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EVALUATION OF ACUTE, SUB ACUTE TOXICITY AND IMMUNOMODULATORY ACTIVITY OF URAI MATHIRAI-SIDDHA HERBAL FORMULATION

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Key Words

Uraimathirai; acute toxicity; sub acute toxicity; herbal formulation; histopathology; immunomodulatory activity.

ABSTRACT

The present study is to evaluate the acute, sub-acute toxicity and its immunomodulatory activity of the *Uraimathirai* in wistar albino rats. ***Methods***: The acute and sub-acute toxicity study was carried out as per (OECD) test guideline 423 and 407 and Central Council for Research in Ayurvedic Sciences (CCRAS) respectively. In acute toxicity study, the dose (10, 50 and100 mg/kg b.wt) of *Uraimathirai* was orally administered to three rat groups in single dose and general behavior, adverse effects and mortality compared to normal group were recorded for 24 hours and once daily further for a period of 14 days. In sub-acute study, the *Uraimathirai* was administered at doses of (10, 50 and 100mg/kg b.wt) for every 24 hours orally for 28 days to three rat groups. At the end of each study, hematological analysis and biochemical parameters were evaluated. Histopathological examination of vital organs of the rats was taken for gross findings, compared to controls. In-vivo immunomodulatory activity of Uraimatirai was evaluated by hypersensitivity reactions using sheep red blood cells (SRBC) as the antigen. Distilled water aided as a control in all the tests. There was no significant difference ($p > 0.05$) observed in the relative organs weight, body weights, hematological, biochemical parameters, and gross abnormalities, compared to the control. No mortality was recorded. Administration of Uraimathirai for seven days has shown a dose related increase in early (4 h) and delayed (24 h) hypersensitivity reaction in rats at a dose of 100 mg/kg, b.wt. and the 4 hour-reaction was found to be of higher magnitude than the 24 hour-reaction. The results may lead that the oral administration of the *Uraimathirai* at doses of 10, 50 and100 mg/kg.b.wt for 28 days does not showed any toxicity effect in wistar rats and at a dose level of 100mg/kg.b.wt has a pronounced immunomodulatory effect.

INTRODUCTION

The traditional system of medicines siddha system, being the oldest ancient ways in which of maintaining a healthy life vogue remains rife and emphasizes the importance of physical, emotional, psychological, social well-being. The real strength of siddha system relies on preventive and encouraging health care

deliveries and additional stress is given towards malady interference than management. The majority of the people in developing countries use various traditional herbal medicines to treat a number of diseases and ailments [(1)]. Although, many studies have been undertaken in the past to investigate the pharmacological potential of such remedies, however, rather little work has been done to assess the potential toxicities of such products. There is now growing evidence that many herbal medicines do cause serious toxicity to their users [(2, 3).] Therefore, much more scientific attention is now being given to assess the potential toxicity of herbal medicines than before. *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[(4)]. Hence the present study was performed to evaluate the acute and sub acute toxicity of the *Siddha* herbal formulation *UraiMathirai* 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

2.1 Composition of *Uraimathirai*

The identified raw drugs as per formula composition are ground together with water and made into tablets. The composition of *Uraimathirai* was presented in **Table 1.**

S. No	Ingredients	Botanical Name	Proportion
1.	Chukku	Zingiber officinale Roscoe	1 part
2.	Adimathuram	Glycyrrhiza glabra L.	1 part
3.	Akkirakaram	Anacyclus pyrethrum (L.) Lag.	1 part
4.	Vashambu	Acorus calamus L.	1 part
5.	Catikkai	Myristica fragrans Houtt.	1 part
6.	Katukkai	Terminalia chebula Retz.	1 part
7.	Masikkai	Quercus infectoria G.Olivier	1 part
8.	Acanam	Allium sativum L.	1 part
9.	Tippili	Piper longum L.	1 part
10.	Perunkayam	Ferula assa-foetida L.	1 part

Table 1: Composition of *Uraimathirai*

2.2 Physical Characterization [(5)]

Colour	Pale green colour
Loss on drying	3.84
Total Ash (%)	4.33
Water soluble Ash (%)	3.17
Acid insoluble Ash (%)	1.05
Ethanol soluble Extractive (%)	9.5
Water soluble Extractive (%)	12.25
pH	5.03

2.3 Test animal

Male and female albino rats weighing 130–250 g were used for the acute and sub acute 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 paddy husk as bedding materials at animal house, in an air conditioned environment with five rats in each cage

and maintained at room temperature of (23 ± 2)ºC with relative humidity (60% ± 10%) under 12 hour dark and light cycle [(6)]. Rats were given free access to standard pellet diet and water ad libitum[(7)]. All experimental procedures were in compliance with the Animal Ethical Committee, Committee for the Purpose of Control and Supervision of Experiments on animals (CPCSEA) and were approved by University Ethical Committee with an approval number 162/Pharma/SCRI/2017.

2.4 Urai mathirai dose calculation[(8)]

The working clinical dose of *Uraimathirai* in children is 50 mg (HED = 2.5mg/kg body weight). The animal (rat) doses are calculated as per the FDA guidelines [1] and the calculated t therapeutic dose (TD) was found to be 10mg/kg of body weight. In this study to evaluate the dose correlated effects, 5 times TD and 10 times TD i.e. 50 mg/kg & 100 mg/kg of bodyweight were chosen correspondingly (4).

2.5 Acute toxicity studies: The oral acute toxicity study was evaluated accordance with to Organization for Economic Cooperation and Development (OECD) guideline 423 and Central Council for Research in Ayurvedic Sciences (CCRAS) guideline [(9, 10)]. Wistar albino Rats of both sexes (over night fasting free excess to water), aged 8–12 weeks old were used. The rats were divided into four groups (Group I, Group II, Group III and Group IV), each group comprising (5males and 5females) total 40 rats. The 1st group served as a negative control, while 2nd, 3rd and 4th was considered as tested groups received orally *Uraimathirai* drug solution at dose based on body weight of (10, 50 and 100mg/kg b.wt). Before drug administration, the body weight of each animal was determined and the dose was calculated according to the body weight. The rats were observed for any toxic effect for first 4 h after the treatment period. Further rats were investigated daily for a period of 14 days for various toxic signs and observation such as physical observations, behavioral changes and other parameters such as body weight, urinations, food intake, water intake, respiration, convulsion, tremor, temperature, constipations, changes in eye and skin colors, etc were recorded**.**

2.6 Sub-acute toxicity study: The oral sub-acute toxicity study was carried out accordance with to Organization for Economic Cooperation and Development (OECD) guideline 423 and Central Council for Research in Ayurvedic Sciences (CCRAS) guideline [9,10]. Adult healthy male and female wistar albino rats aged 8–12 weeks were used. The rats were divided into four groups (Group I, Group II, Group III and Group IV) each group comprising (6males and 6females) total 48 rats. The 1st group served as a negative control, while 2nd, 3rd and 4th was considered as tested groups received orally *Uraimathirai* drug solution at dose of (10, 50 and 100mg/kg b.wt). Before drug administration, the body weight of each animal was determined and the dose was calculated according to the body weight of each sex every week for 28 consecutive days [(11)]. Various toxic signs and observation, such as body weight, mortality, and food and water intake was monitored. After 28 days, all surviving rats were fasted overnight and anesthetized. The serum from non-heparinized blood was collected for determining clinical blood biochemistry. Rats were then euthanized after blood collection and the internal organs (brain, heart, thymus, lungs, liver, stomach, spleen, kidney, adrenal gland and sex organs) were removed and weighed to determine the relative organ weights and observed for any gross lesions. The internal organs were preserved in 10% buffered formaldehyde solution for histopathological examination.

2.6.1 Relative organ weight: The internal organs (brain, heart, thymus, lungs, liver, stomach, spleen, kidney, adrenal gland and

sex organs) excised from all the experimental rats after 28th day .Organ-to-body weight ratio was calculated by dividing the weight (g) of each organ by the weight (g) of rats before sacrifice.

2.6.2 Biochemical parameters: In sub-acute study after 28th day 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, and thyroid profile level (T3, T4, TSH) were determined for both control and *Uraimathirai* treated groups by the standard method of practical in biochemistry.

2.6.3 Histopathological examination:

The internal organs (brain, heart, thymus, lungs, liver, stomach, spleen, kidney, adrenal gland and sex organs) excised from all the experimental rats were fixed in 10% buffered formalin in labeled bottles, and processed for histological examination. Tissues embedded in paraffin wax were sectioned 5 mm thick, stained with haematoxylin and eosin, mounted on glass slides and examined under a standard light microscope [(12)].

2.7immunomodulatory activity [(13)]

Immunomodulatory activity was estimated in-vivo by delayed type hypersensitivity reactions using standard protocols and procedures. The animals for the experimental studies were grouped into 5 groups containing 6 animals each, where group-I serves as normal control, Group-II as standard group and Group-III, IV and V for the three test doses (10, 50 and 100mg/kg b.wt). Sheep red blood cells (SRBCs) was used as inducing agent for Hypersensitivity reaction in rats, following the prescribed method (S.Y. Gabhe *et.al*). The *uraimathirai* (10, 50, and 100 mg/kg, body weight) was administered to the animals (test group) orally for 7 days, Cyclophosphamide (30mg/kg *i.p.*) for group-II and the vehicle was used for control animals. Animals were immunised by 1% SRBC (20 µL) on 7th day into the right hind footpad (3), right hind footpad thickness was measured at 0,1, 2, 4, and 24 hour using digital water plethysmometer (Panlab LE 7500). % Inhibition of paw oedema using the formula below given:

% Inhibition of paw oedema = Vc – Vt/ Vc X 100 , Where, Vc = Paw oedema of control animals, Vt = Paw oedema of drug treated animals

2.8 Statistical analysis:

All the data was expressed as mean ± SEM. Statistical significance between more than two groups was tested using one-way ANOVA using Graph pad prism version-8

3. RESULTS

3.1. Acute toxicity study: No treatment related toxic symptom or mortality were observed after oral administration of the tested *Uraimathirai* drug solution at dose of (10, 50 and 100mg/kg b.wt). The general behavioral of *Uraimathirai* treated rats and control group was observed first for short period (4 hours) followed by 14 days, did not display any drug related changes in behavior, breathing, skin effects, water consumption, impairment in food intake and temperature compared to control group. Therefore, the extract seems to be safe at a dose level of 100 mg/kg, and the LD50 was considered be >100 mg/kg b.wt. The parameters observed for acute toxicity study after the administration of the test *Uraimathirai* compared with normal group were presented in **Table 2**.

Table 2: General appearance and behavioral observations of acute toxicity study for 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				
Fur	N	N	N	N
Eyes	N	N	N	N
Mucous Membrane	N	N	N	N
Urine	N	N	N	N
Behavioral observations				
Mood	N	N	N	N
CNS Excitation	NO	NO	NO	NO
CNS Depression	NO	NO	NO	NO
Motor Indication				
Abnormal gait	NO	NO	NO	NO
Righting reflex	N	N	N	N
Posture	N	N	N	N
Sensory Responses				
Touch & pain response	N	N	N	N
Straube"s phenomenon	NO	NO	NO	NO
Reflexes				
Pinnna & corneal	N	N	N	N
Autonomic effects				
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				
Apnoea & dysponea	NO	NO	NO	NO
Death	NO	NO	NO	NO

3.2. SUB-ACUTE TOXICITY STUDY

All the tested group rats treated with *Uraimathirai* at a dose of (10, 50 and 100mg/kg b.wt) daily survived throughout the 28 days. No clinical toxicity signs such as physical observations, behavioral changes and other parameters such as body weight, urinations, food intake, water intake, respiration, convulsion, tremor, temperature, constipations, changes in eye and skin colors, etc were observed in the treated group compared to the control group.

3.2.1. EFFECT OF *URAI MATHIRAI* ON RELATIVE ORGAN BODY WEIGHT

The average and relative organ weight of tested *Uraimathirai* at a dose of (10, 50 and 100mg/kg b.wt) and control treated 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 by *Uraimathirai*. The effect *Uraimathirai* on principal organ weights relative to body weight were presented in **Tables 3** and graphical representation presented in **Figure 1**.

Table 3 Effect of Oral administration of *Uraimathirai* 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	9.15 ± 0.33	11.45±0.52	9.40±0.51	11.80±0.76
Heart	4.15±39.12	4.75±0.20	3.90±0.34	5.69±0.43
Thymus	0.90±3.15	2.30±0.12	1.45±0.15	2.55±0.20
Lungs	6.30±78.39	9.10±0.38	8.70±0.92	23.55±3.29
Liver	34.30±1.37	36.10±1.21	35.05±1.43	37.65±2.03
Stomach	7.75±0.21	7.90±0.30	7.60±0.44	10.15±0.68
Spleen	3.50±0.26	4.45±0.30	5.00±0.38	6.45±0.48
Kidney	8.30±0.24	7.90±0.17	9.95±0.39	12.30±1.20
Adrenal gland	0.60±5.03	0.25±0.06	0.90±0.08	2.05±0.32

All values are expressed as mean ± SEM (n=12). No significant difference since p > 0.05, as compared to control group.

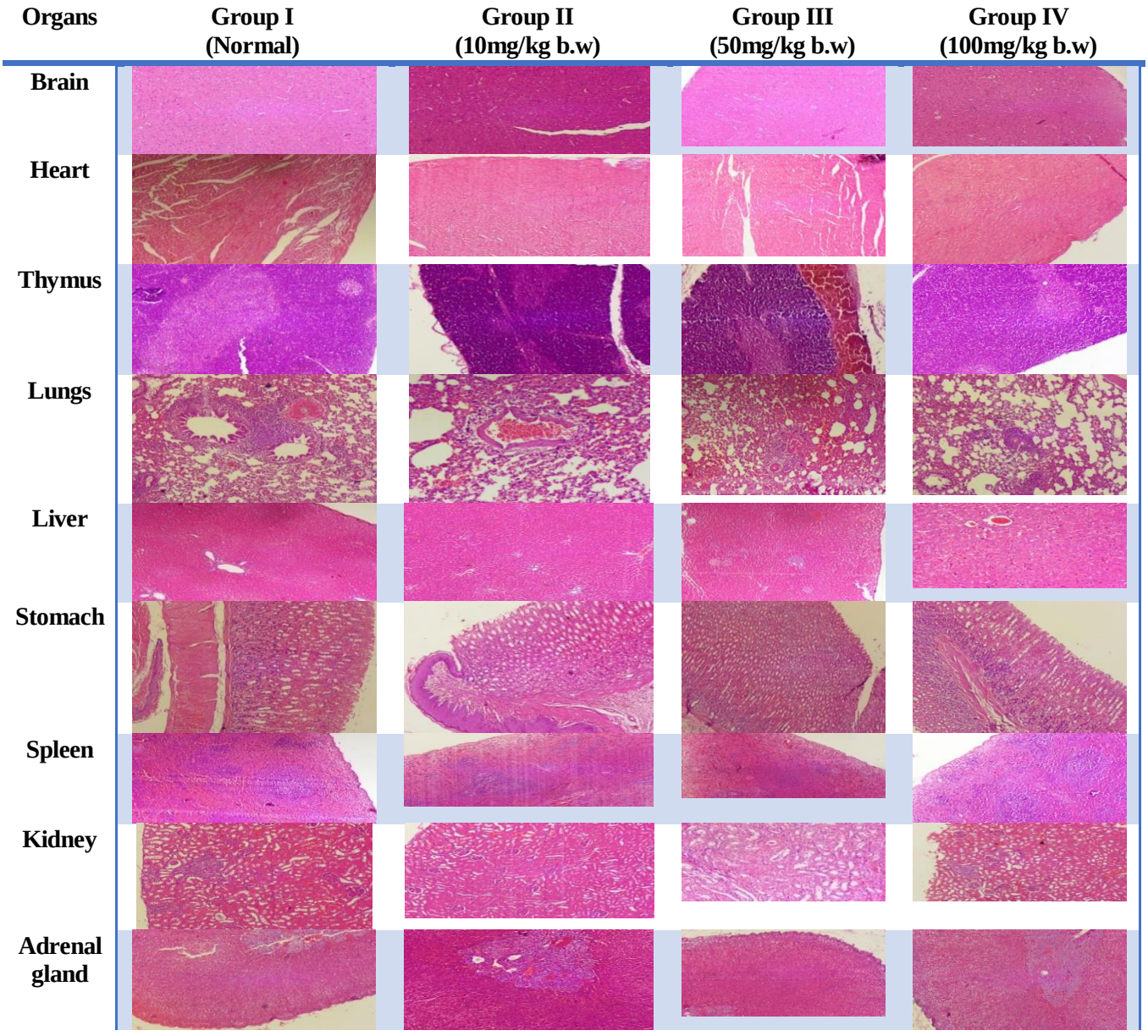

Figure1. Histopathological examination rats organs

3.2.2 Effect of urai mathirai on biochemical parameters: The results of the various biochemical parameters on the experimentally treatedrats with the oral administration of the *Uraimathirai* 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, T-BIL, urea, sodium, creatinine and uric acid levels etc., when compared to control group. The effect of *Uraimathirai* on biochemical parameters are summarized in **Table 4** and graphical representation presented in **Figure 2**.

Table 4 Effect of oral *Uraimathirai* 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)	43.67±5.37	59.17±6.69	54.00±6.11	71.78±1.61
Total Cholesterol levels (mg/dl)	81.58±3.95	80.73±2.45	70.11±2.47	77.44±3.13
Triglyceride levels (mg/dl)	86.75±8.44	81.18±6.67	83.22±7.54	97.25±11.73
HDL levels (mg/dl)	29.92±2.01	28.27±0.84	24.44±1.39	30.38±1.16
LDL levels (mg/dl)	36.08±3.45	36.36±1.91	28.44±2.37	24.75±1.75
SGOT levels (U/L)	305.83±9.64	315.42±15.25	338.45±27.60	286.00±18.32
SGPT levels (U/L)	63.33±2.66	63.08±1.99	70.36±1.47	64.78±3.01
ALP levels (U/L)	3.92±0.31	2.86±0.48	4.67±0.53	4.56±0.24
GGT levels (U/L)	207.58±15.49	214.42±17.41	283.45±27.03	265.56±20.59
Total Protein levels (g/dl)	3.66±0.10	3.58±0.07	3.22±0.15	3.68±0.08
Albumin levels (g/dl)	439.00±50.75	681.09±119.70	854.78±215.87	1263.44±187.62
LDH levels (U/L)	0.46±0.10	0.71±0.13	0.72±0.16	0.74±0.13
CRP levels (mg/L)	7.08±0.09	7.06±0.12	11.85±4.97	7.27±0.19
Creatinine Kinase levels (U/L)	596.42±19.85	536.82±34.93	530.00±59.37	426.33±26.95
Creatinine Kinase – MB levels (U/L)	1465.00±58.50	1382.18±111.35	1411.43±158.93	1370.00±139.61
Urea levels (mg/dl)	32.91±2.78	29.67±0.98	34.09±2.39	33.67±2.01
Serum creatinine levels (mg/dl)	0.18±0.02	0.18±0.01	0.17±0.02	0.16±0.02
Total Bilirubin levels (mg/dl)	0.38±0.03	0.38±0.02	0.45±0.02	0.46±0.04
Uric acid levels (mg/dl)	1.23±0.17	1.13±0.11	1.28±0.23	1.36±0.22
Serum Calcium levels (mg/dl)	9.10±0.14	10.65±1.71	8.61±0.18	9.59±0.22
T3 levels	0.13±0.02	0.10±0.00	0.11±0.01	0.59±0.32
T4 levels	2.09±0.22	1.44±0.14	1.10±0.11	1.24±0.20
TSH levels	11.41±1.42	8.44±0.44	8.99±0.49	10.77±1.48

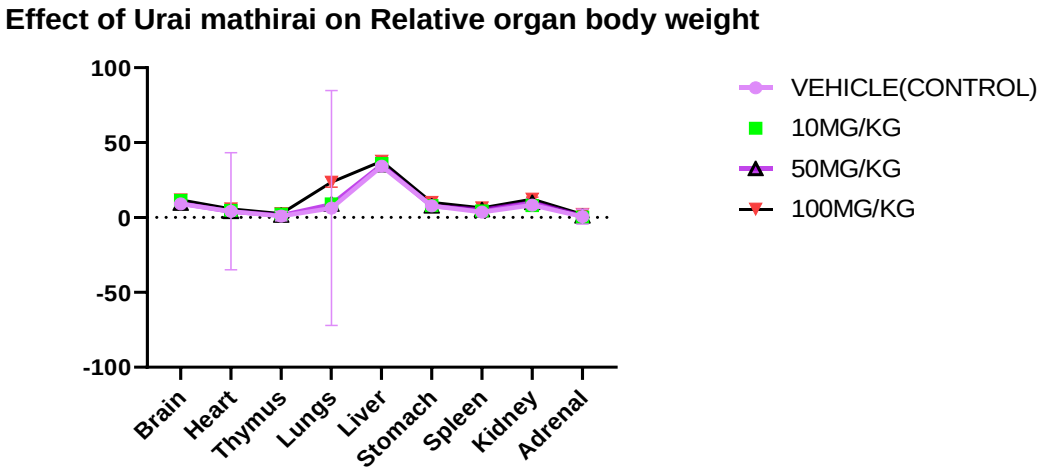

Figure 2: Relative organ weight of rats

3.2.3. Effect of *Uraimathirai* in Histopathological Study

Light microscopic examination sections of various organs like brain, heart, thymus, lungs, liver, stomach, spleen, kidney and adrenal gland of control and treated groups showed a normal histology and absence of any gross pathological lesions. Additionally, 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 which are peribronchial and interstitial mononuclear cell infiltration and pulmonary congestions mild biliary epithelial cell hyperplasia, mild kupffer cell hyperplasia and multifocal hepato cellular degenerations which are all not related with test compound *Uraimathirai*.The histopathological slides were presented in **Figure 3.**

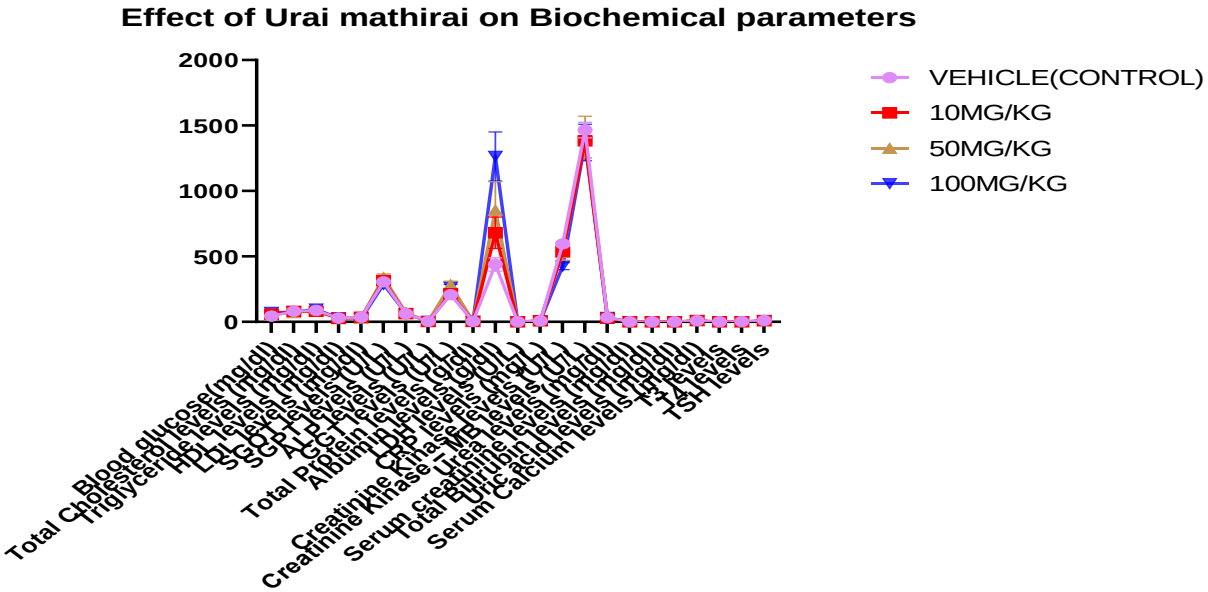

Figure 3: Biochemical parameters

3.3 Effect of *Uraimathirai* in Immunomodulatory Activity

All the test groups (III, IV and V) at three doses (10, 50 and 100 mg/kg, b.wt) for seven days produced a dose related increase in early (4 h) and delayed (24 h) hypersensitivity reaction in rats at a dose of 100 mg/kg, b.wt. The 4 hour-reaction was found to be of higher magnitude than the 24 hour-reaction. From the results it is evident that the Uraimathirai at a dose of 100mg/kg.bt has shown a pronounced effect on hypersensitivity reaction. The recorded results were given in **Table.5**.

Table 5 Effect of *Uraimathirai* on SRBC induced Immunomodulatory activity in rats

S. No	Groups	Paw Volume in ml Mean + SEM (%Inhibition of Paw edema)				
		Individual control	0th hour	1st hour	2nd hour	4th hour
1	SRBC	0.85 ± 0.07	1.04 ± 0.09	1.66 ± 0.04***	1.93 ± 0.03***	2.13 ± 0.05***
2	Urai mathirai 10 mg/kg.b.wt	0.93 ± 0.03	0.87± 0.11	1.49 ± 0.05***	1.33 ± 0.05**	1.35 ± 0.09**
3	Urai mathirai 50 mg/kg. b.wt	0.94 ± 0.03	1.15± 0.04	1.38± 0.05***	1.25± 0.06**	1.21± 0.06**
4	*Urai mathirai* 100mg/kg. b.wt	0.84 ± 0.06	1.03 ± 0.08	1.41 ± 0.05***	1.28 ± 0.06**	1.23 ± 0.06**

*All values are expressed as mean ± SEM (n=6).more significant difference *P<0.05, **P<0.01 and ***P<0.001, as compared to control group.*

4. DISCUSSION

In general, the safety studies on herbal medicines have been carried out by performing acute and sub-acute toxicity tests in laboratory rats (e.g. rodents and non-human primates). In the present study, we investigated the acute and sub-acute oral toxicity of *Uraimathirai*in wistar albino rats. The acute and sub- acute toxic effects differ principally from each other with respect the time intervening before the effects are observed. While, the acute effects are normally observed soon after a single exposure of test drug, the sub-chronic effects are usually monitored over an extended period of 28 days during which there is repeated exposure of test drug.

The acute toxicity study on *Uraimathirai suggest that there was no* mortality in the entire drug treated group which indicates that *UraiMathirai*- Siddha Formulationis safe up to 100 mg/kg b.wt, which is ten times of therapeutic dose. In sub acutetoxicity study reveals that no abnormal behavioral activity and pre/post terminal deaths were recorded (therapeutic dose10 mg/kg b.wt, average dose 50 mg/kg b.wt and high dose 100mg/kg b.wt) in the rats exposed to the test compound up to 10 times of the intended therapeutic dose. No significant difference was observed with respect to body weight gain, feed/water consumption, biochemical, hormonal and histological changes between the test groups and control group. NOAEL was found to be 100mg/kg. All relative organ weights and biochemical parameters were analyzed statistically. All the data was expressed as mean ± SEM. Statistical significance between more than two groups was tested using one-way ANOVA 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 [14]. The hypersensitivity reaction of Immunomodulatory study suggests that the 4th hour-reaction was found to be of higher magnitude than the 1st hour-reaction. It indicates that *Uraimathirai* has a greater

effect on the delayed hypersensitivity reaction (Immunomodulatory). The p value indicates *P<0.05, **P<0.01 and ***P<0.001when more significant when compared with respective control group.

5. CONCLUSION

In conclusion, the present investigations demonstrates that at doses consumed in the traditional medicine of *Uraimathirai*upto 100mg/kg b.wtas showed good immunomodulatory effect and relatively safe, as it did not cause either mortality or significant drug treatment related effects on clinical signs or behavioral activity, biochemical, hormonal and histological changes etc., were observed in all the groups of rats that were survived during the experimental period. This study provides very important data on the acute and sub acute toxicity profile that should be very useful for any future in vivo and clinical study in siddha system of medicine.

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