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# TOXICITY STUDY ON SIDDHA FORMULATION MEGA SANJEEVI MATHIRAI IN ALBINO RATS

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## ABSTRACT

The present study was carried out to investigate the toxicity of Siddha formulation Mega Sanjeevi Mathirai in albino rats. The animals were maintained on a standard laboratory conditions. Acute toxicity study and repeated dose oral toxicity study was done on different dose levels. The experiments were carried out in four groups of animals. Each group contains 6 animals. Among them were 3 males and 3 females. Histopathological studies, hematological investigations, biochemical parameters and ICP-OES studies were done to find out abnormality if any. LD50 cut off value was calculated. The safe dose of Mega Sanjeevi Mathirai was recommended to be 100mg/kg or less than 200 mg/kg body weight.

**KEYWORDS; Mega Sanjeevi Mathirai, LD50, Histopathological studies, Hematological studies, ICP-OES studies.**

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# INTRODUCTION

Indian system of medicine has been widely used for thousands of years in India. Now a day's acceptance of traditional system of medicine in development world is sharply increasing.[1-3]Siddha system is unique among the Indian system of medicine. It is believed to have been developed by the siddhar's the ancient supernatural spiritual saints of India. In Siddha system of medicine the drug sources are obtained from plant, mineral, metal and animals.[4]Metals have been used in disease treatment since time immemorial. Siddha medicine has immense faith in the miracles of mercurial drugs and in the prolongation of life through rejuvenating treatments and intense yogic practices.[5]Several others have also worked on the efficacy and safety aspects of mercurial preparations in such traditional drugs.[6, 7]Role of these herbo-mineral and herbo-metallic preparations for treating diseases like diabetes, STD, AIDS, Leprosy, psoriasis etc. which are very harmful to human.[8] Mega Sanjeevi Mathirai, a Herbo metallic formulation to treat Male genital disorders, sexually transmitted diseases, which is used in Siddha system of medicine (Anupoga Vaithiya NavaneethamPart-VII).Proper standardization techniques on these medicines to meet the criteria to support its use worldwide. Now this day and age several Toxicological evaluations and standardization were done on traditional medicine systems in India.[9]

# MATERIAL AND METHODS

**(*i) Preparation of Mega Sanjeevi Mathirai*:**
Purified Lingam-8 ¾ gm, Purified Veeram-8 ¾ gm, Purified Pooram-8 ¾ gm, Purified Rasa chenduram-8 ¾ gm, *Terminalia chebula*- 35 gm, and Extract of Lemon- Required amount. The aforementioned drugs were grind for 3 hours duration continuously. Extract of lemon was slowly added into it and again it was grinded for a period of 12 hours. The grinded outcome was made as 65 mg pills.Dosage of the pill was ½ to 1 pill. Sugar and butter was used as adjuvant.

***(ii) Animals:***
Male wistar rats weighing about 200-220gms were selected and kept under standard laboratory conditions. The animals were allowed free accessto standard pellet diet and water ad libitum. The blood samples were drawn after application of topical lignocaine anaesthesia to minimize pain tothe animals. This study protocol was approved bythe Institutional Animal Ethics Committee (IAEC).

***(iii) Acute toxicity studies:***
The Acute toxicity study of Mega SanjeeviMathirai was evaluated in rats as per the OECD guide line 423.[10]It is the principle of the test that based on stepwise procedure with the use of the minimum number of animal per step. Three animals were used for each step. The dose level of 5, 50,300 and 2000 mg/kg body weight was administered stepwise. Observations were made and recorded systematically and continuously observed as per the guideline after substance administration. The number of survivors was noted after 24 hours and these animals were then maintained for a further 14 days and observations made daily.

***(iv) Repeated dose oral toxicity study:***
Repeated dose oral toxicity studies were conducted as per OECD guideline 407on four groups of rats (0 mg/kg body weight, 50 mg/kg body weight-low dose, 100 mg/kg body weight-middle dose, 200 mg/kg body weight- higher dose in the volume of 10ml/kg). Each group was containing 6 rats. Of these were 3 males and 3 females. The test substance suspensions were freshly prepared every 2 days once for 28 days. The control animals were administered vehicle only. Administration was by oral (gavage) once daily for 28 consecutive days. Experimental animals were kept under observations throughout the course of study for the following: Clinical signs and mortality, body weight, food and water consumption;Hematological parameters were determined using Hematology

analyzer; Bio chemical parameters were determined using auto analyzer; Gross necropsy: All the animals were sacrificed on day 29. Necropsy of all animals was carriedout and the weights of the organs including Liver, Spleen, Kidney, Brain, Lung, Pancreas, Heart, Stomach, Testis were recorded; Histopathology: Liver, Spleen, Kidney, Brain, Lung, Pancreas, Heart, Stomach, Testis were fixed in 10% formalin for routine histopathological examination. Embedding was done by passing the cleared samples through three cups containing molten paraffin at 50 $^{0}$ C and then in a cubical block of paraffin made by the "L" moulds. It was followed by microtome and the slides were stained with haematoxyline and eosin.

### *(v) Estimation of Heavy metals by the technique ICP-OES:*

Analysis of Mega Sanjeevi Mathirai was performed using Optima 5300 DV ICP-OES equipped with a Sea Spray concentric nebulizer (Glass Expansion, Pocasset, MA) and cyclonic spray chamber. Following parameters were introduced: nebulizer flow, 0.8 l min$^{-1}$; radiofrequency power, 1450 W; sample introduction, 1.5 ml min$^{-1}$; flush time, 20 s; delay time, 10 s; read time, 10 s; wash time, 30 s; and replicates, three. Standards were prepared by dilution of 1000 mg l$^{-1}$ stock solutions and the calibration curve was obtained using five to ten points including the blank.[11]

### *(vi) Evaluation of powder properties of Mega Sanjeevi Mathirai:*

JEOL ASM 3500 SEM was used for the analysis. A representative portion of each sample was sprinkled onto a double side carbon tape and mounted on aluminum stubs in order to get a higher quality secondary electron image for SEM examination.

### *(vii) Statistical analysis:*

Data were expressed as mean ±SEM (n=6) forstatistical evaluation of data. One-way ANOVAand student t-test were performed by using Dunnett test statistical software program.

## RESULTS

### *(i) Acute toxicity studies:*

At the dose level of 2000 mg/ kg body weight 2 animals were died. And behavioral changes observed at every dose level and body weight was increased (Table1). From these findings as per the OECD guidelines the LD 50 cutoff value was concluded as 100 mg/kg body weight in rats.

**Table 1**
***Dose finding experiment and its behavioral signs of toxicity***

| No | Dose mg/kg | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 5 | + | - | - | + | - | + | - | - | - | - | - | - | - | - | - | - | - | - | ↑ | 0 |
| 2 | 50 | + | - | - | - | - | - | + | - | - | - | + | + | + | - | - | - | - | - | ↑ | 0 |
| 3 | 300 | - | - | - | + | - | - | + | - | - | - | + | + | + | + | + | - | - | - | ↑ | 0 |
| 4 | 2000 | - | - | - | + | - | - | + | - | - | - | + | + | + | + | + | - | - | + | ↑ | 2 |

***1. Alertness, 2. Aggressiveness, 3. Pile erection 4. Grooming, 5. Gripping,6. Touch Response, 7. Decreased Motor Activity, 8. Tremors, 9. Convulsions, 10. Muscle Spasm, 11. Catatonia, 12. Muscle relaxant, 13. Hypnosis, 14. Analgesia, 15.Lacrimation, 16. Exophthalmos, 17. Diarrhoea, 18. Writhing, 19. Body weight, 20. Number of deaths. (+)=Positive response, (-)=Negative response, (↑)=Increased.***

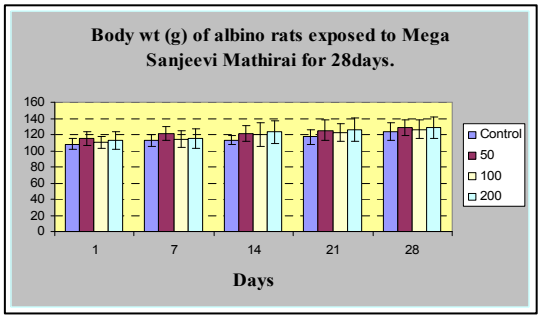
***(ii) Repeated dose oral toxicity study:***

**Body weight, Food and water consumption**

Body weight was gain (Graph 1) and Food (Graph 2)and water consumption (Graph 3) was found to be normal throughout the dosing period of 28 days when compared the treatment groups with control.

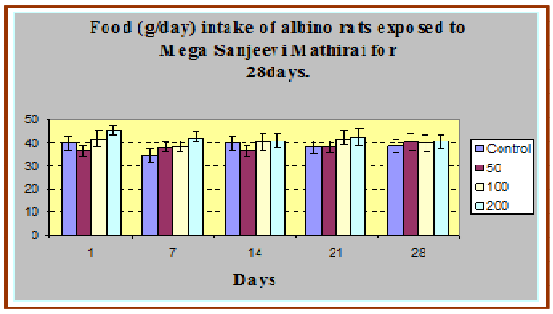
**Graph 1**

***Body weight gain***



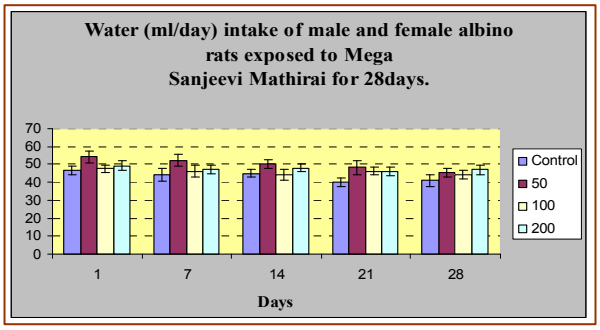

**Graph 2**

***Food Consumption***




**Graph 3**

***Water Consumption***



***(iii) Hematological and Biochemical parameters:***

Except decreased values of MCHC, increased values of MCV and MCH there were no significant differences observed in any of the hematological parameters examined in either the control or treated group of animals.

Biochemical parameters were presented in (Table 3). Except elevated level of ALP and urea level, there were no significant differences observed in any of the biochemical parameters examined in either the control or treated group of animals.

**Table 2**
***Hematological parameters after 28days treatment with MSM in rats***

| Parameter | Control | 50 mg/kg | 100 mg/kg | 200 mg/kg |
|---|---|---|---|---|
| Red blood cell ($mm^3$) | 7.41±0.16 | 6.88±0.31** | 6.50±0.22** | 6.24±0.11** |
| HB (%) | 15.20±0.41 | 15.00±0.36 | 15.10±0.68 | 15.9±0.68 |
| Leukocyte($x10^6$/mL) | 10139±126.53 | 10154±321.14** | 12200±556.77** | 11485±388.4** |
| Platelets/ul | 1368±39.67 | 1146±78.10** | 1297±101.35 | 1189±28.55** |
| MCV (ul) | 59.77±4.46 | 55.42±3.74 | 54.28±2.12* | 54.20±2.90* |
| ESR(mm) | 1±00 | 1±00 | 1±00 | 1±00 |
| PCV | 48.10±1.88 | 44.17±2.00 | 45.34±1.88 | 43.20±2.13 |
| MCH (mg) | 18.28±0.45 | 18.45±1.46 | 18.69±0.27 | 18.60±0.50 |
| MCHC g/dl | 30.66±0.98 | 31.00±0.51 | 30.51±1.32 | 30.85±0.54 |

***Values are mean of 6 animals MEAN± SEM (Dunnett's test). *P<0.05; **P<0.01. N=6.***

**Table 3**
***Biochemical parameters after 28days treatment with MSM in rats***

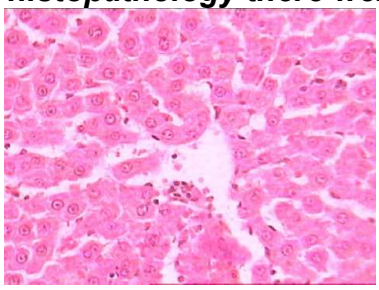
| PARAMETER | CONTROL | 50mg/kg | 100 mg/kg | 200 mg/kg |
|---|---|---|---|---|
| Total bilirubin (mg/dl) | 0.209±0.05 | 0.212±0.06 | 0.218±0.05 | 0.215±0.04 |
| ALP (U/L) | 382.34±10.16 | 374.21±12.32 | 386.38±10.30 | 294.1±12.32** |
| SGOT (U/L) | 168.24±6.21 | 160.67±6.58 | 166.38±5.80 | 154.01±6.57* |
| SGPT (U/L) | 46.4±2.34 | 44.8±3.20 | 45.08±2.58 | 46.62±4.19 |
| TOTAL PROTEINS(g/dl)) | 10.02±1.30 | 9.17±0.30 | 8.52±0.27 | 9.12±0.46 |
| GGT (U/L) | 7.4±0 | 7.2±0 | 7.8±0 | 8±0 |
| UREA (mg/dl) | 58.19±1.56 | 61.21±3.45 | 60.2±2.12 | 0.78±0.06 |
| CREATININE (mg/dl) | 0.82±0.06 | 0.74±0.05 | 0.80±0.07 | 0.78±0.06 |
| URIC ACID (mg/dl) | 1.5±0.10 | 1.5±0.24 | 1.6±0.20 | 1.62±0.30 |
| TOTAL CHOLESTEROL (mg/dl) | 41.98±2.78 | 40.2±2.59 | 44.10±3.23 | 46.24±2.98* |
| TRIGLYCERIDE (mg/dl) | 82.15±3.38 | 81.32±2.65 | 90.10±4.62** | 90.22±2.89** |
| BLOOD GLUCOSE (mg/dl) | 112.16±8.62 | 118.2±5.38 | 112.83±7.49 | 130.5±2.35** |

***Values are mean of 6 animal'sMEAN± S.D. (Dunnett's test). *P<0.05; **P<0.01.vs. control group N=6***

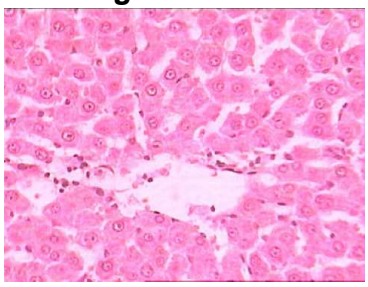
***(iv) Histopathological examination:***

**(Figure 1 A-l)**

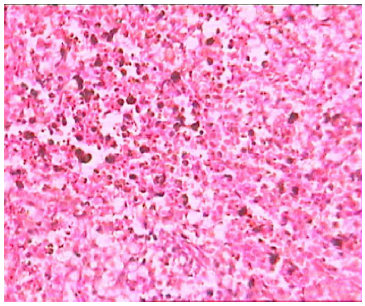
***for histopathology there were no changes to be observed.***



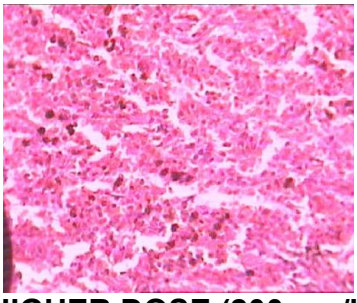
**A. CONTROL**



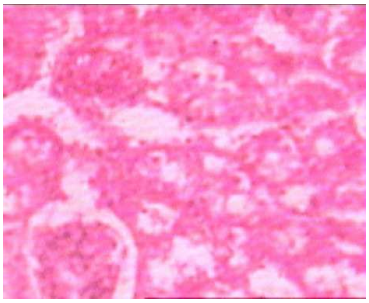
**A. HIGHER DOSE (200 mg/kg)**



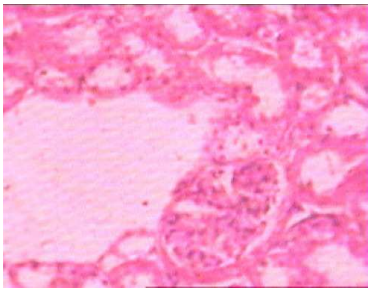
**B. CONTROL**



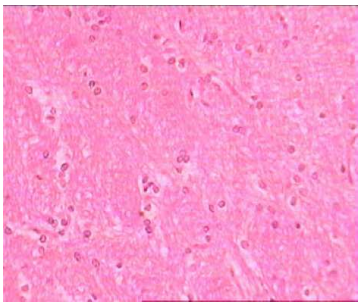
**B. HIGHER DOSE (200 mg/kg)**



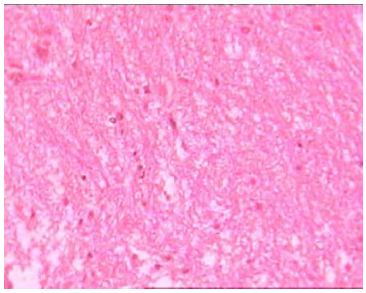
**C. CONTROL**



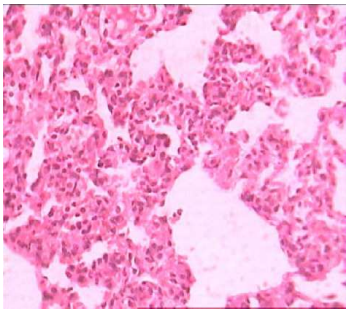
**C. HIGHER DOSE (200 mg/kg)**



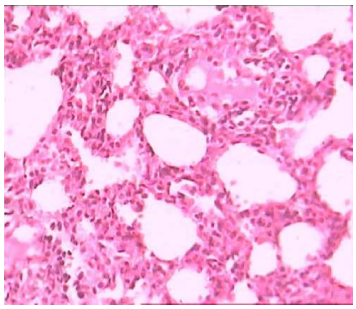
**D. CONTROL**



**D. HIGHER DOSE (200 mg/kg)**



**E. CONTROL**



**E. HIGHER DOSE (200 mg/kg)**

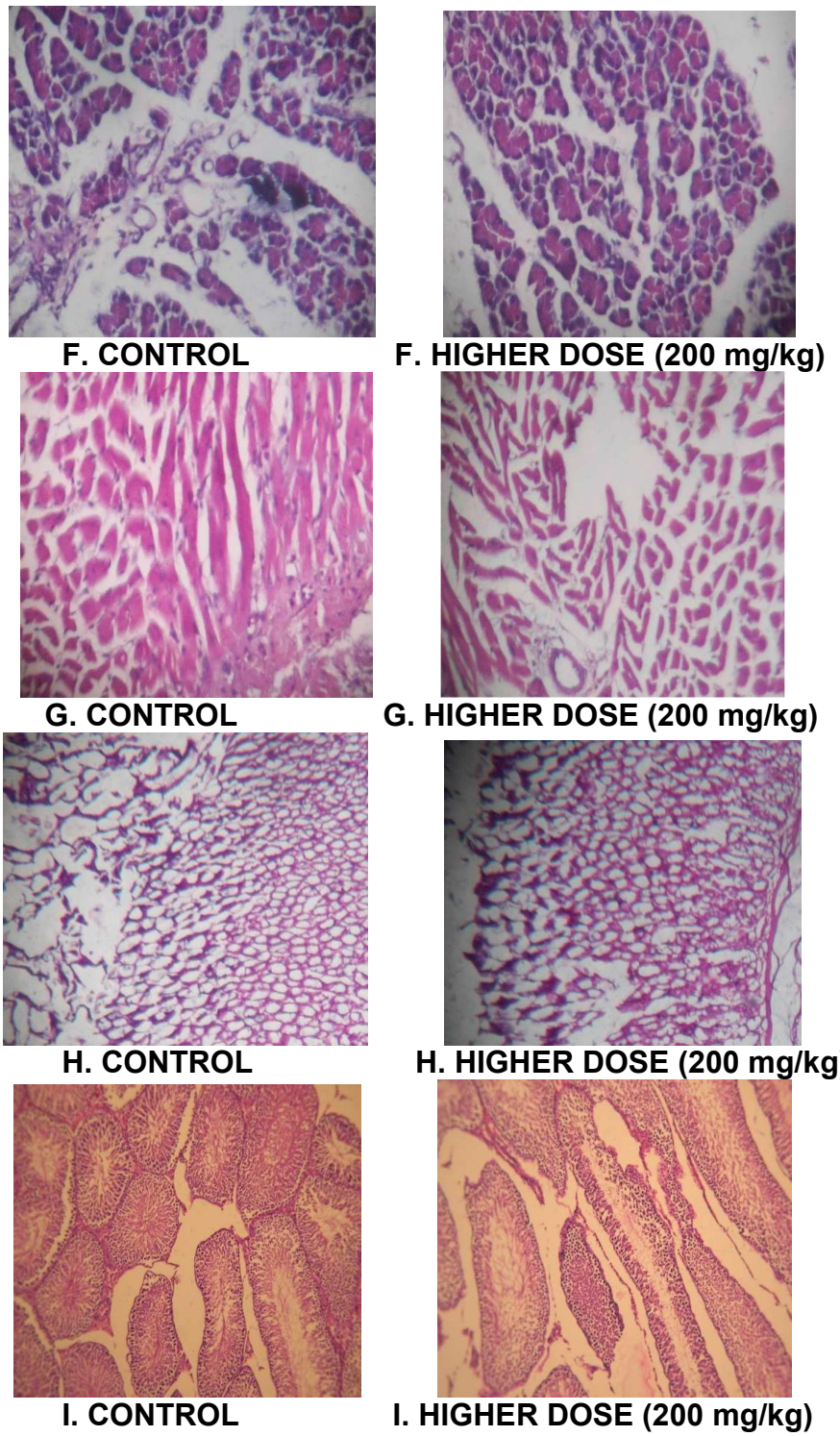



***Figure1(A-I): Histopathological Analysis of Mega Sanjeevi Mathirai (Liver, Spleen, Kidney, Brain, Lung, Pancreas, Heart, Stomach, and Testis)***

### *(v) ICP-OES analysis:*

ICP-OES analysis revealed the presence of mercury level of Mega Sanjeevi Mathirai was within the ppm level (Table 4). Other heavy metals were below the detection limit.

**Table 4**
***ICP-OES Analysis of Mega Sanjeevi Mathirai***

| S. NO. | ELEMENTS | DETECTABLE LIMIT IN ppm |
|---|---|---|
| 1. | Arsenic | *BDL |
| 2. | Mercury | 0.960 |
| 3. | Lead | *BDL |
| 4. | Cadmium | *BDL |
| 5. | Sodium | 6.26 |
| 6. | Potassium | 4.22 |
| 7. | Sulphur | 6.25 |

***BDL- Below Detection Level***

***(vi) HR SEM analysis:***

HR SEM analysis revealed the particle size of Mega Sanjeevi Mathirai was 3 to 10 µm. Shape of the particles were more or less cubical in shape and the surface was smooth. (Figure 2)

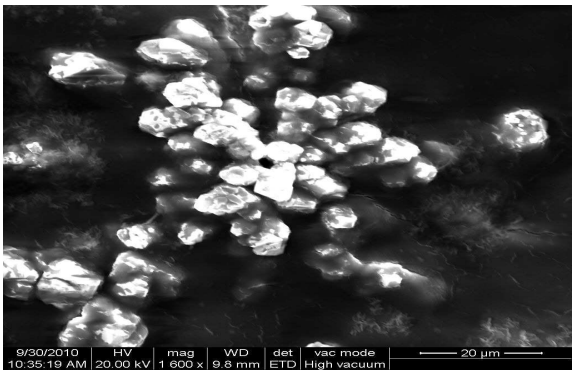



**Figure 2**
***SEM of Mega Sanjeevi Mathirai***

# DISCUSSION

A world health organization survey indicates that about 70-80% of the world's populations rely on non-conventional medicine mainly of herbal source in their primary healthcare.[12] To determine the safety of drugs and plant products for human use, toxicological evaluation was carried out in various experimental animals to predict toxicity and to provide guidelines for selecting a safe dose in human.As there was no previous report on toxicity of Mega Sanjeevi Mathirai as per the OECD guidelines, 5, 50,300 and 2000 mg/kg body weight were selected for acute toxicity study.2 animals were died at the dose level of 2000mg/kg body weight. Thus the LD50 cut off value was 1000mg/kgas per the OECD-423 based on acute toxicity study the dose level of then one twenty, one tenth and one fifth was selected in repeated dose oral toxicity study.

In Repeated dose oral toxicity study, mild toxic signs observed at the higher dose level of 200mg/kg body weight.Since the changes in body weight have been used as an indicator of adverse effects of drugs and chemicals.[13, 14]The present results suggested

that higher dose level shows mild toxic symptoms in rats. In addition determination of food consumption was important in the study of safety of a product with therapeutic purpose as proper intake of nutrients are essential to the physiological status of the animals and to the accomplishment of the proper response to the drug tested instead of a false response due to improper nutritional conditions.In the present study Mega Sanjeevi Mathirai treated rats did not show significant differences in food and water consumption. Significant changes in

enzymes like ALP, AST and ALT represent liver impairment, since they are important indices of liver toxicity.[15]200 mg/dl dose level of Mega SanjeeviMathirai shows elevated level of urea. Increased level of urea represents the renal impairment.

Except urea, renal function markers like creatinine and uric acid levels were normal when compared with control groups. [16, 17]Biochemical parameter showed elevated level of MCV and MCH. It represented the occurrence of anemia.[18] Other biochemical parameters were normal when compared with control groups. But the increased level of ALP,

MCV, MCH and UREA were within the laboratory limits.ICP-OES analysis indicated the presence of mercury was 0.960 ppm in mega SanjeeviMathirai.It represented the preparation process was done properly as per the literature. SEM analysis indicates the particle size of mega SanjeeviMathirai is 3-10 µm.The shape is cubical and the surface is smooth. Due to its smooth surface flowability is possible.

## CONCLUSION

Based on these findings no toxic effect was observed up to 200 mg/kg body weight of Mega Sanjeevi Mathirai treated via oral route over a period of 28 days. But, the maximum dose caused mild signs of toxicity in animal models. So it can be concluded that the Mega Sanjeevi Mathirai is safewith the dosage recommendations of 100mg/kg or less than 200 mg/kg body weight. And the heavy metal analysis indicates, it does not produce mercurial toxicity. Powder property indicates no evidence of nanotoxicity. Overall, this study provides valuable data on toxicity profile of Mega Sanjeevi Mathirai.

## ACKNOWLEDGEMENTS

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