EVALUATION OF CHRONIC TOXICITY OF GOWRI CHINTHAMANI CHENDURAM IN WISTAR ALBINO RATS

P. Elankani¹, G. Dayanand Reddy², R. Ganesan², B. Rama Devi², G.V. Narasimha Kumar³

¹. Siddha Clinical Research Unit, Palayamkottai, Tirunelveli, Tamilnadu-627002.
². Siddha Central Research Institute, Arumbakkam, Chennai, Tamilnadu -600106.
³. Dr. Anjali Chatterjee Regional Research Institute for Homoeopathy, Kolkata, West Bengal-700035.

(Received on Date: 4th April 2020      Date of Acceptance: 28th May 2020      Date of Publish: 01st July 2020)

Email id: ramasamba527@gmail.com

ABSTRACT

Aim and Objectives: The aim of the present study was to carry out 90 days repeated oral toxicity of Gowri chinthamani chenduram (GCC) in Wistar albino rats and to assess in a 30 days recovery period for delayed onset of any toxicity of Gowri chinthamani chenduram. Methods: Cronic toxicity of Gowri chinthamani chenduram was evaluated in wistar albino rats with reference to haematological, bio-chemical and histopathological studies. Results: No GCC-related changes in body weights, food 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: Based on the results of this study, the no-observed-adverse-effect-level (NOAEL) of Gowri chinthamani chenduram in rats is >400 mg/kg/day when administered orally for 90 days

Key words: Cronic toxicity, Gowri chinthamani chenduram, metallomineral, Siddha.

No: of Tables: 13                              No: of Figures : 02                              No: of References: 09
Introduction

India has different recognized systems of medicine. They are Ayurveda, Siddha, Unani, Yoga, Naturopathy Homoeopathy and Sowa Rigpa. Among them Siddha is the unique system of medicine “Siddha” means “established truth.” Siddha system of medicine is claimed to alleviate the root cause of the diseases by maintaining the ratio of Vatham, Pitham and Kapham. In this system of medicine the commonly used formulations in combination with minerals are Parpam (mineral/metallic oxides), Chendhooram (mineral/metallic sulphides), Chunnam (caustic or major oxides) and Pathangam (sublimation). Among them Parpam and Chenduram type of medicines are widely used, having potential therapeutic values.

Gowri chinthamani chenduram (GCC) is a Siddha metallominal formulation and it contains Mercury, Sulphur and Borax. Gowri chinthamani chenduram at a dose of 100 – 200 mg thrice a day with honey is one of the best and potent compound drugs for osteoarthritis mentioned in Agasthiar Vaidhya Kaviyam – 1500. It has a long history in Siddha system of medicine for various ailments especially for the treatment of inflammation. GCC is also known to cure 18 types of colic, 16 types of gastritis, chronic fevers, rat bite, pneumonia, bronchitis, dyspnoea, Tuberculosis (TB), bronchial asthma, piles, jaundice, inflammation of male and female genital organs accompanied by severe pain, pain in the tongue and ulcerative bites. The duration of the treatment is depends on the severity from 30 – 60 days. As mentioned earlier, GCC is a metallominal formulation and is being used for prolong period to treat several diseases. Chronic usage of these metal formulation may grounds toxic effects, hence, the need for its safety has to be ascertained. So the present work was carried out in wistar albino rats by following OECD guideline 408.

Materials and Methods:

Procurement of Gowri chinthamani chenduram: Gowri chinthamani chenduram was collected from The Indian Medical Practitioner’s Co-operative Pharmacy & Stores LTD (GMP certified) Thiruvaanmiyur, Chennai.

Preparation of Vehicle: The vehicle in the study was prepared as follows:

5 ml of dabur honey diluted with 95 ml R.O water and made the volume up to 100 ml (5% v/v). 0.5% CMC was prepared by triturating the 500 mg of CMC in 100 ml of 5% v/v honey till formation of a clear solution.

Preparation of Gowri chinthamani chenduram: The 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 to obtain a concentration of 40 mg/kg, 200 mg/kg and 400 mg/kg.

Animals and Husbandry: A total of 80 wistar albino rats (40/sex) of 5 to 6 weeks age were received from TANUVAS, Chennai, Tamil Nadu. The body weight variation of the animals selected for the study on the day of randomization did not exceed ± 20 % of the mean body weight of each sex. This study was 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. The study was approved by the Institutional Ethical Committee (164/PHARMA/SCRI/2017).

Feed and water were provided ad libitum, except on study day (SD) 30-31, 60-61, 90-91 (before terminal sacrifice) and SD 119-120 (before recovery sacrifice) when food-fasting was implemented and rats were fasted for 12 hours before termination at those 4 occasions. The animals were maintained in polypropylene cages at room temperature 18-25°C, humidity 30 to 65% and light cycle 12-hour light/12-hour dark.

Chemicals: The chemicals used in this study such as Carboxy methyl cellulose, Sodium chloride, Formaldehyde, Anesthetic ether and Sodium ethylenediaminetetraacetic acid (sodium-EDTA) of analytical grade were purchased from Therese scientific works, Chennai.

Dose calculation of Gowri chinthamani chenduram: The clinical dose of Gowri chinthamani chenduram is 200 mg. The animal (rat) doses are calculated as per the FDA guidelines and the calculated therapeutic dose (TD) was found to be 40mg/kg of body weight. In the present study to evaluate the dose correlated effects, 5 times TD and 10 times TD i.e. 200 mg/kg & 400 mg/kg of bodyweight were chosen correspondingly.

Experiment Design: The present study was carried out according to OECD 408 guidelines. Animals were initially divided into four groups, each group contains 20 animals (10/sex) based upon descending body weights and physical examinations. Male and females were randomized into groups separately based on bodyweight. After the randomization process, each study animal was assigned a unique number and identified by a picric acid mark. Group-I served as normal control, Group-II, Group-III and Group-IV administered with test drug at a doses of 40 mg/kg, 200 mg/kg and 400p.o. mg/kg respectively for 90 days. At 90th day 50% of the experimental animals (40 animals) in each group were subjected to euthanasia. The necropsy was carried out on all euthanized animals and the organs were isolated and observed macroscopically for abnormalities. After 30days of post treatment the remaining half animals (40animals) were euthanized and organs were collected and observed macroscopically for abnormalities. The organs of treatment and post treatment were preserved in 10% neutral formalin and were subjected for histopathology.

Observations: Cage side observations included observation for 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.

**Hematology:** The hematology parameters viz., hemoglobin concentration (HB), packed cell volume (PCV), total red blood cell count (RBC), total white blood cell count (WBC) and platelet count (PLT) were analyzed.

**Serum Biochemistry:** Following serum biochemical parameters were estimated using RA-50 auto analyzer(Bayer). Glucose, Alkaline phosphatase Total proteins, Albumin, Creatine phosphotase, Uric acid and Calcium.

**Histopathology:** All tissue samples from each group viz., brain, pancreas, adrenal glands, heart, thymus, liver, kidneys, spleen, stomach, testes / ovaries, epididymides/ uterus, sciatic nerve, skin and femur bone from each group test animals(1/sex) were 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.

**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.

**Results and Discussions:**
The dose volume was 1ml/100g body weight per day for all animals. The total volume of administration was calculated based on the weekly body weights of the animals. In the present study changes in body weight, feed and water consumption, biochemical parameters and histopathological studies were carried out and the results are expressed in the form of tables and figures.

Blood urea, Creatinine, Total Cholesterol,Triglycerides, urea, creatinine, cholesterol, triglycerides, were measured using the methods described in the previous section. All the results were expressed in mean ± SEM. The statistical significance was determined using one-way ANOVA followed by Tukey’s post hoc test. The statistical significance level was set at P<0.05. The results are presented in Table 1 and 2.

No compound-related mortality or signs of toxicity were noted. Other observations noted included alopecia, excessive grooming, Straube’s phenomenon, abscess formation and hyperactivity; these observations were considered unrelated to treatment because they occurred in both the compound-treated and control groups or only appeared sporadically in low incidence throughout the study with no correlation to treatment or sex.

No compound-related body weight changes were noted. No significant differences were noted in total body weight change over the course of the study for either sex. Group summary of body weight data for males and females are presented in table 1 and 2 respectively.

A significant change in the feed intake was not observed in animals during treatment period and post recovery period between the groups. The data are presented in table 3.

No significant change in the water intake was observed in animals during treatment
period and post recovery period between the groups. The data are presented in table 4.

The clinical pathology evaluation and data reports are presented in table 5-12. No compound-related changes in hemoglobin concentration, packed cell volume, total red blood cell count, total white blood cell count and platelet count were noted (table 5-8). 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 (table 9-12) were noted.

Macroscopic observations were listed in table 13. All findings listed in table 13 were considered incidental because they occurred in frequently, in both treated and control animals, exhibited no dose relationship.

The histopathology of brain and stomach (glandular & non glandular) shown normal characteristic features with regular cell arrangements at the end of the treatment (90th day) and post recovery period (120th day) in both control and GCC treated animals. Liver histopathology showed congestion, multifocal moderate vesicular (micro to macro) fatty degeneration of hepatocytes. The histopathology of heart in all groups shown normal characteristic features at the end of the treatment (90th day) and post recovery period (120th day). Congestion and mild tubular epithelial cell degeneration was noticed in the histopathology of kidneys of control and test drug GCC treated animals. Pulmonary congestion, haemorrhages, peribronchial and interstitial mononuclear cell infiltration was observed in the lungs histopathology of both control and test drug treated animals at end of the treatment (90th day) and post recovery period (120th day). Congestion was noticed in the spleen of group-II (animal No .GCC20; dose 40mg/kg) at post recovery period (120th day). The histopathology of thymus has shown mild lymphoid cell depletion in group-III (animal No.66; dose 200mg/kg) and group-IV (animal No.37; dose 400mg/kg) animals at end of the treatment. No abnormalities and damaged cells were noticed in the histopathology of sciatic nerve, adrenal gland, spleen, skin, femur bone, eye, ovary and uterus. Images of the organs were shown in figure 1 and 2 at end of the treatment and post recovery period respectively.
Table 1. Effect of GCC on body weight (g) of male rats

| S.NO. | Group | Treatment Period | Post recovery Period |
|-------|-------|------------------|----------------------|
|       |       | 0\textsuperscript{th} Day | 30\textsuperscript{th} Day | 60\textsuperscript{th} Day | 90\textsuperscript{th} Day | 120\textsuperscript{th} Day |
| 1     | I     | 216.0±11.77      | 246.1±28.35          | 272.5±30.98          | 304.5±34.20          | 325.5±12.95          |
| 2     | II    | 224.3±9.804      | 269.2±12.02          | 312.4±15.01          | 333.2±14.81          | 351.6±23.57          |
| 3     | III   | 224.7±4.847      | 271.1±4.927          | 314.8±6.751          | 336.4±6.822          | 353.5±11.59          |
| 4     | IV    | 221.3±6.683      | 276.9±4.334          | 321.5±4.865          | 338.3±5.880          | 362.0±6.988          |

All values were expressed as Mean ± S.E.M; n=10

Table 2. Effect of GCC on body weight (g) of female rats

| S.NO. | Groups | Treatment Period | Post Recovery Period |
|-------|--------|------------------|----------------------|
|       |        | 0\textsuperscript{th} Day | 30\textsuperscript{th} Day | 60\textsuperscript{th} Day | 90\textsuperscript{th} Day | 120\textsuperscript{th} Day |
| 1     | I      | 171.1±6.92       | 201.0±5.90          | 207.8±5.08          | 215.9±5.982          | 229.3±14.40          |
| 2     | II     | 168.9±6.16       | 201.4±5.02          | 211.2±4.99          | 222.2±5.921          | 232.0±5.779          |
| 3     | III    | 166.8±3.99       | 185.9±21.2          | 171.3±30.2          | 160.0±35.55          | 229.3±16.25          |
| 4     | IV     | 183.6±8.88       | 195.4±4.76          | 183.3±20.8          | 190.0±21.67          | 225.0±5.447          |

All values were expressed as Mean ± S.E.M; n=10
Table 3. Effect of GCC on Feed intake (g) of experimental animals

| Weeks       | Treatment Groups |                   |                   |                   |
|-------------|------------------|-------------------|-------------------|-------------------|
|             | Group I           | Group II          | Group III         | Group IV          |
| Treatment Period |                   |                   |                   |                   |
| Week 1      | 235±34.74        | 255±45.43         | 242±38.49         | 302±9.09          |
| Week 2      | 323.5±6.15       | 344±4.28          | 344±6.95          | 331.5±3.47        |
| Week 3      | 343.5±6.15       | 361.5±2.41        | 366.5±1.34        | 351±4.81          |
| Week 4      | 342.5±0.27       | 362.5±1.87        | 335.5±5.08        | 356±1.60          |
| Week 5      | 322.5±1.87       | 341.5±2.41        | 334±2.14          | 301.5±9.34        |
| Week 6      | 338±3.21         | 368.5±2.41        | 326.5±0.27        | 343±0.53          |
| Week 7      | 338.5±6.68       | 364.5±0.80        | 336±3.21          | 340.5±9.35        |
| Week 8      | 338.5±5.61       | 368.5±1.34        | 332.5±0.27        | 343.5±7.75        |
| Week 9      | 351.5±1.34       | 365±2.67          | 315±4.28          | 350±1.60          |
| Week 10     | 326±2.67         | 377.5±4.54        | 327±3.74          | 347.5±7.75        |
| Week 11     | 335±9.62         | 367.5±1.34        | 305±3.21          | 346.5±3.47        |
| Week 12     | 344±8.02         | 366.5±2.94        | 311.5±9.35        | 338.5±5.08        |
| Week 13     | 247.5±43.56      | 276±46.5          | 217±48.11         | 253.5±48.37       |
| Post Recovery Period |                   |                   |                   |                   |
| Week 14     | 166.5±2.94       | 192.5±1.34        | 128±0.53          | 156.5±4.01        |
| Week 15     | 171±3.21         | 181±3.74          | 132±0             | 159.5±1.87        |
| Week 16     | 156.5±6.15       | 180.5±2.41        | 151.5±0.27        | 142.5±1.87        |
| Week 17     | 160.9±1.60       | 190.5±0.80        | 136.5±0.27        | 170.5±0.80        |

All values were expressed as Mean ± S.E.M; n=10
Table 4. Effect of GCC on water intake (ml) of experimental animals

| Weeks       | Treatment Groups |         |         |         |
|-------------|------------------|---------|---------|---------|
|             | Group I          | Group II| Group III| Group IV|
| Treatment Period |                   |         |         |         |
| Week 1      | 592.5±6.68       | 600±0   | 630.5±18.98 | 625±2.67 |
| Week 2      | 615±29.40        | 630±42.76 | 567.5±14.70 | 636±8.55 |
| Week 3      | 616.5±48.91      | 674±23.52 | 612.5±22.72 | 664±34.21 |
| Week 4      | 747.5±14.70      | 782.5±9.35 | 690±21.38  | 742.5±14.7 |
| Week 5      | 697.5±25.39      | 670±42.76 | 655±61.47  | 705±45.43 |
| Week 6      | 775±8.02         | 735±13.36 | 725±13.36  | 760±0    |
| Week 7      | 785±8.02         | 780±10.69 | 757.5±6.68 | 775±13.36 |
| Week 8      | 795±2.67         | 760±10.69 | 730±16.04  | 770±5.35 |
| Week 9      | 770±5.35         | 730±10.69 | 695±8.02   | 730±0    |
| Week 10     | 795±2.67         | 790±5.35  | 765±2.67   | 780±0    |
| Week 11     | 750±0            | 760±0    | 770±5.35   | 760±10.69|
| Week 12     | 925±24.05        | 955±13.36 | 835±29.40  | 935±2.67 |
| Week 13     | 675±168.37       | 675±136.30 | 660±165.7  | 645±157.68|
| Post Treatment Period |             |         |         |         |
| Week 14     | 395±13.36        | 435±8.02 | 290±5.35  | 335±2.67 |
| Week 15     | 305±8.02         | 305±8.02 | 270±0     | 310±5.35 |
| Week 16     | 385±8.02         | 425±2.67 | 300±10.69 | 315±2.67 |
| Week 17     | 355±18.71        | 385±24.05 | 260±5.35  | 320±0    |

All values were expressed as Mean ± S.E.M; n=10
### Hematology results:

Table 5. Effect of GCC on Hematological parameters at 30th day

| Parameter     | Group I       | Group II      | Group III     | Group IV      |
|---------------|---------------|---------------|---------------|---------------|
| Hb (g/dl)     | 12.66±0.559   | 12.71±0.398   | 11.79±0.374   | 12.43±0.191   |
| PCV (%)       | 33.63±1.448   | 33.77±1.085   | 31.32±0.954   | 32.94±0.507   |
| RBC (m/µL)    | 6.906±0.282   | 6.936±0.206   | 6.520±0.209   | 6.827±0.106   |
| WBC (/cmm)    | 10285±900.6   | 8881±687.7    | 9800±660.1    | 8553±575.8    |
| Platelets (L / µL) | 7.564±0.451 | 7.038±0.491   | 8.000±0.307   | 7.888±0.244   |
| Neutrophils (/cmm) | 20.74±0.801 | 18.50±1.072   | 16.72±0.726   | 18.84±0.693   |
| Lymphocytes (/cmm) | 74.11±0.884 | 76.65±1.208   | 79.63±0.780   | 76.21±0.890   |
| Monocytes (/cmm)    | 2.737±0.365   | 2.550±0.328   | 1.842±0.115   | 2.526±0.377   |
| Eosinophils (/cmm) | 2.556±0.166   | 2.350±0.181   | 2.105±0.169   | 2.474±0.177   |

All values were expressed as Mean ± S.E.M; n=10

Table 6. Effect of GCC on Hematological parameters at 60th day

| Parameter     | Group I       | Group II      | Group III     | Group IV      |
|---------------|---------------|---------------|---------------|---------------|
| Hb (g/dl)     | 12.43±0.425   | 11.47±0.235   | 39.07±6.366   | 38.05±8.067   |
| PCV (%)       | 33.55±1.224   | 30.81±0.675   | 29.73±1.037   | 29.89±0.562   |
| RBC (m/µL)    | 6.702±0.258   | 6.198±0.140   | 6.029±0.209   | 5.951±0.121   |
| WBC (/cmm)    | 9050±1087     | 8055±494.5    | 7595±421.9    | 7625±647.0    |
| Platelets (L / µL) | 7.903±0.398 | 8.219±0.277   | 8.337±0.376   | 8.389±0.254   |
| Neutrophils (/cmm) | 23.60±1.072 | 21.75±1.135   | 22.7±1.363    | 24.38±1.064   |
| Lymphocytes (/cmm) | 69.80±2.677 | 73.00±1.277   | 72.75±1.442   | 71.81±1.141   |
| Parameter              | Group I     | Group II    | Group III   | Group IV   |
|-----------------------|-------------|-------------|-------------|------------|
| Monocytes (/cmm)      | 2.80±0.277  | 2.90±0.190  | 2.85±0.283  | 2.25±0.111 |
| Eosinophils (/cmm)    | 1.750±0.123 | 1.850±0.150 | 1.700±0.105 | 1.800±0.144|

All values were expressed as Mean ± S.E.M; n=10

Table 7. Effect of GCC on Hematological parameters at 90th day

| Parameter              | Group I     | Group II    | Group III   | Group IV   |
|-----------------------|-------------|-------------|-------------|------------|
| Hb (g/dl)             | 11.21±0.381 | 11.96±0.327 | 11.92±0.207 | 11.73±0.329|
| PCV (%)               | 31.48±1.055 | 33.92±0.945 | 33.72±0.504 | 33.12±0.863|
| RBC (m/µl)            | 5.955±0.210 | 6.286±0.218 | 6.405±0.114 | 6.248±0.178|
| WBC (/cmm)            | 9620±881.2  | 8605±685.8  | 8118±591.2  | 15606±4731 |
| Platelets (L / µL)    | 7.791±0.281 | 8.481±0.287 | 8.700±0.198 | 8.549±0.340|
| Neutrophils (/cmm)    | 26.20±1.167 | 42.25±18.75 | 21.76±0.779 | 26.50±1.049|
| Lymphocytes (/cmm)    | 69.4±1.148  | 70.35±1.487 | 73.94±0.829 | 69.83±1.055|
| Monocytes (/cmm)      | 2.550±0.245 | 3.400±0.515 | 2.353±0.331 | 4.167±1.681|
| Eosinophils (/cmm)    | 1.850±0.150 | 2.100±0.160 | 1.941±0.159 | 1.313±0.150|

All values were expressed as Mean ± S.E.M; n=10

Table 8. Effect of GCC on Hematological parameters at 120th day

| Parameter              | Group I     | Group II    | Group III   | Group IV   |
|-----------------------|-------------|-------------|-------------|------------|
| Hb (g/dl)             | 10.42±0.494 | 11.03±0.209 | 10.84±0.527 | 10.06±0.395|
| PCV (%)               | 28.80±1.357 | 31.27±0.322 | 30.31±1.412 | 28.65±1.057|
| RBC (m/µl)            | 5.591±0.296 | 5.851±0.156 | 5.825±0.318 | 5.450±0.240|
| WBC (/cmm)            | 7313±841.6  | 8800±801.9  | 7500±847.7  | 8163±731.4 |
| Platelets (L / µL)    | 7.274±0.447 | 8.179±0.203 | 7.575±0.436 | 7.149±0.367|
| Neutrophils (/cmm)    | 23.75±1.556 | 24.40±1.157 | 22.50±0.823 | 23.63±1.569|
| Lymphocytes (/cmm)    | 69.00±1.822 | 68.80±1.519 | 72.50±1.052 | 69.88±1.608|
| Monocytes (/cmm)      | 5.500±0.566 | 5.100±0.622 | 3.375±0.532 | 4.500±0.731|
|                  | G-I           | G-II          | G-III         | G-IV          |
|------------------|--------------|--------------|---------------|--------------|
| Glucose (mg/dl)  | 83.28±5.42   | 87.83±5.22   | 98.63±4.20    | 87.28±5.19   |
| ±1.27           | ±1.21        | ±1.22        | ±1.24         | ±1.24        |
| 9               | 8            | 5            | 4             | 5            |
| Creatinine (mg/dl) | 0.43±0.018   | 0.43±0.016   | 0.38±0.024    | 0.42±0.018   |
| ±0.018          | ±0.016       | ±0.024       | ±0.018        | ±0.018       |
| 9               | 8            | 5            | 4             | 5            |
| T. Cholesterol  (mg/dl) | 79.17±3.853 | 71.76±2.838 | 72.37±3.424  | 66.89±2.175  |
| ±6.02           | ±0.97        | ±0.79        | ±0.55         | ±0.55        |
| 9               | 3           | 9            | 9             | 9            |
| Triglyceride (mg/dl) | 25±1.281    | 23.88±0.97   | 23.63±0.79    | 23.68±0.78   |
| ±3.91           | ±0.72        | ±0.79        | ±0.78         | ±0.78        |
| 9               | 6           | 9            | 7             | 7            |
| HDL (mg/dl)     | 32.22±3.91   | 32.29±2.72   | 30.84±3.22    | 28.32±3.02   |
| ±0.01           | ±0.01        | ±0.00        | ±0.01         | ±0.01        |
| 9               | 0           | 1            | 0             | 0            |
| LDL (mg/dl)     | 227.6±14.1   | 214.6±11.2   | 183.9±5.41    | 175.5±7.60   |
| ±0.14           | ±0.16        | ±0.14        | ±0.16         | ±0.16        |
| 7               | 2           | 9            | 6             | 5            |
| T. bilirubin (mg/dl) | 0.127±0.012  | 0.138±0.011  | 0.121±0.009   | 0.131±0.012  |
| ±0.01           | ±0.01        | ±0.00        | ±0.01         | ±0.01        |
| 9               | 0           | 1            | 0             | 0            |
| SGOT (U/L)      | 54.61±1.94   | 51.35±3.58   | 53.05±1.48    | 50.05±2.08   |
| ±0.17           | ±0.16        | ±0.17        | ±0.17         | ±0.17        |
| 2               | 4           | 7            | 6             | 5            |
| SGPT (U/L)      | 167.2±12.06  | 194.8±14.46  | 205.4±15.54   | 197.7±12.41  |
| ±0.17           | ±0.17        | ±0.17        | ±0.17         | ±0.17        |
| 4               | 2           | 5            | 8             | 2            |
| ALP (U/L)       | 6.889±0.09   | 6.876±0.11   | 6.868±0.11    | 6.879±0.10   |
| ±0.09           | ±0.09        | ±0.09        | ±0.09         | ±0.09        |
| 0               | 4           | 5            | 8             | 2            |
| T. proteins (g/dl) | 6.889±3.67   | 6.876±0.11   | 6.868±0.11    | 6.879±0.10   |
| ±0.09           | ±0.09        | ±0.09        | ±0.09         | ±0.09        |
| 0               | 8           | 8            | 8             | 8            |
| Calcium (mg/dl) | 8.367±0.09   | 8.341±0.07   | 8.374±0.09    | 8.379±0.09   |
| ±0.09           | ±0.07        | ±0.09        | ±0.09         | ±0.09        |
| 0               | 0           | 0            | 0             | 0            |

All values were expressed as Mean ± S.E.M; n=10

* indicates p<0.05 when compared to vehicle control
Table 10. Effect of GCC on serum biochemistry (60th day):

| Group | Glucose (mg/dl) | Urea (mg/dl) | Creatinine (mg/dl) | T. Cholesterol (mg/dl) | Triglyceride (mg/dl) | HDL (mg/dl) | LDL (mg/dl) | T. bilirubin (mg/dl) | SGOT (U/L) | SGPT (U/L) | ALP (U/L) | T. proteins (g/dl) | Calcium (mg/dl) |
|-------|-----------------|--------------|--------------------|------------------------|---------------------|-------------|-------------|-------------------|--------|----------|---------|----------------|-----------------|
| G-I   | 109.4±3.363     | 32.68±1.47   | 0.494±0.02         | 75.32±3.35             | 103.6±5.77          | 25.26±1.03  | 29.16±3.13  | 0.105±0.00        | 191.7±6.77 | 63.26±2.34 | 225.6±10.8 | 7.437±0.08        | 9.57±9.08       |
| G-II  | 96.43±3.310     | 32.05±1.22   | 0.476±0.01         | 68.33±2.69             | 100.1±4.29          | 22.76±1.29  | 25.52±2.02  | 0.110±0.01        | 221.7±13.0 | 66±3.10   | 242.8±13.5 | 7.310±0.26        | 9.55±0.54       |
| G-III | 101.3±3.314     | 34.44±1.65   | 0.455±0.02         | 67±3.16                | 91±4.16             | 22.3±1.07   | 26.4±2.92   | 0.105±0.00        | 193.8±7.91 | 57.11±1.61 | 246.7±10.8 | 7.050±0.17        | 9.45±6.01       |
| G-IV  | 93.68±3.295     | 32.16±1.19   | 0.421±0.02         | 61.16±1.65             | 85.37±4.69          | 21.11±0.88  | 24.7±1.73   | 0.100±0.00        | 177.5±6.84 | 57.74±2.76 | 234.2±21.1 | 7.121±0.07        | 9.50±0.08       |

All values were expressed as Mean ± S.E.M; n=10
Table 11. Effect of GCC on serum biochemistry (90th day):

| Group | Glucose (mg/dl) | Urea (mg/dl) | Creatinine (mg/dl) | T. Cholesterol (mg/dl) | Triglyceride (mg/dl) | HDL (mg/dl) | LDL (mg/dl) | T. bilirubin (mg/dl) | SGOT (U/L) | SGPT (U/L) | ALP (U/L) | T. proteins (g/dl) | Calcium (mg/dl) |
|-------|----------------|--------------|-------------------|-----------------------|---------------------|-------------|-------------|---------------------|------------|-------------|-----------|-------------------|----------------|
| G-I   | 99±3.43        | 29.5         | 73±3.1            | 93.89±6.43            | 28.84±1.70          | 0.115±0.00  | 0.172±0.01  | 1.32±6.19          | 268.3±1    | 324.3±10.1  | 156.4±0.06   | 7.521±0.06       | 9.39±5.00      |
|       | 8±1.143        | 163           |                   |                       |                     |             |             |                     |            |             |           |                   | 144            |
| G-II  | 95.25±3.298    | 27±0.74      | 66.75±3.10        | 73.3±3.608            | 26.30±1.51          | 0.120±0.00  | 182.9±6.31  | 51.85±1.55         | 6.31±1     | 154.2±9.35  | 7.315±0.11   | 8.87±0.00        | 3.98±3.00     |
|       | 8±0.29         | 16            |                   |                       |                     |             |             |                     |            |             |           |                   | 398            |
| G-III | 91.06±6.025    | 28.7         | 65.65±2.76        | 84.82±9.78            | 27.18±1.80          | 0.118±0.01  | 173.3±8.02  | 52.19±2.01         | 11.73±3    | 171±11.73  | 7.281±0.10  | 8.98±2.00        | 6.44±2.00     |
|       | 1±1.07         | 107           |                   |                       |                     |             |             |                     |            |             |           |                   | 644            |
| G-IV  | 100.6±1.09     | 32.1         | 61.79±2.10        | 76.72±4.79            | 26.47±1.97          | 0.126±0.01  | 182.6±2.33  | 54.11±2.33         | 14.8±0.07  | 182.5±0.07  | 7.368±0.07  | 9.50±0.00        | 0.88±0.06     |
|       | 6±2.410        | 410           |                   |                       |                     |             |             |                     |            |             |           |                   | 0.088          |

All values were expressed as Mean ± S.E.M; n=10
Table 12. Effect of GCC on serum biochemistry (120th day):

| Group | Glucose (mg/dl) | Urea (mg/dl) | Creatinine (mg/dl) | T. Cholesterol (mg/dl) | Triglyceride (mg/dl) | HDL (mg/dl) | LDL (mg/dl) | T. bilirubin (mg/dl) | SGOT (U/L) | SGPT (U/L) | ALP (U/L) | Calcium (mg/dl) |
|-------|----------------|--------------|-------------------|-----------------------|---------------------|-------------|-------------|-------------------|-------------|-------------|-----------|----------------|
| G-I   | 97.67±5         | 29.8         | 0.422±0.01        | 74.56±3.57            | 93.22±10.4          | 25.78±1.16   | 30±4.327    | 0.144±0.01        | 186.6±7.68  | 5311±3.195 | 189.8     | 6.456±0.76     |
|       | 9.180          | 620          | 4                  | 1                     | 4                   | 4           | 7           | 6                 | 6           | 6           | 6         | 6±0.146       |
| G-II  | 113.1±9         | 29.3         | 0.480±0.02        | 76±2.996              | 105.7±9.43          | 27±1.011     | 27.5±3.468  | 0.100±0.00        | 206.7±13.3  | 56.7±1.535  | 227.3     | 7.390±0.13     |
|       | 390            | 1.5          | 0.02              | 96                   | 111                 | 111         | 111         | 111               | 111         | 111         | 111       | 111           |
All values were expressed as Mean ± S.E.M; n=10

**Macroscopic Observations:**

| S. No | Group | Total No. of Animals | Macroscopic Findings | Terminal sacrifice | Post recovery sacrifice |
|-------|-------|-----------------------|-----------------------|--------------------|------------------------|
| 1     | G-I   | 20                    | NAO                   | A cyst in left kidney (animal No. GCC50; male). Presence of black pores, pale colored liver and kidneys (animal No. GCC58; female). |
| 2     | G-II  | 20                    | Cystic ovary (animal No. GCC18; female) | Presence of black pores, pale colored liver and kidneys (animal No. GCC55; male). |
| 3     | G-III | 20                    | Nodules at the base of the pancreas (animal No. GCC22; male) | Cyst in left kidney (animal No. GCC54; male). |
| 4     | G-IV  | 20                    | NAO                   | NAO                |

NAO= No Abnormality Observed
Figure 1. Effect of GCC on histopathology of rat organs (at end of the treatment)

| Effect of GCC on Histopathology of Different Organs (End of the treatment) |
|-----------------------------------------------|
| Brain                                      | Liver          |
| ![Brain (a)](image1)                       | ![Liver (a)](image2) |
| ![Brain (b)](image3)                      | ![Liver (b)](image4) |
| Lungs                                      | Thymus         |
| ![Lungs (a)](image5)                       | ![Thymus (a)](image6) |
| ![Lungs (b)](image7)                       | ![Thymus (b)](image8) |
| Testis                                     | Epididymis     |
| ![Testis (a)](image9)                      | ![Epididymis (a)](image10) |
| ![Testis (b)](image11)                     | ![Epididymis (b)](image12) |
| Stomach                                    | Spleen         |
| ![Stomach (a)](image13)                    | ![Spleen (a)](image14) |
| ![Stomach (b)](image15)                    | ![Spleen (b)](image16) |
| Skin                                       | Sciatic nerve  |
| ![Skin (a)](image17)                       | ![Sciatic nerve (a)](image18) |
| ![Skin (b)](image19)                       | ![Sciatic nerve (b)](image20) |
Microscope magnification: 10X

(a) denotes organs of the normal control animals

(b) denotes organs of the animals treated with high dose of *Gowri chinthamani chenduram*
Figure 2. Effect of GCC on histopathology of rat organs (Post recovery period)

| Effect of GCC on Histopathology of Different Organs (Post recovery period) |
|----------------------------------------------------------|
| **Brain**                                                 |
| ![Image of brain histopathology (a)](image1)               |
| ![Image of brain histopathology (b)](image2)               |
| **Liver**                                                  |
| ![Image of liver histopathology (a)](image3)               |
| ![Image of liver histopathology (b)](image4)               |
| **Lungs**                                                  |
| ![Image of lung histopathology (a)](image5)                |
| ![Image of lung histopathology (b)](image6)                |
| **Thymus**                                                 |
| ![Image of thymus histopathology (a)](image7)              |
| ![Image of thymus histopathology (b)](image8)              |
| **Testis**                                                 |
| ![Image of testis histopathology (a)](image9)              |
| ![Image of testis histopathology (b)](image10)             |
| **Epididymis**                                             |
| ![Image of epididymis histopathology (a)](image11)         |
| ![Image of epididymis histopathology (b)](image12)         |
| **Stomach**                                                |
| ![Image of stomach histopathology (a)](image13)            |
| ![Image of stomach histopathology (b)](image14)            |
| **Spleen**                                                 |
| ![Image of spleen histopathology (a)](image15)             |
| ![Image of spleen histopathology (b)](image16)             |
| **Skin**                                                   |
| ![Image of skin histopathology (a)](image17)               |
| ![Image of skin histopathology (b)](image18)               |
| **Sciatic nerve**                                          |
| ![Image of sciatic nerve histopathology (a)](image19)      |
| ![Image of sciatic nerve histopathology (b)](image20)      |
Rama Devi et al.,

Microscope magnification: 10X

(a)- denotes organs of the normal control animals

(b)- denotes organs of the animals treated with high dose of *Gowri chinthamani chenduram*
ACKNOWLEDGEMENT

The present IMR-Project was sponsored by Central Council for Research in Siddha, Ministry of AYUSH, Arumbakkam, Chennai, Tamil Nadu and the work has been carried out at Department of Pharmacology, Siddha Central Research Institute, Arumbakkam, Chennai.

CONCLUSION:

Under the conditions of the present study, daily oral administration of Gowri chinthamani chenduram at doses of up to 400 mg/kg/day was well tolerated in rats. The no-observed-adverse-effect-level (NOAEL) of Gowri chinthamani chenduram in rats is >400 mg/kg/day when administered orally for 13 consecutive weeks (90 days).

REFERENCES

Madhavan R, Sathish R and Murugesan M. standardization of Sangu parpam a herbo marine siddha drug. International Journal of Current Research in Chemistry and 1. Pharmaceutical Sciences. 2016; 3: 77-84.

Thas JJ. Siddha medicine-background and principles and the application for skin diseases. Clindermatol. 2008; 26:62-78.

Ravishankar B. and Shukla VJ. Indian systems of medicine: a brief profile. African Journal of Traditional, Complementary and Alternative medicines. 2007; 4: 319 –337.

Therapeutic index-Siddha. Published by SKM centre for Ayush Research and Education, 1st ed. 2010; 134-144.

Ramachandran S.P, Agasthiar Vaidhya Kaviyam – 1500. Published by Thamarai Publication, Chennai, 1st ed. 1992; 195.

Rafael Henrique Oliveira Lopes, Luis Fernando Benitez Macorini, Katia Ávila Antunes, Priscilla Pereira de Toledo Espindola, Tamaeh Monteiro Alfredo, Paola dos Santos da Rocha et al. “Antioxidant and Hypolipidemic Activity of the Hydro ethanolic Extract of Curatella americana L. Leaves”. Oxidative Medicine and Cellular Longevity 2016.