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Research article

EVALUATION OF SAFETY OF VETTUMARAN GUTIKA THROUGH SUB ACUTE TOXICITY STUDY IN WISTAR RATS

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ABSTRACT:
The study aimed to the study sub acute toxicity evaluation of Vettumaran gutika a Compound Ayurvedic formulation. The test drug was administered to Wistar rats at doses 30 mg, 150 mg and 300 mg for 28 days and observations pertaining to general behavior, adverse effects, and mortality were recorded during this period followed by blood collection and sacrifice at the termination of the trial. Vattumaran gutika did not produce any death or hazardous symptoms during sub acute study. Sub acute treatment with the test drug did not show any change in body weight, food and water consumption, Hematological, serum biochemical and histopathological finding also supported the findings. From the present study it was evident that Vettumaran gutika was found to be safe at the tested dose levels in Wistar Rats.
Key words: Vettumaran Gutika, sub acute toxicity, Wistar Rats, hematology, histopathology

INTRODUCTION

Herbomineral preparations are in use for treatment of various diseases with proven efficacy and Vettumaran gutika (VG) is one such medicine. It is antipyretic, carminative, anticonvulsive, anti emetic, diuretic as well as analgesic. As per the illustration in Sahasrayogam, an important hand book on Ayurveda, it is used in conditions such as fever, Gas trouble, emesis, pyrexia , dysuria with different vehicles [1]. By virtue of antipyretic and arthralgic effect, Vettumaran gutika in combination of other medicine has proved to be effective in alleviating symptoms of chikungunya [2]. The test drug at single exposure (acute toxicity study) at dose of 2000 mg/kg body weight was found to be safe in Swiss Mice [3]. Treatment of sandhisula (joint pain) and after effects of viral infections necessitates the use of VG for longer durations. Hence the present study has been undertaken to assess the safety of VG upon prolonged use.

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MATERIALS AND METHODS

Test drug

Vettumaran gutika (VG) was prepared at Pharmacy of National research institute for Panchakarma, Cheruthuruthy as described in Sahasrayoga. The ingredients of the preparation have been enlisted in Table 1.

Dose calculation

The human dose of the Vettumaran gutika is 300 mg per day and the dose equivalent to rats was calculated as per the method of Ghosh [4]. The test drug was administered at test dose level (test dose), 5 times of the test dose (Average dose) and 10 times of the test dose (High Dose).

Animals

Wistar Rats of either sex were procured from Veterinary College, Mannuthy, Thrissur, Kerala were used in the trial. After quarantine period, animals were caged individually as per Committee for the purpose of Control and supervision of experiments on animals (CPCSEA) guidelines.

Table 1. Ingrediants of Vettumaran gulika

Scientific name	Sanskrit name
Aconitum ferox Wall.	Vatsanabhi
Piper nigrun Linn.	Maricha
Trachyspermum ammi (Linn.) Sprague	Ajamoda
Cinnabar (Mercuric Sulfide)	Hingula
Borax (Sodium borate)	Tankana
Zingiber officinale Rosc.	Fresh ginger juice

Ethical Clearance

The present trial was conducted with the approval of Institutional animal Ethics Committee
(IAEC) meeting held at National Research Institute for Panchakarma, Cheruthuruthy, Thrissur, Kerala.

Experimental design

The rats were divided into four groups namely Vehicle Control (VC) group, test dose (TD) group, Average dose (AD) group and high dose (HD) group each comprising of 12 animals (6 Male and 6 Female). While the VC group received distilled water, the test drug was administered orally at dose of 30 mg, 150 mg and 300 mg per kg body weight in TD, AD and HD groups respectively for 28 consecutive days. Animals were observed for signs of mortality and clinical signs of toxicity during the study period. Behavioral and physiological responses were monitored daily and weekly feed consumption and body weight gain were recorded as per OECD guideline 407 [5].

After the dosage period of 28 days, animals were fasted and blood samples were collected by retro orbital plexus puncture under ether anesthesia for hematology and serum biochemistry.

Total Leucocyte Count (TLC), Polymorph Percentage, Lymphocyte percentage, Packed Cell Volume , Hemoglobin (HB)levels, Total red Cells Count (TRC) and platelets Count, Serum Glucose, Serum glutamic oxaloacetic transaminase (SGOT), Serum glutamic pyruvic transaminase (SGPT), Creatinine, Total Protein (TP) and Prothrombin Time (PT) were analysed at Biochemistry division of the Institute.

Animals were sacrificed by cervical dislocation and detailed post mortem examination was carried out. Vital organs were individually weighed and tissue samples of the same were stored in 10% formalin for histopathology studies.

Statistical analysis

The data obtained during the trial was compared between groups through Analysis of variance (ANOVA) followed by Dunnet's test at P 0.05 significance.

RESULTS AND DISCUSSION

Oral administration of Vettumaran Gutika at doses 30,150 and 300 mg per kg body weight for 28 days did not produce any mortality or clinical signs of toxicity. There were no adverse physical and behavioral changes in the animals receiving test drug at different dose levels.

There were no significant changes pertaining to body weight gain and feed intake between animals in different groups (Tables 2 & 3). Hematological parameters and biochemical parameters did not differ significantly except for an increase in hemoglobin per cent age and total red cell count in high dose group as compared to vehicle control group (Tables 4 & 5).

The test drug did not affect the normal development of internal organs and significant differences were not observed with respect to the relative organ weights (Table 6).

Histopathology studies did not reveal any pathological changes in the internal organs of animals at any of the dose levels (Fig. 1 – 5)

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Table 2. Effect of Vettumaran gutika on Weekly % body weight gain as compared to day 1 in Wistar rats.

Weeks	0-1	0-2	0-3	0-4
VC	67.65±7.15	107.86±9.46	145.08±16.03	174.02±19.59
TD	64.81±6.50	101.87±8.31	144.95±13.08	171.55±16.30
AD	70.21±7.26	110.69±10.287	144.91±15.24	173.02±19.74
HD	73.98±6.82	114.63±10.21	159.06±15.28	186.58±18.44
F value	0.3152	0.3125	0.2219	0.1393

(Average of 12 Values)

Table 3. Effect of Vettumaran gutika on Weekly % feed intake in Wistar rats

Weeks	0-1	0-2	0-3	0-4
VC	137.58±8.22	73.77±4.12	74.40±3.35	56.20±1.83
TD	130.25±8.031	71.53±3.60	74.78±3.50	59.38±1.87
AD	139.45±9.41	73.95±3.10	72.95±2.93	59.47±1.80
HD	144.67±8.428	70.70±3.30	75.54±2.98	60.90±1.58
F value	0.5883	0.2088	0.1160	1.250

(Average of 12 Values)

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Table 4. Effect of Vettumaran gutika on hematological parameters in Wistar rats

	TLC ($x10^3$)	POLY (%)	LYM (%)	PCV (%)	HB (g %)	TRC (10^6)	Platelets (10^5)
VC	5.53±0.33	27.25±1.498	72.75±1.498	35.42±0.87	13.79±0.41	3.93±0.19	2.11±0.03
TD	5.39±0.36	26.83±1.15	73.16±1.15	36.33±1.00	14.3±0.39	4.18±0.13	2.06±0.09
AD	5.30±0.38	29.25±1.54	70.75±1.54	37.67±1.20	14.63±0.34	4.40±0.15	2.06±0.05
HD	532±0.37	27.17±1.12	72.83±1.12	35.25±0.99	15.19±0.21*	4.52±0.17*	2.03±0.03
F Value	0.080	0.6690	0.6690	1.172	2.908	2.606	0.3273

* P<0.05 (Average of 12 Values)

Table 5. Effect of Vettumaran gutika on serum Biochemical parameters in Wistar rats

	Glucose (mg %)	SGOT (IU/L)	SGPT (IU/L)	Creatinine (mg %)	Total Protein (g %)	Prothrombine Time (Sec.)
VC	43.08 ± 3.01	130.08 ± 7.613	110 ± 15.2	1.05 ± 0.04	5.891 ± 0.10	17.67 ± 0.87
TD	47.17 ± 2.57	112.17 ± 7.18	76.67 ± 7.35	0.94 ± 0.04	5.683 ± 0.10	17.08 ± 1.26
AD	50.5 ± 3.62	126.83 ± 4.1	89.17 ± 12.87	0.90 ± 0.04	5.77 ± 0.11	19 ± 2.81
HD	51.17 ± 4.2	133.3 ± 6.8	71.6 ± 7.9	0.91 ± 0.05	5.641 ± 0.132	24.08 ± 3.511
F Value	1.185	2.024	2.336	2.486	0.9351	1.0797

(Average of 12 Values)

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Table 6. Effect of Vettumaran gutika on relative organ weights in Wistar rats

	Hear	Lung	Liver	Spleen	Kidneys	Testes#	Ovaries #	Brain
VC	0.39 ± 0.01	0.70 ± 0.02	3.36 ± 0.08	0.28 ± 0.01	0.88 ± 0.05	0.99 ± 0.08	0.05 ± 0.00	1.05 ± 0.03
TD	0.39 ± 0.01	0.78 ± 0.03	3.32 ± 0.08	0.25 ± 0.01	0.79 ± 0.02	1.18 ± 0.07	0.05 ± 0.00	1.05 ± 0.05
AD	0.48 ± 0.01	0.61 ± 0.05	3.30 ± 0.08	0.26 ± 0.01	0.81 ± 0.02	1.10 ± 0.07	0.06 ± 0.00	1.07 ± 0.04
HD	0.43 ± 0.02	0.74 ± 0.07	3.27 ± 0.13	0.26 ± 0.02	0.80 ± 0.03	1.07 ± 0.04	0.05 ± 0.00	1.01 ± 0.05
F Value	1.147	2.313	1.492	1.336	1.730	1.522	1.638	0.4154

P<0.05 (Average of 12Values) # (Average of 6 values)

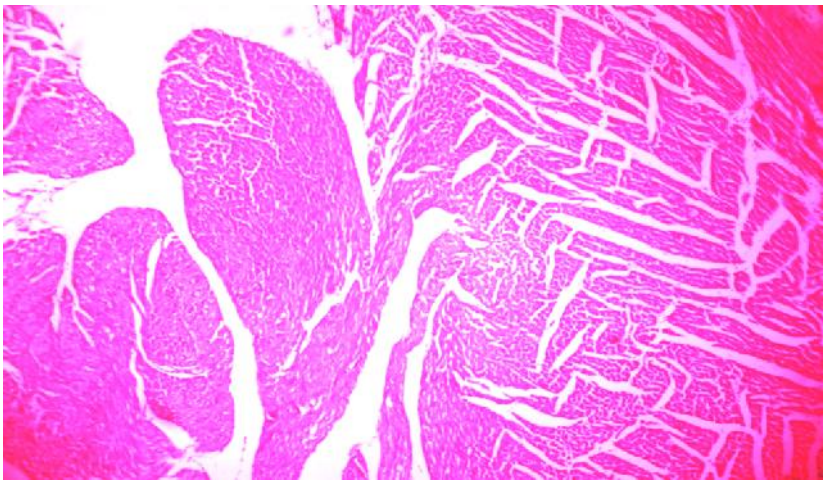

Fig 1. Histopathology of section Heart of a female rat showing normal endicardium and myocardium (VG: 300 m/kg B.W.).

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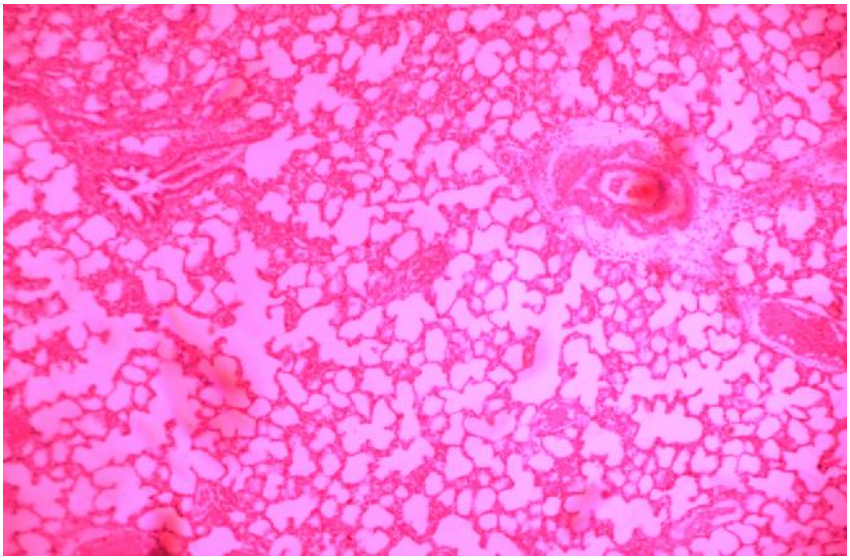

Fig 2. Histopathology of section of Lung of a male rat showing normal bronchioles and alveoli (VG: 300 m/kg B.W.).

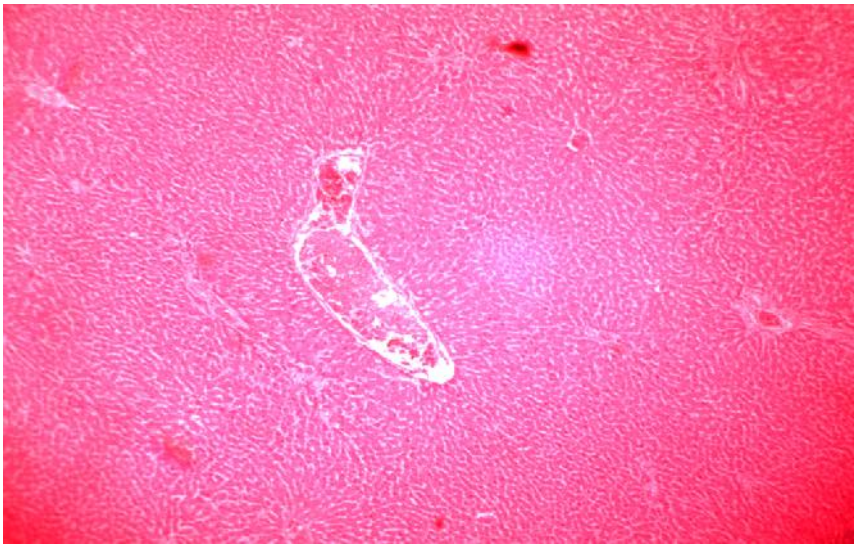

Fig 3. Histopathology of section Liver of a female rat showing normal hepatocytes and hepatic veins (VG: 300 m/kg B.W.).

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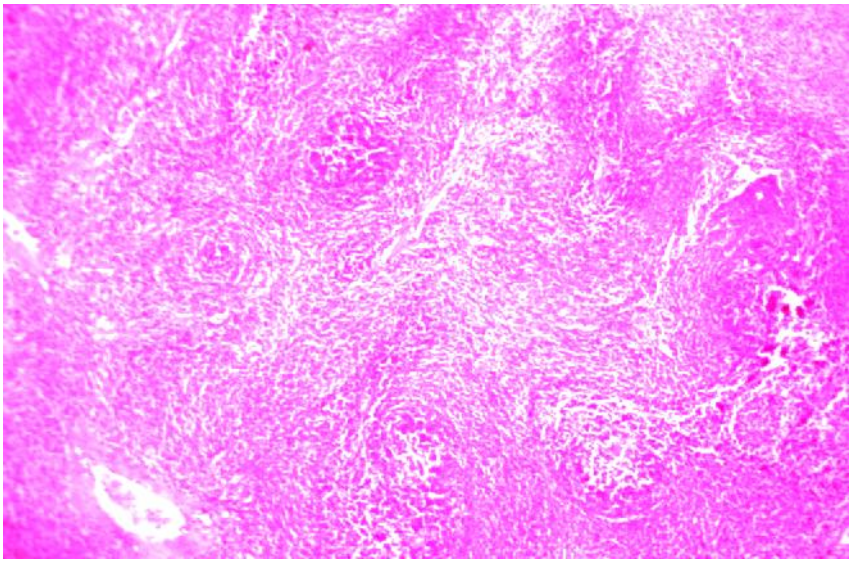

Fig 4. Histopathology of section of Spleen of a female rat showing normal lymphoid follicles and germinal centres (VG: 300 m/kg B.W.).

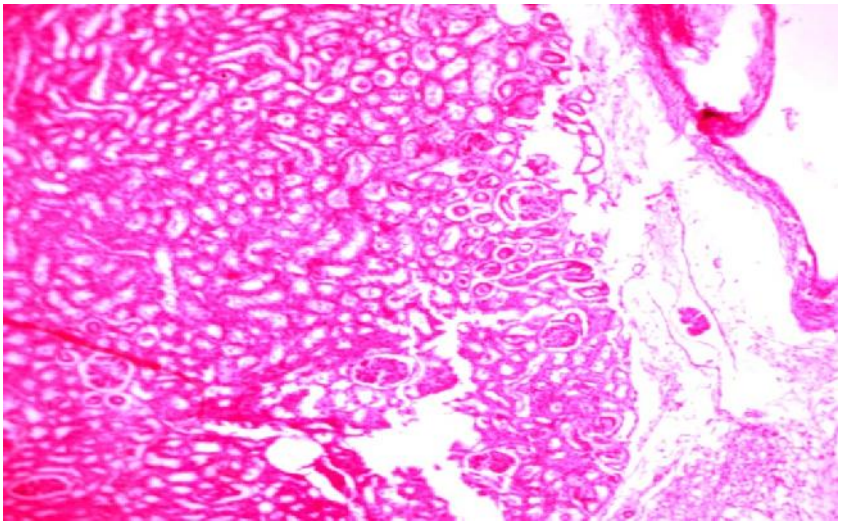

Fig 5. Histopathology of section of Kidney of a female rat showing normal renal tubules and Bowman's capsule (VG: 300 m/kg B.W.).

CONCLUSION

Findings of the study suggest that consecutive oral of Vettumaran gutika up to dose of 300 mg per kg body consecutively for 28 days is safe in Wistar rats . The safety of the Vettumaran gutika which is widely used in traditional Ayurvedic medicine was confirmed by physiological, hematological, biochemical and histopathology findings.

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