamilnadu, India
[2]Siddha Regional Research Institute, Ministry of AYUSH, Thiruvananthapuram, Kerala, India
[3] St Mary's Group of Institutions, Guntur, Patha Reddy Palem, Chebrolu, A.P, India

***Corresponding author Email**: ramasamba527@gmail.com

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ABSTRACT

Aim: The present study was aimed to evaluate the acute and sub-acute toxicities of Siddha herbal formulations Aya poonaga chenduram (APNC), Seenthil choornam (SC) and their Combination (APNC+SC)as per OECD test guideline 423 and 407 respectively. Methods: The study was carried out in healthy albino rats of wistar strain. For acute toxicity study a single dose of test drugs at a concentration of 2000 mg/kg body weight were administered orally and animals were observed for mortality for 14 days. For sub- acute toxicity study the test drugs at a concentration of 1000 mg/kg body weight were administered orally for 28 days and to assess in a 14 days recovery period for the delayed onset of any toxicity. At the end of the study, haematological and biochemical parameters were evaluated. Histopathological examination of vital organs of experimental rats was taken for gross findings. Results: In acute toxicity study, no treatment related death or toxic signs were observed with APNC, SC and their combination up to 2000 mg/kg body weight in female albino rats. In sub acute toxicity study no compound-related changes in body weights, feed and water intake, cage side observations, clinical observations, clinical pathology, mortality, macroscopic examinations and organ histopathology were noted during treatment period and post recovery period. **Conclusion:** Acute toxicity study reveals that the LD_{50} of APNC, SC and their combination was greater than 2000 mg/kg body weight, and the no-observed-adverse-effect-level (NOAEL) of APNC, SC and their combination in rats is >1000 mg/kg/day when administered orally for 28 days.

INTRODUCTION

Approximately 70-80% of the world population depends on traditional medicine for their primary health care needs[1]. Herbs are the principal form of medicine in traditional medical systems[2]. India has several recognized traditional systems of medicine like Ayurveda, Siddha, Unani, Yoga, Naturopathy Homoeopathy and Sowa Rigpa[3]. Siddha system of medicineis one of the oldest unique systems of medicine which is originated from Tamil Nadu[4]. Most of the Siddha formulations are prepared by using Mooligai (herbs), Thathu (minerals) and Jeeva (animal products) as their sources[5].*Seenthil choornam and Aya poonaga chenduram* are two poly herbal widely used Siddha formulations.

Seenthil choornam is a siddha polyherbal formulation containing the powder mixtures of *Tinospora cordifolia* stem, *Eclipta alba,* and purified earth worm[6]. This is widely used in the treatment of rheumatism, tuberculosis, cough, and skin diseases given with ghee. In sinusitis and ulcer of the nasal passage, it is given with honey and in dandruff and

alopecia it is given with sugar. It is also used to treat fever[7].SC can be used as a feed supplement for poultry[8]. *Aya poonaga chenduram* is a siddha herbo-mineral formulation containing ingredients of Iron fillings and Earthworm. It is used in the treatment of sinusitis[9]. The presence of limited quantities of metals/minerals in Siddha formulations, SSM should undergo scientific validation to establish the safety level for global acceptance[10].In the present study we are evaluating the single dose and 28-days repeated dose toxicities of APNC, SC and their combination in albino rats to assess the safety profile of each formulation by individual and combination form.

MATERIAL AND METHODS:

1.1. Selection and Preparation of Test doses:

As the ingredients present in *Aya poonaga chenduram* and *seenthil chooranam* had the information indicating that the test material is likely to be nontoxic, i.e., having toxicity only above regulatory limit doses[11]. So a limit test at one dose level of 2000 mg/kg body weight for acute toxicity and 1000 mg/kg *p.o* for 28 days for sub-acute toxicity study was selected. Test doses were prepared by triturating a weighed quantity of test drug in required volume of 0.5 % carboxy methyl cellulose (CMC) prepared in 5% v/v honey.

1.2. Animals and Husbandry:

For acute oral toxicity study 21 young, healthy, adult female nulliporus and non-pregnant albino rats weighing 120-150 g were used. Animals were administered with APNC, SC and their combination (APNC+SC) at a dose of 2000 mg/kg *p.o*. Before dosing, animals were fasted overnight and food was withheld for further 3-4 hrs after dosing.In Sub-acute toxicity study 40 young, healthy, adult male and female rats of Wistar strain weighing 150-280 g were used. Feed and water were provided *ad libitum*, except on Study day (SD) 14-15 and 28-29 (before terminal sacrifice) and SD 42-43 (before recovery sacrifice) when food-fasting was implemented and rats were fasted for 12 hours before termination at those 3 occasions. Except control animals, all thegroupsare administered with APNC, SC and their combination (APNC+SC) at a dose of 1000 mg/kg *p.o* for 28 days. The studies were performed as per the recommendations of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines for Laboratory Animal Facility after approval of Institutional Animal Ethics Committee (IAEC) of Siddha Central Research Institute, Central Council for Research in Siddha (Ministry of AYUSH), Arumbakkam, Chennai-106 with an approval number 199/PHARMA/SCRI/2018.

1.3. Instruments:

The instruments used are Electronic weighing balance (Shimadzu-model No. AUX-220), Refrigerator (BPL- model No. F272MVX), Auto analyzer (Bio system - model No. BA400), Centrifuge (REMI-model No.R8M)

1.4. Chemicals:

Carboxy methyl cellulose, Sodium chloride, Formaldehyde, Anesthetic ether and Sodium ethylenediaminetetraacetic acid (sodium-EDTA) of analytical grade were purchased from Theres scientific works, Chennai and used.

1.5. Experimental Procedure

Acute oral toxicity study was conducted as per OECD guideline 423[12-14]. Animals were divided into four groups. Group-I(Vehicle control, n=3) received honey diluted with 0.5%w/v CMC and group II-IV(n=6) administered APNC, SC and their combination (APNC+SC) at a dose of 2000 mg/kg *p.o*. respectively. Before dosing, animals were fasted overnight and food was withheld for further 3-4 hrs after dosing. Sub-acute oral toxicity study was conducted as per OECD guideline 407[15]. Animals were divided into four groups each contains 5 animals. Group-I (Vehicle control) received honey diluted with 0.5%w/v CMC and group II-IV administered APNC, SC and their combination (APNC+SC) respectively at a dose of 1000 mg/kg *p.o* for 28 days.

1.6. Observations:

Animals were observed individually after dosing at least once during first 30 min, 1st hr, 2nd hr, 4th hr and 8th hr periodically during the first 24 hours, with special attention given during the first 4 hours and daily thereafter, for a total of 14 days for the mortality. All the rats were observed at least twice daily with

the purpose of recording any symptoms of ill-health or behavioral changes. Observations recorded are changes in skin, fur, eyes, mucous membranes and also respiratory, circulatory, autonomic and central nervous systems, and somatomotor activity and behavior pattern. Attention was directed for observations of tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma. The time of death, if any, was recorded. Body weight of the animals was recorded on 1st day (before drug administration), 2nd day, 7th day and 14th day. All surviving animals were sacrificed on 15th day of the study using Carbon dioxide (CO_2) asphyxiation method of euthanasia. Complete necropsy was carried out on all animals.

In sub- acute toxicity studyall the experimental animals were observed daily for case side observations like mortality, morbidity, general health, and signs of toxicity. Clinical observations included evaluation of skin and fur characteristics, eye and mucous membrane, sensory responses and reflexes, respiratory and autonomic effects, motor activity and behavior patterns.

1.7. Clinical parameters:

The hematological parameters like hemoglobin concentration (HB), packed cell volume (PCV), total red blood cell count (RBC), total white blood cell count (WBC) and platelet count (PLT), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC), Mean corpuscular volume (MCV) and Mean platelet volume (MPV) were analyzed.

BiochemicalParameters like Glucose, Blood urea, Creatinine, Total Cholesterol, Tri glycerides, High density lipoproteins, Low density lipoproteins, Total bilirubin, Alanine aminotransferase, Aspartate aminotransferase, Alkaline phosphatase, Total proteins, Albumin
, Creatine phosphotase, Uric acid, Calcium, C-reactive protein, Creatine Kinase, Creatine Kinase-MB, Lactate dehydrogenase, Gamma-glutamyl transferase were measured in all experimental animals of sub-acute toxicity study.

1.8. Histopathology:

All tissue samples from each group viz., brain, pancreas, adrenal glands, heart, thymus, liver, kidneys, spleen, stomach, testes / ovaries and epididymides/ uterus from each group of sub-acute toxicity studywere processed and evaluated. Those tissue samples were embedded in paraffin, sectioned, stained with hematoxylin and eosin and examined microscopically by a board-certified veterinary pathologist.

3. STATISTICAL ANALYSIS

Body weights, food intake, water intake, relative organ weights, and clinical pathology data were analyzed statistically. All the data was expressed as mean ± SEM. Statistical significance between more than two groups was tested using one-way ANOVA followed by Tukey's post hoc using Graph pad prism version-5. The significance level was set at $P<0.05$ for all tests. Group II, III, and IV will be statistically compared with Group I to find the treatment related effects[16].

4.RESULTSAND DISCUSSION:

4.1. Acute toxicity study:

The body weights of experimental animals were increasing throughout the study. No significant difference ($P>0.05$)in body weight gain was observed between group I and other groups. There were no variations in physical observations,behavioral changes and central nervous system excitations and depressions. The autonomic effects are normal in all experimental animals and all sensory responses and reflexes are found to be normal. Somatomotor effects were observed in all animals that showed normal effects and no abnormal gait was observed. No compound related respiratory effects were observed.

4.2. Sub-acute toxicity study:

No compound-related mortality or signs of toxicity were noted. Significant changes in body weight and relative organ weight were not observed. Compound related significant change in the feed intake and water intake was also not observed in animals during treatment period and post recovery period between the groups.

4.2.1. Clinical parameters:

The clinical pathology evaluation and data reports are presented in the following tables. No compound-related changes in hemoglobin concentration (HB), packed cell volume (PCV), total red blood cell count

(RBC), total white blood cell count (WBC) and platelet count (PLT), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC), Mean corpuscular volume (MCV) and Mean platelet volume (MPV) were noted. No compound-related changes in serum glucose, serum Urea, serum Creatinine, serum Total Cholesterol, serum Triglycerides, serum HDL, serum LDL, serum Total bilirubin, serum SGOT, serum SGPT,serum ALP, serum Total proteins, serum Albumin, serum CRP, serum Uric acid, serum Calcium**,** serum CK, and CK-MB, serum LDH, and GGT levels were noted.No compound-related macroscopic findings were noted.

4.2.2. Histopathology:

Liver histopathology of group-I (control) showed very mild hepatocellular degeneration and mild kupffer cell hyperplasia and group-II-IV animals showed congestion, multifocal moderate vesicular (micro to macro) fatty degeneration of hepatocytes, very mild periportal mononuclear cell infiltration. Mild tubular epithelial cell degeneration was noticed in the histopathology of kidneys of control and test drugs treated animals.Stomach (glandular and non-glandular) histopathology of control and test drugs treated animals has shown normal features with regular cell arrangements.Pulmonary congestion, peribronchiolar mononuclear cell infiltration was observed in the lungs histopathology control animals, congestion, peribronchiolar mononuclear cell infiltration, perivascular and interstitial mononuclear cell infiltration was observed in test drug treated animals.No abnormalities were noticed in the histopathology of testis and epididymis of test drugs treated animals.Histopathology of heart, brain, spleen, thymus, pancreas and adrenal glands shown normal characteristic features in all experimental animals.

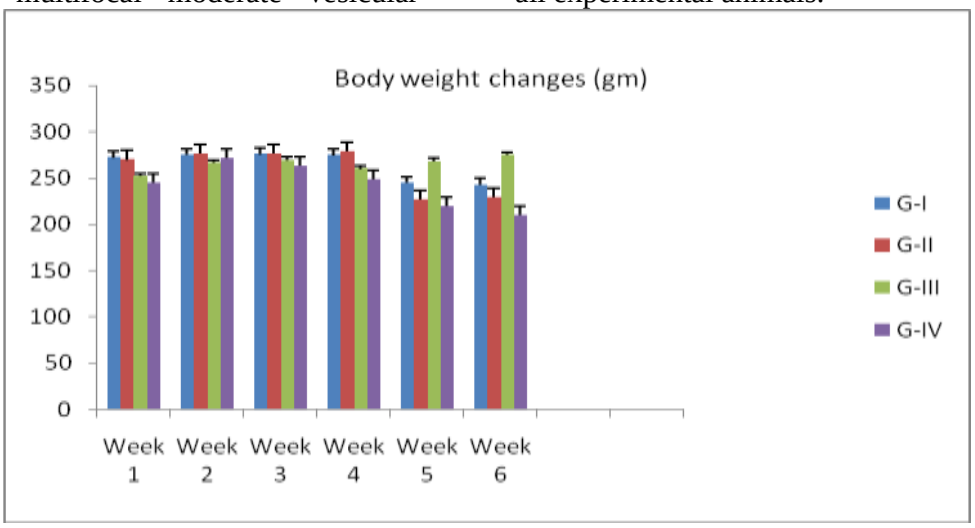


Fig. 1. Body weight (gm) changes in experimental animals

Table 1. Observations for acute toxicity study of APNC, SC and their combination in experimental rats

Observations	0 min	30 min	1 hr	2 hr	4 hr	8 hr	24 hr	7th day	14th day
Colour									
Skin color	N	N	N	N	N	N	N	N	N
Fur color	N	N	N	N	N	N	N	N	N
Eyes color	N	N	N	N	N	N	N	N	N
Urine color	N	N	N	N	N	N	N	N	N
Observations	0 min	30 min	1 hr	2 hr	4 hr	8 hr	24 hr	7th day	14th day
Behavioural observations (Mood)									
Alertness – Exploratory activity	N	N	N	N	N	N	N	N	N
Eyes opened/closed	N	N	N	N	N	N	N	N	N
Grooming	N	N	N	N	N	N	N	N	N
Restlessness	NOA	NOA	NOA	NOA	NOA	NOA	NOA	NOA	NOA
Irritability	NOA	NOA	NOA	NOA	NOA	NOA	NOA	NOA	NOA

Reactivity (Environment)	N	N	N	N	N	N	N	N	N
CNS Excitation									
Tremors	NOA	NOA	NOA	NOA	NOA	NOA	NOA	NOA	NOA
Twitches	NOA	NOA	NOA	NOA	NOA	NOA	NOA	NOA	NOA
Convulsions	NOA	NOA	NOA	NOA	NOA	NOA	NOA	NOA	NOA
CNS Depression									
Sedation	NAO	NAO	NAO	NAO	NAO	NAO	NAO	NAO	NAO
Sleep	N	N	N	N	N	N	N	N	N
Catatonia	NAO	NAO	NAO	NAO	NAO	NAO	NAO	NAO	NAO
Ataxia	NAO	NAO	NAO	NAO	NAO	NAO	NAO	NAO	NAO
Autonomic effects									
Defecation	N	N	N	N	N	N	N	N	N
Lacrimation	NAO	NAO	NAO	NAO	NAO	NAO	NAO	NAO	NAO
Urination	N	N	N	N	N	N	N	N	N
Salivation	N	N	N	N	N	N	N	N	N
Piloerection	NAO	NAO	NAO	NAO	NAO	NAO	NAO	NAO	NAO
Mydriasis	NAO	NAO	NAO	NAO	NAO	NAO	NAO	NAO	NAO
Meiosis	NAO	NAO	NAO	NAO	NAO	NAO	NAO	NAO	NAO
Emesis	NAO	NAO	NAO	NAO	NAO	NAO	NAO	NAO	NAO
Diarrhoea	NAO	NAO	NAO	NAO	NAO	NAO	NAO	NAO	NAO
Sensory Responses									
Touch responses	N	N	N	N	N	N	N	N	N
Pain responses	N	N	N	N	N	N	N	N	N
Pain Responses									
Pinna	N	N	N	N	N	N	N	N	N
Corneal	N	N	N	N	N	N	N	N	N
Motor Indication									
Abnormal Gait	NAO	NAO	NAO	NAO	NAO	NAO	NAO	NAO	NAO
Righting Reflex	N	N	N	N	N	N	N	N	N
Body Posture									
Body position	N	N	N	N	N	N	N	N	N
Limb position	N	N	N	N	N	N	N	N	N
Respiratory effects									
Apnoea	NAO	NAO	NAO	NAO	NAO	NAO	NAO	NAO	NAO
Dyspnoea	NAO	NAO	NAO	NAO	NAO	NAO	NAO	NAO	NAO

N-Normal; NAO-No Abnormality Observed

Table 2. Effect of APNC, SC and their combination on Hematological parameters at 28th day

Parameters	Group I	Group II	Group III	Group IV
Hb (g/dl)	13.54±0.56	14.32±0.52	13.64±0.47	13.34±0.43
PCV (%)	35.00±1.43	36.52±1.62	34.92±1.24	38.27±1.50
RBC (10^6/µl)	7.58±0.31	7.88±0.24	7.55±0.32	7.49±0.27
WBC (10^3/ µl)	11.08±9.05	12.50±1.49	10.30±1.37	11.44±2.21
Platelets(10^5/ µL)	7.95±3.56	7.99±7.91	9.69±6.76	10.24±1.47
MCV	46.12±0.44	46.22±0.88	46.30±0.47	51.12±0.74
MCH	17.92±0.15	17.94±0.23	18.10±0.18	17.79±0.22
MCHC	38.78±0.09	39.30±0.44	39.10±0.19	35.22±0.50
MPV (fl)	3.76±0.05	9.06±2.21	7.38±2.34	4.67±0.19
Neutrophils (%)	27.40±3.66	25.20±3.50	21.60±2.44	25.30±2.24
Lymphocytes (%)	63.40±4.30	65.60±4.45	69.00±3.27	69.20±2.63
Monocytes (%)	6.40±1.29	6.60±1.66	6.20±1.16	3.10±0.38
Eosinophils (%)	2.80±0.37	2.60±0.24	2.80±0.37	2.40±0.27

All values are expressed as Mean ± SEM; n=5. No significant difference since $p > 0.05$, when compared with control group.

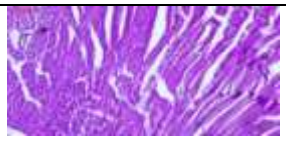
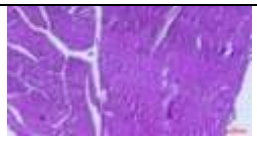
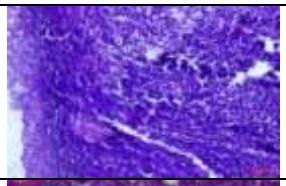
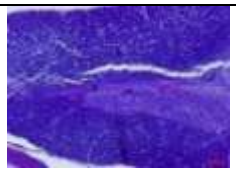
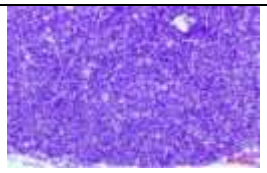
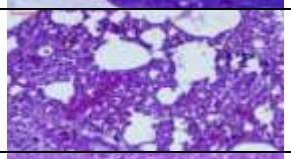
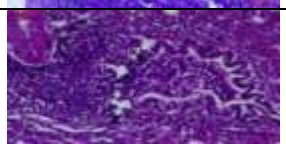
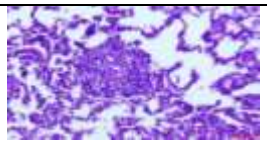
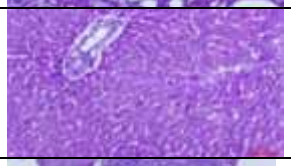
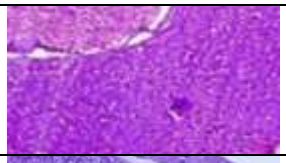
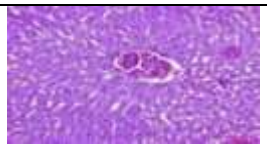
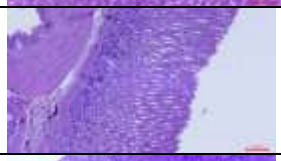
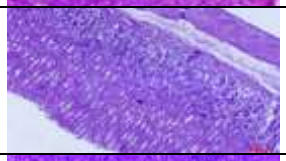
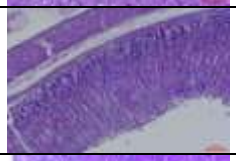
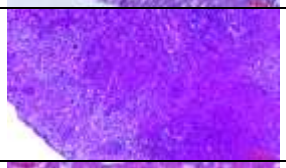
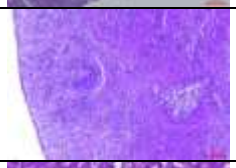
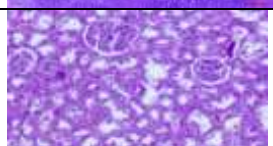
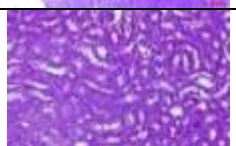
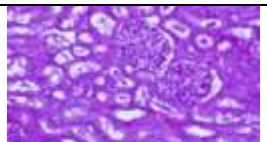
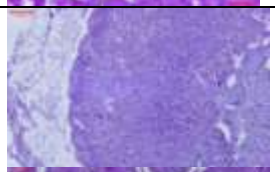
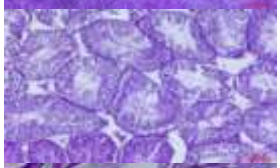
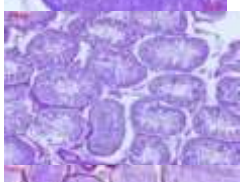
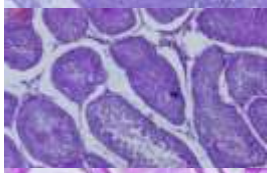
Organs	**Group I (Normal)**	**Group II APNC (1000 mg/kg b.w)**	**Group III SC (1000 mg/kg b.w)**	**Group IV APNC+SC (1000mg/kg b.w)**
Brain				
Heart				
Thymus				
Lungs				
Liver				
Stomach				
Spleen				
Kidney				
Adrenal gland				
Testis				
Epidedimis				

Fig.2. Effect of APNC, SC and their combination on histopathology of rat organs

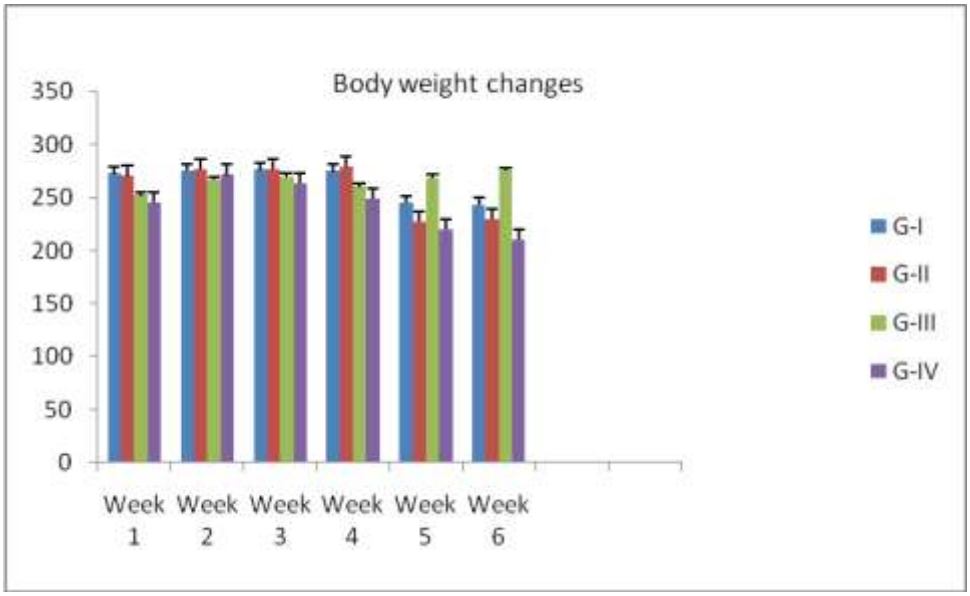


Fig.3. Effect of APNC, SC and their combination on body weight (g)

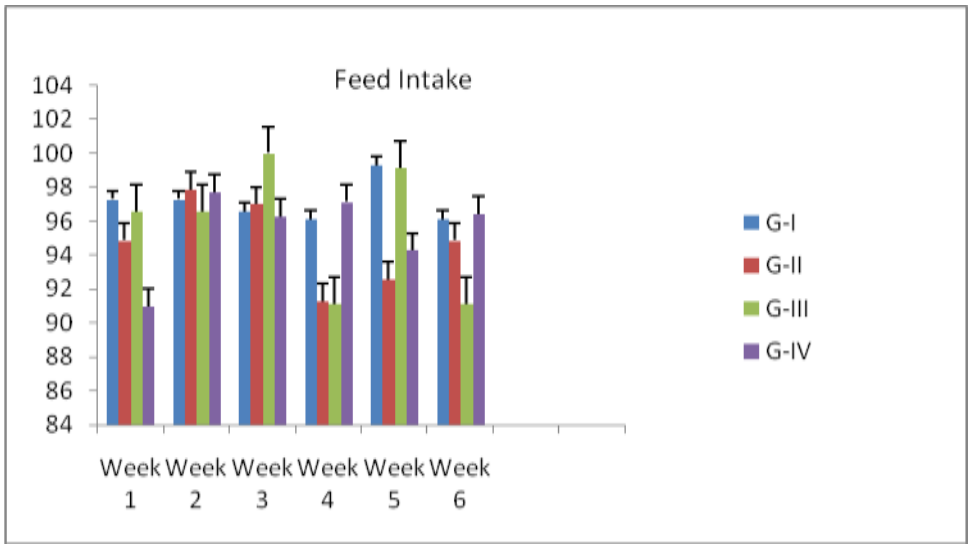


Fig 4. Effect of APNC, SC and their combination on Feed intake (g)

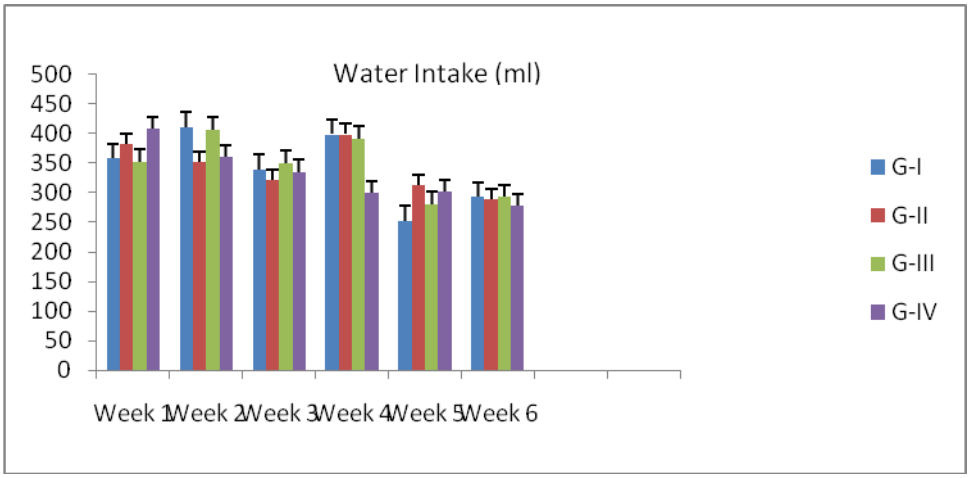


Fig.5. Effect of APNC, SC and their combination on water intake (ml)

Table 3: Effect of APNC, SC and their combination on serum parameters at 28th day

Parameter	Group I	Group II	Group III	Group IV
Total cholesterol (mg/dl)	84.60±4.58	70.60±5.84	80.40±3.78	68.50±0.96
Triglyceride (mg/dl)	45.80±4.34	35.00±2.35	47.40±8.24	33.75±6.38
HDL (mg/dl)	20.40±0.68	18.20±2.13	19.80±0.66	15.75±0.75
LDL (mg/dl)	52.60±3.66	43.60±4.71	46.20±3.25	46.25±1.03
Total bilirubin (mg/dl)	0.18±0.02	0.26±0.04	0.22±0.04	0.23±0.02
SGOT (U/L)	242.80±11.84	229.60±8.27	199.60±10.69	212.25±12.17
SGPT (U/L)	50.60±4.65	42.60±3.31	42.40±4.06	50.50±5.63
ALP (U/L)	145.40±16.17	141.00±15.22	150.80±16.10	144.00±25.53
Total protein (g/dl)	6.92±0.20	6.86±0.22	6.82±0.18	6.73±0.11
Albumin (g/dl)	2.60±0.07	2.56±0.13	2.66±0.18	3.28±0.78
GGT (U/L)	4.00±0.63	4.60±1.89	2.80±0.58	*3.50*±0.65
Creatinine kinase (U/L)	908.00±67.64	1005.20±61.77	724.20±111.35	812.50±115.89
Creatinine kinase –MB (U/L)	590.80±48.52	642.00±46.19	465.60±57.40	500.25±91.96
LDH(U/L)	1058.00±193.26	758.00±213.44	1487.60±57.40	1507.00±539.06
Blood urea (mg/dl)	27.40±1.81	26.00±1.52	24.80±1.24	26.50±3.01
Serum creatinine (mg/dl)	0.32±0.04	0.28±0.02	0.26±0.04	0.30±0.04
Serum calcium (mg/dl)	9.44±0.19	9.00±0.09	9.02±0.12	8.85±0.13
Uric acid (mg/dl)	1.64±0.47	1.44±0.14	1.68±0.17	1.38±0.22
CRP (mg/dl)	0.96±0.27	1.44±0.39	0.86±0.21	1.20±0.14
Blood glucose (mg/dl)	95.00±6.20	69.20±5.83	103.00±5.78	107.25±5.96

All values are expressed as Mean ± SEM; n=5. No significant difference since p> 0.05, when compared with control group.

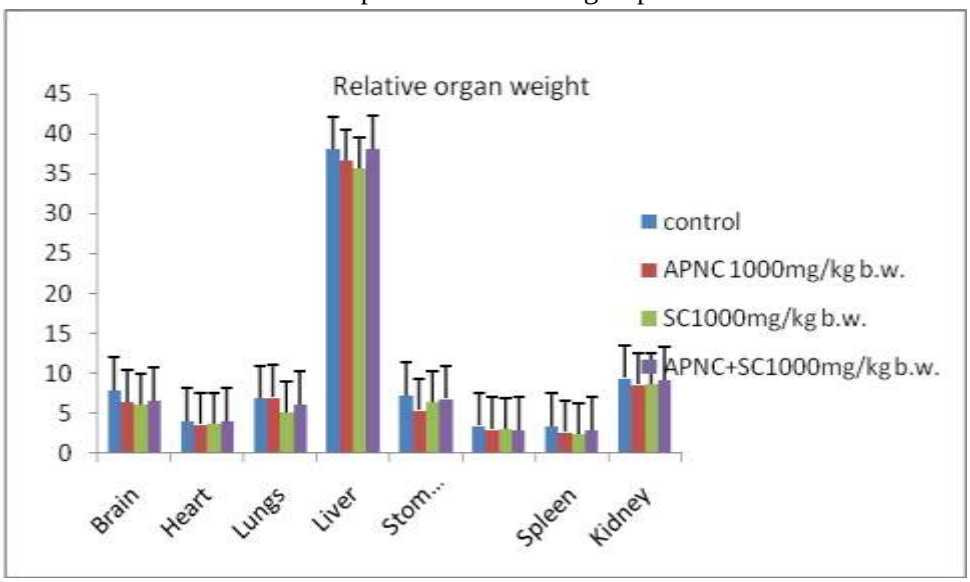


Fig. 6. Effect of APNC, SC and their combination on Relative Organ Weight of Male rats:

CONCLUSION:

In the summary, *Ayapoonaga chenduram, Seenthil choornam* and their combination (APNC+SC) are safe and not providing any mortality or other toxicity symptoms in both single and 28-days repeated oral toxicity studies. Acute toxicity study reveals that the LD_{50} of*Aya poonaga chenduram, Seenthil choornam* and their combination was greater than 2000 mg/kg body weight in fasted female rats and can be classified as category 5. Under the conditions of the present study, daily oral administration of *Ayapoonaga chenduram, Seenthil*

choornam and their combination at doses of up to 1000 mg/kg/day was well tolerated in rats. The no-observed-adverse-effect-level (NOAEL) of APNC, SC and their combination in albino rats is >1000 mg/kg/day when administered orally for 28 days.

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