Evaluation of Chronic toxicity of Urai mathirai- Siddha Herbal Formulation

Dayanand Reddy Gaddam[1], Sathiyarajeswaran P[2], Patturayan R[3], Ganesan R[2], Narasimha Kumar G .V.[4], Pullaiah C.P.[2], and DHANARAJ K[2]

[1]Siddha Central research Institute
[2]Siddha Central Research Institute
[3]National Institute of Siddha
[4]Dr. Anjali Chatterjee Regional Research Institute For Homoeopathy

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Abstract

Background &objectives:The present study was to carry out 90 days repeated oral toxicity of Urai mathirai in wistar albino rats. Methods:The chronic toxicity study was carried out as per (OECD) test guideline 408 and Central Council for Research in Ayurvedic Sciences (CCRAS) respectively. In this study, theUrai mathirai drug solution was administered at dose of (10, 50 and 100mg/kg b.wt) for every 24 hours orally upto 90 days for all the three groups. At the end of each study, hematological and biochemical analysis were evaluated. Histopathological examination of vital organs of the rats were taken for gross findings, compared to controls. Distilled water aided as a control in all the tests. Results: There was no significant difference ($p > 0.05$) observed in the relative organs weight, body weight, hematological, biochemical parameters, and gross abnormalities when compared to the control. No mortality was recorded with respect drug effect. Interpretation&conclusions: The results may lead that the oral administration of the Urai mathiraiat doses of 10, 50 and100 mg/kg.b.wtdoes not showedany toxicity effect in wistar rats during the experimental period. Keywords: Urai mathirai; chronic toxicity; herbal formulation; histopathology.

What is already known about this subject:

- In Siddha system, Urai mathirai has been prescribed by doctors to improve the immunity in children and there is lack of scientific evidence (Toxicity and safer dose levels).
- In order to ensure the scientific data i.e chronic toxicity (NOAEL) effect has been evaluated with the help of animal models.

What this study adds :

- This Chronic toxicity study reveals about the maximum dose level of 100 mg/kg body weight was found to be safe and it doesn't produce any toxic effects related to our drug over the animal model.
- Hence our study ensures Urai mathirai was found to be safe upto 100 mg/kg body weight.

INTRODUCTION

Traditional Systems of medicines are playing a key role in meeting the global health care needs. 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 which is originated from Tamil nadu and has its origins in Tamil language. Literally the word "Siddha" means "established truth". Siddha system of medicine was claimed to alleviate the root cause of the diseases by maintaining the ratio of Vatham, Pitham and Kapham. The origin of the Siddha system of medicine is attributed from ancient saint called Siddhars(1).

Urai Mathirai is a drug used for the past three decades in the form of long finger size bullets which are rubbed and administered through breast milk with children's to improve immunity to get free from health hazards such as frequent respiratory infections /gastrointestinal infections and anorexia. For global acceptance, this system of medicine should undergo scientific validation, i.e., upgrading the one of the levels is safety of the dose[(2)].

The acute and sub acute toxicity of the *Siddha* herbal formulation urai mathirai was administered orally at a dose of 10, 50 and100 mg/kg.b.wt for 28 days does not showed any toxicity effect in wistar rats.

Hence the present study was performed to evaluate the chronic toxicity of the *Siddha* herbal formulation *Urai Mathirai* in experimental wistar rats. Through this study the safety of this herbal drug can be established for the clinical use of this traditional formulation among the children's to improve immunity.

2. MATERIAL AND METHODS

2.1Test animal

Wistar albino rats of both sex (Male and female) weighing about130–250 g were used in the chronic toxicology studies. The rats were housed in the Department of Pharmacology, Siddha central research institute, Chennai. The rats were kept in sanitized polypropylene cages housed with sterile corn cob as bedding materials at animal house, in an air conditioned environment with four rats in each cage and maintained at room temperature of (23 ± 2)ºC with relativehumidity (60% ± 10%) under 12 hourdark and light cycle[(3)]. Rats were given free access to standard pellet diet and water ad libitum[(4)]. All experimental procedures were in compliance with the Committee for the Purpose of Control and Supervision of Experiments on animals (CPCSEA) and were approved by Institutional Animal Ethical Committee, with an approval number

162/Pharma/SCRI/2017.

2.2Dose calculation [(5)]

The clinical dose of *Urai mathirai* in children is 50 mg (HED = 2.5mg/kg body weight). The animal (rat) doses are calculated as per the FDA guidelines and the calculated therapeutic dose (TD) was found to be 10mg/kg of body weight. In this study to determine the dose correlated effects, 5 times TD and 10 times TD i.e. 50 mg/kg & 100 mg/kg of bodyweight were chosen correspondingly.

2.3Experimental design

The oral chronic toxicity study was evaluated accordance with to Organization for Economic Cooperation and Development (OECD) guideline 408 and Central Council for Research in Ayurvedic Sciences (CCRAS) guideline [(6, 7)]. Wistar albino Rats of both sexes (over night fasting free access to water), aged 8–12 weeks old were used. In 80 rats (40Male and 40females) 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 with vehicle as normal control, Group-II, Group-III and Group-IV administered with test drug orally at a dose of 10 mg/kg, 50 mg/kg and 100 mg/kg respectively for 90 days. Before drug administration, the body weight of each animal was determined and the dose was calculated according to the body weight for every week consecutively for 90 days [(8)]. Rat general health, and signs of toxicity, body weight, mortality, food and water intake was monitored. At 90th day 50% of the experimental animals (40 animals form both sex) 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 collected organs during the treatment and post treatment were preserved in 10% formaldehyde solution for histopathological examination.

2.3.1 Relative organ weight

The internal organs (brain, heart, thymus, lungs, liver, stomach, spleen, kidney, adrenal gland, pancreas and sex organs) excised from all the experimental rats after 90th day and 120th day.Organ-to-body weight ratio

was calculated by dividing the weight (g) of each organ by the weight (g) of rats before sacrifice.

2.3.2Biochemical parameters [9]

The biochemical analysis were done on serum after centrifugation of collected blood and the following parameters like Blood glucose, Total Cholesterol, Triglyceride, HDL, LDL, SGOT, SGPT, ALP, GGT, Total Protein, Albumin, LDH, CRP, Creatinine Kinase levels, Creatinine Kinase – MB, Urea, Serum creatinine, Total Bilirubin, Uric acid, Serum Calcium were determined for both control and *Urai mathirai* treated groups by using standard biochemical method.

2.3.3Haematological parameters [9]

The hematological parameters such as white blood cell (WBC), red blood cell (RBC),lymphocyte (LYMP), monocyte (MON), granulocyte (GRAN),hemoglobin (HGB), andHematocrit (Hct) Levels were evaluated.

2.3.3 Histopathological examination

The organs (brain, heart, thymus, lungs, liver, stomach, spleen, kidney, adrenal gland, pancreas and sex organs) excised from all the experimental rats were fixed in 10% formalin in labeled 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[10].

2.4 Statistical analysis : All the data was expressed as Mean ± SEM. Statistical analysis was tested by using One-way ANOVA followed by (Dunnett's test) using Graph pad prism version-8.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.

3. RESULTS

3.1.Chronic toxicity study

All the treatment group ratswere administered with *Urai mathirai*drug solution at a dose of (10, 50 and 100mg/kg b.wt) throughout the 90 days found to be no clinical toxicity signs such as physical observations, behavioral changes and other parameters such as body weight, food intake, water intake, respiration, convulsion, tremor, changes in eye and skin colors, etc were observed in the treated group compared to the control group. The observations were measured and summarized in **Table 1.**

3.1.1. Effect of*Urai mathirai* on relative organ body weight

The average and relative organ weight of *Urai mathirai* orally treated group of animals (at dose of 10, 50 and 100mg/kg b.wt) and control groups showed statistically non-significant differences (P > 0.05). The results revealed that, the internal organs of rats were not adversely affected throughout the treatment. The effect of*Urai mathirai* on principal organ weights relative to body weight were presented in **Table 2** .

3.2.2 Effect of*Urai mathirai* on biochemical parameters

The results of the various biochemical parameters on the experimentally treated rats with the oral administration of the *Urai mathirai* at a dose of (10, 50 and 100mg/kg b.wt) and normal groups showed statistically non-significant differences (P > 0.05). The results revealed that no abnormal changes in serum biochemical parameters such as albumin, total protein, globulin, Total bilirubin, urea, sodium, creatinine and uric acid levels etc., when compared to control group. The effect of *Urai mathirai* on biochemical parameters measured and summarized in **Table 3**

3.2.3.Effect of *Urai mathirai* on Hematological parameters

The hematological parameters white blood cell (WBC), red blood cell (RBC),lymphocyte (LYMP), monocyte (MON), granulocyte (GRAN),hemoglobin (HGB), andHematocrit (Hct) Levelswere within normal limits compared to control group. No significant differences (P > 0.05) between treated animals with the *Urai*

mathirai orally at dose of 10 mg/kg, 50 mg/kg and 100 mg/kg and control group rats were found. The hematological parameters were measured and summarized in **Table 4.**

3.2.4.Effect of *Urai mathirai*in Histopathological Study

Sections of lung, liver, kidney, spleen, heart, and brain tissues were perfused with 10% formalin and stored in the same andused for histopathological studies. The tissues were then embedded in molten paraffin wax. Sections were cut at 5μm thickness and stained with haematoxylin and eosin. The sections were then viewed under light microscope. The macroscopic examination of organs of treated rats revealed no abnormalities in the colour or texture when compared with the organs of the control group. Although some differences have been observed in the histopathological slides were presented in**Figure 1-8** .

Histopathology

Histopathological findings of lungs showed mild pulmonary oedema, multi focal mild mononuclear cell infiltration in peribronchiolar and alveolar region of control rats (Group I) and also treated rats groups (Group II to Group IV) of *Urai mathirai* orally at a dose level of(10 mg/kg, 50 mg/kg and 100 mg/kg).It revealed that no significant changes were observed related to drug toxicity effect and pictures were presented in the **Figure1** .

A. Group I	B.Group II
C. Group III	D.Group IV

Figure 1 (A-D) Histopathological findings of Lungs in different groups of rats.

Histopathological findings of liver showed sinusoidal congestion, very mild hepatocellular degeneration and focal hepatocellular necrosis in both control rats (Group I) and also in treated rats groups (Group II to Group IV) of *Urai mathirai* orally at a dose level of(10 mg/kg, 50 mg/kg and 100 mg/kg). It revealed that no significant changes were observed related to drug toxicity effect and pictures were presented in the **Figure 2** .

A. Group I	B.Group II
C. Group III	D.Group IV

Figure 2 (A-D) Histopathological findings of Liver in different groups of rats.

Histopathological findings of kidney showed mild tubular epithelial cell degeneration and focal mild interstitial mononuclear cell infiltration in both control rats (Group I) and also in treated rats groups (Group II to Group IV) of *Urai mathirai* orally at a dose level of(10 mg/kg, 50 mg/kg and 100 mg/kg).It revealed that no significant changes were observed related to drug toxicity effect and pictures were presented in the **Figure 3** .

A. Group I	B.Group II
C. Group III	D.Group IV

Figure 3 (A-D) Histopathological findings of Kidney in different groups of rats.

Histopathological findingsof spleen showed no abnormality in both control rats and also in treated rats groups (Group II to Group IV) of*Urai mathirai* orally at a dose level of(10 mg/kg, 50 mg/kg and 100 mg/kg).It

revealed that no significant changes were observed related to drug toxicity effect and pictures were presented in the**Figure 4** .

A. Group I	B.Group II
C. Group III	D.Group IV

Figure 4 (A-D) Histopathological findings of Spleen in different groups of rats.

Histopathological findingsof heart showed no abnormality in both control rats and also in treated rats groups (Group II to Group IV) of*Urai mathirai* orally at a dose level of(10 mg/kg, 50 mg/kg and 100 mg/kg).It revealed that no significant changes were observed related to drug toxicity effect and pictures were presented in the**Figure 5** .

A. Group I	B.Group II
C. Group III	D.Group IV

Figure 5 (A-D) Histopathological findings of heart in different groups of rats.

Histopathological findingsof brain showed no abnormality in both control rats and also in treated rats groups (Group II to Group IV) of*Urai mathirai* orally at a dose level of(10 mg/kg, 50 mg/kg and 100 mg/kg).It revealed that no significant changes were observed related to drug toxicity effect and pictures were presented in the**Figure 6** .

A. Group I	B.Group II
C. Group III	D.Group IV

Figure 6 (A-D) Histopathological findings of brain in different groups of rats.

Histopathological findings of glandular and non glandular stomach showed no abnormality in both control rats and also in treated rats groups (Group II to Group IV) of *Urai mathirai* orally at a dose level of(10 mg/kg, 50 mg/kg and 100 mg/kg).It revealed that no significant changes were observed related to drug toxicity effect and pictures were presented in the **Figure 7** .

A. Group I	B.Group II
C. Group III	D.Group IV

Figure 7 (A-D) Histopathological findings of stomach in different groups of rats.

Histopathological findings of pancreas showed no abnormality in both control rats and also in treated rats groups (Group II to Group IV) of*Urai mathirai* orally at a dose level of(10 mg/kg, 50 mg/kg and 100 mg/kg).It revealed thatno significant changes were observed related to drug toxicity effect and pictures were presented in the**Figure 8** .

A. Group I	B.Group II

C. Group III D.Group IV

Figure 8 (A-D) Histopathological findings of pancreas in different groups of rats.

4. CONCLUSION

Urai mathirai administered in 3 doses by oral route (at therapeutic dose of 10 mg/kg, average dose 50 mg/kg and high dose 100mg/kg) regularly for 90 days did not produce any toxicity in animal models. There were no statistically significant alterations found in behavior, biochemistry, hematological parameters, organ weight and histopathology during the experimental period.

5. ARCHIVING

All the raw data related to this study has been archived in Department of Pharmacology, Siddha Central Research Institute, Chennai, Tamilnadu, India.

5.1 Availability of data

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

6. ACKNOWLEDGEMENT

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6.1 Author Contribution

Dr.P.Sathiyarajeswaran	Principal Investigator; Urai mathirai drug formulation
Dr.G.Dayanand Reddy	Co-Investigator; Study design, monitoring, compilation of the data and manuscript reviewer.
Dr. R. Patturayan	Overall Advisor
Dr. R. Ganesan	Co-Investigator; Biochemical Investigations
Mr.G.V.Narasimha kumar	Randomization and monitoring of the animal health status
Mr. C.P. Pullaiah	Drug administration and macroscopical examination
Mr. K. Dhanaraj	Execution of the study and manuscript preparation

6.2 Conflict of interest

All the authors declare that have no conflict of interest in this manuscript.

7. REFERENCES

1. B. Rama Devi, G. Dayanand Reddy, G.V. Narasimha Kumar, P. Sathiyarajeswaran and P. Elankani.Herbomineral formulation's safety and efficacy employed in siddha system ofmedicine: a review:Int. Res. J. Pharm. 2019;10:16-24.

2. Sathiyarajeswaran P, Dhanaraj K, Shree Devi MS, Muthu Tamizh M, Patturayan R, Design of solid oral dosage form and its quality control assessment of urai mathirai – A tablet from siddha formulation for immuno modulation in pediatric community. http://wjpr.net. 2018; 7: 514-522.

3. S. Muralidhara, R. Ramanathan, S. M. Mehta, L. H. Lash, D. Acosta*etal.* Acute, Sub-acute, and Sub-chronic Oral Toxicity Studies of 1, 1-Dichloroethane in Rats: Application to Risk Evaluation. Toxicological sciences 2001;64:135-145.

4. Purobi Nath, Arun K. Yadav. Acute and sub-acute oral toxicity assessment of the methanolic extract from leaves of Hibiscus rosa-sinensis L. in mice: J Intercult Ethnopharmacol 2015;04:70-73.

5. Dayanand Reddy G, Sathiyarajeswaran P, Pattarayan R, Narasimha Kumar GV, Chitikela P Pullaiah, Experimental Evaluation of Analgesic and Anti-Inflammatory Potential of Urai mathirai – A Siddha Formulation. J. Global Trends Pharm Sci, 2018; 9: 4839 – 4844.

6. Organization for Economic Co-operation and Development. OECD guidelines for the testing of chemicals. Paris: Organization for Economic Co-operation and Development; 1998. [Online] Available from: https://www.oecd.org/chemicalsafety/testing/Revision-OECD-TG408-repeated-dose-90-day-oral-toxicity-study-in-rodents.pdf [Accessed on 21 Feb, 2020].

7. Central Council for Research in Ayurvedic Sciences. General guidelines for Safety/toxicity Evaluation of Ayurvedic formulations.2008; 1: 5-8.

8.Pillai P, Suresh P, Mishra G, Annapurna M. Evaluation of the acute and sub-acute toxicity of the methanolic leaf extract of Plectranthus amboinicus (Lour) Spreng in Balb C mice. Eur J Exp Biol 2011; 1: 236-45.

9. Bigoniya, P.; Sahu, T.; Tiwari, V. Hematological and biochemical effects of sub-chronic artesunate exposure in rats. Toxicol. Rep. 2015, 2, 280–288. [CrossRef].

10. Rafael Henrique Oliveira Lopes, Luis Fernando BenitezMacorini, Katia Ávila Antunes, Priscilla Pereira de Toledo Espindola, Tamaeh Monteiro Alfredo, Paola dos Santos da Rocha, Zefa Valdivina Pereira, Edson Lucas dos Santos, and Kely de Picoli Souza, "Antioxidant and Hypolipidemic Activity of the Hydro ethanolic Extract of Curatella americana L. Leaves". Oxidative Medicine and Cellular Longevity, 2016.

Table 1: General appearance and behavioral observations of control and treated groups.

Response	Group I (Normal)	Group II (10mg/kg b.w)	Group III (50mg/kg b.w)	Group IV (100mg/kg b.w)
Colour	**Colour**	**Colour**	**Colour**	**Colour**
Fur	N	N	N	N
Eyes	N	N	N	N
Mucous Membrane	N	N	N	N
Urine	N	N	N	N
Behavioral observations	**Behavioral observations**	**Behavioral observations**	**Behavioral observations**	**Behavioral observations**
Mood	N	N	N	N
CNS Excitation	NO	NO	NO	NO
CNS Depression	NO	NO	NO	NO
Motor Indication	**Motor Indication**	**Motor Indication**	**Motor Indication**	**Motor Indication**
Abnormal gait	NO	NO	NO	NO
Righting reflex	N	N	N	N
Posture	N	N	N	N
Sensory Responses	**Sensory Responses**	**Sensory Responses**	**Sensory Responses**	**Sensory Responses**
Touch & pain response	N	N	N	N
Straube"s phenomenon	NO	NO	NO	NO
Reflexes	**Reflexes**	**Reflexes**	**Reflexes**	**Reflexes**
Pinnna & corneal	N	N	N	N
Autonomic effects	**Autonomic effects**	**Autonomic effects**	**Autonomic effects**	**Autonomic effects**

Response	Group I (Normal)	Group II (10mg/kg b.w)	Group III (50mg/kg b.w)	Group IV (100mg/kg b.w)
Defecation &Lacrimation	N	N	N	N
Urination &Salivation	N	N	N	N
Piloerection	N	N	N	N
Miosis & Mydriasis	NO	NO	NO	NO
Diarrhoea	NO	NO	NO	NO
Respiratory effect	**Respiratory effect**	**Respiratory effect**	**Respiratory effect**	**Respiratory effect**
Apnoea & dysponea	NO	NO	NO	NO
Death	NO	NO	NO	NO

N-Normal No-No Abnormality Observed

Table 2Effect of Oral administrationof *Urai mathirai* on Relative organs weight (g) of rats.

Organs	Group I (Normal)	Group II (10mg/kg b.w)	Group III (50mg/kg b.w)	Group IV (100mg/kg b.w)
Brain	6.88±1.11	7.14±0.59	6.58±0.58	7.17±0.49
Heart	3.90±3.73	3.81±0.72	3.75±0.12	3.80±0.45
Thymus	3.41±1.83	2.23±1.11	0.75±0.14	0.89±0.19
Lungs	7.53±0.47	6.61±0.36	7.01±0.44	7.88±1.22
Liver	39.60±4.11	34.43±1.46	36.04±1.32	39.06±5.04
Stomach	6.14±0.86	6.48±0.42	5.73±0.18	6.60±0.45
Spleen	2.42±0.18	2.91±0.29	2.94±0.16	2.56±0.47
Pancreas	1.64±0.95	3.30±0.36	4.35±1.64	3.19±0.38
Kidney	8.26±1.05	8.55±0.96	7.92±0.19	8.53±0.78
Adrenal gland	0.34±1.20	0.27±0.07	0.20±0.04	0.22±0.05

All values are expressed as mean ± SEM (n=8). No significant difference since $p > 0.05$, as compared to control group .

Table 3 Effect of oral administration *Urai mathirai* on biochemical parameters at the end of the treatment period

Organs	Group I (Normal)	Group II (10mg/kg b.w)	Group III (50mg/kg b.w)	Group IV (100mg/kg b.w)
Blood glucose(mg/dl)	72.47±6.83	71.00±5.97	75.36±6.88	72.71±6.12
Total Cholesterol levels (mg/dl)	73.13±3.78	71.64±3.04	68.86±4.49	70.65±3.57
Triglyceride levels (mg/dl)	103.00±10.05	121.64±14.94	109.93±9.90	101.29±13.96
HDL levels (mg/dl)	30.87±6.70	25.07±1.41	25.36±1.76	23.47±1.26

Organs	Group I (Normal)	Group II (10mg/kg b.w)	Group III (50mg/kg b.w)	Group IV (100mg/kg b.w)
LDL levels (mg/dl)	28.27±3.65	23.00±3.36	21.43±3.41	27.56±2.89
SGOT levels (U/L)	164.40±12.31	190.43±14.45	194.29±12.72	162.53±4.64
SGPT levels (U/L)	62.60±2.07	64.79±4.10	64.50±3.17	67.76±9.74
ALP levels (U/L)	200.60±19.15	183.21±16.86	220.36±20.74	165.47±14.62
GGT levels (U/L)	4.60±0.46	4.92±0.47	4.64±0.37	4.71±0.32
Total Protein levels (g/dl)	7.67±0.11	7.50±0.10	7.59±0.17	7.64±0.18
Albumin levels (g/dl)	3.41±0.07	3.44±0.10	3.37±0.13	3.45±0.06
LDH levels (U/L)	1234.13±65.36	1184.50±82.60	1232.50±91.74	14090.12±18.76
CRP levels (mg/L)	0.48±0.06	0.54±0.09	0.50±0.08	0.58±0.11
Creatinine Kinase levels (U/L)	814.87±70.65	904.79±124.68	887.07±139.57	651.65±41.45
Creatinine Kinase – MB levels (U/L)	507.80±43.57	496.50±50.51	446.14±36.92	369.76±18.04
Urea levels (mg/dl)	30.67±1.68	32.57±2.26	32.57±1.25	34.71±1.57
Serum creatinine levels (mg/dl)	0.53±0.02	0.53±0.02	0.56±0.02	0.54±0.03
Total Bilirubin levels (mg/dl)	0.12±0.01	0.11±0.01	0.11±0.01	0.10±0.00
Uric acid levels (mg/dl)	0.13±0.02	0.12±0.01	0.13±0.02	0.12±0.01
Serum Calcium levels (mg/dl)	9.98±0.12	9.89±0.12	9.12±0.68	14.22±4.49

All values are expressed as mean ± SEM (n=15, 14, 15,17). No significant difference since p > 0.05, as compared to control group.

Table 4: Effect of oral *Urai mathirai* on haematological parameters at the end of the treatment period

Parameters	Group I (Normal)	Group II (10 mg/kg b.w)	Group III (50 mg/kg b.w)	Group IV (10 mg/kg b.w)
WBC (10^9/L)	12.10±0.64	11.59*±0.99*	*10.94±0.71*	*10.52±0.80*
RBC (10^{12}/L)	7.42±0.17	7.78*±0.31*	*7.66±0.21*	*7.76±0.24*
LYMP (10^9/L)	8.13*±0.40*	9.36*±1.10*	*7.54±0.55*	*7.11±0.55*
MON (10^9/L)	0.31*±0.02*	0.31*±0.03*	*0.32±0.03*	*0.31±0.03*
GRAN (10^9/L)	3.68*±0.28*	3.21*±0.38*	*3.08±0.24*	*2.81±0.29*
HGB (g/dL)	12.00*±0.27*	12.44*±0.47*	*12.19±0.26*	*19.61±7.41*
HCT (%)	38.44*±0.87*	39.36*±1.56*	*38.17±0.87*	*39.32±1.37*

All values are expressed as mean ± SEM (n=13, 14,15,17).No significant difference since $p > 0.05$, as compared to control group, except $p < 0.05$ in group,

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