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Toxicological evaluation of ethanolic extract of *Anacyclus pyrethrum* in albino wistar rats

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ABSTRACT

Objective: To evaluate the sub chronic toxicity of ethanolic extract of *Anacyclus pyrethrum (A. pyrethrum)* in albino wistar rats. **Methods:** In sub chronic toxicity study ethanolic extract of *A. pyrethrum* prepared in 2%v/v tween 80 was administered to rats at the dose of 1000 mg/kg per day for 90 days by oral gavage. A control group received only 2%v/v tween 80. During study period the rats were observed for changes body weight. At the end of dosing period rats relative organ weight of the liver, kidney, brain, lungs and spleen in rats treated with *A. pyrethrum* extract and control group were examined and also rats were subjected to haematological, biochemical and histopathological examination. **Results:** The administration of ethanolic extract of *A. pyrethrum* had no effect on body weight, growth and survival. There was no significant difference in the relative organ weight of the liver, kidney, brain, lungs and spleen in rats treated with *A. pyrethrum* extract and control group. In the present study, all the haematological and biochemical parameters at the end of dosing and observation period did not reveal difference between drug treated and control groups. Studies on histopathological examination of vital organs showed normal architecture suggesting no evidence of pathological lesions. **Conclusions:** The studies on sub chronic toxicity reveals that no mortalities or evidence of adverse effects on oral administration of extract. The findngs of the study indicate that ethanolic extract of *A. pyrethrum* had no treatment related toxicological abnormalities and can be considerd as safe for long-term treatment.

1. Introduction

Medicinal herbs are indispensible part of traditional medicine practiced all over the world due to easy access, low cost, least risk and low side effect profile. *Anacyclus pyrethrum (A. pyrethrum)*, family Asteraceae, is used in traditional system of medicine, and it is regarded as a tonic to the nervous system[1]. The roots contain anacyclin, pellitorine, hydrocarolin, inulin, traces of volatile oil and seasamin. *A. pyrethrum* is a perennial, procumbent herb, which is found throughout India. The plant roots is reported for antibacterial, antidepressant[2], anti-inflammatory[3], immunostimulating[4] and aphrodisiac activities[5]. There were no scientific reports on sub chronic toxicity data for *A. pyrethrum* roots. As root of this plant has potential health benefits, it can be better utilized for nutraceutical and functional food formulations. Hence toxicological evaluation of ethanolic extract of *A. pyrethrum* roots has been conducted to optimize the safe use as plant based medicine.

2. Materials and methods

2.1. Plant material and extraction

The roots of *A. pyrethrum* used for investigation was collected from hilly regions of Pathanamthitta district of Kerala, and the roots of *A. pyrethrum* was identified and authenticated by Professor Jayaraman, National Institute of Herbal Science, Chennai and voucher specimen (no. 0997) of the plant has been be deposited in the herbarium of the department. The roots of *A. pyrethrum* was powdered (500 g) and ethanolic extract was prepared by simple maceration process using 2 L of ethanol. The ethanolic extract was evaporated under reduced pressure using rotavapor evaporator. The yield of the extract was 5.6 g. A suspension was prepared using 2%v/v tween 80 and administered orally.

2.2. Animals

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Albino wistar rats of either sex approximately of same age group having weight 150-200 g were used after they were acclimatized for a week under laboratory conditions. They were provided standard rodent pellet diet (Lipton India) and water ad libitum. The animals had free access to food and water and were maintained under 12:12 hour light and dark cycle. All experiments were carried out during daytime. The institutional animal ethical committee approved the protocol (no. 290/04/V/CPCSEA/IAEC/PHA-24-29) and care of animals was taken as per guidelines of committee for the purpose of control and supervision in experiments on animals.

2.3. Sub chronic toxicity

Sub chronic toxicity studies were carried out according to Organization for Economic Cooperation and Development guideline (OECD408 (1995) repeated dose 90-day oral toxicity study in rodents)[6]. Forty-eight age and weight matched-rats were randomly divided into control and treatment groups (12 male and 12 female rats/group). The extracts of *A. pyrethrum* was administered to the treatment group of rats at the dose of 1 000 mg/kg per day by oral gavage, in a volume of 0.5 mL/100 g body weight for 90 days, whereas an equal volume of vehicle was given to the control group. All the animals were weighed once a week. During the period of administration, toxic manifestation such as signs of toxicity, mortality was monitored daily.

2.4. Hematological and biochemical parameters of rats

At the end of period of study, all surviving animals were fasted overnight before anesthetization with ether. The blood sample was carefully collected for blood chemistry and enzyme analysis in to heparinized and dry non-herparinized tubes. Heparinized blood samples were used for hematological study such as total red blood cell count, total white blood cell count, platelet count, hemoglobin, hematocrit, differential leukocyte count[7]. The serum separated from non-heparinized samples were used for the estimation of biochemical parameters like creatinine[8], urea[9], triglycerides, total cholesterol[10], total protein[11], albumin[12], Aspartate transaminase (AST), Alanine transaminase (ALT)[13-16], Alkaline phosphatase (ALP)[17] and total bilirubin[18,19].

2.5. Histopathological examinations

After blood collection rats were sacrificed for tissue studies. The internal organs like liver, kidney, lungs, brain and spleen were isolated and blotted free of blood weighed immediately to determine relative organs weights and observed for gross lesions. Histological examination were performed on the tissue preserved in 10% buffered formalin solution with particular emphasis on those which showed gross pathological changes[20-22].

2.6. Statistical analysis

Statistical analysis was carried out by one way ANOVA and the results were expressed as mean±SEM. The data obtained from the toxicity study was analyzed. $P<0.05$ were considered as significant.

3. Results

In the sub chronic toxicity studies, *A. pyrethrum* extracts at dose of 1 000 mg/kg, given orally for 90 days did not produce any mortality in rats. No signs of toxicity were observed during the experimental period. Changes in general behavior or other physiological abnormalities were not observed at any point in the present study. Body weight changes were monitored at weekly intervals till 90 days. Change in body weights in treated and control female group during the 90-day sub chronic toxicity studies are summarized in (Figure 1) and body weights changes in treated and control male group during the 90-day sub chronic toxicity studies are summarized in Figure 2. Percentage increase in weight of treatment group and control group was found to be

Table 1
Relative organ weight(g/100g) of control and *Anacyclus pyrethrum* ethanolic extract treated rats.

Organs	Control		*A. pyrethrum* (1 000 mg/kg)	
	Female	Male	Female	Male
Liver	2.66±0.02	2.69±0.06	2.65±0.15	2.63±0.03
Kidney	0.62±0.03	0.60±0.12	0.63±0.02	0.61±0.12
Brain	0.53±0.10	0.49±0.14	0.52±0.10	0.55±0.16
Lungs	0.45±0.03	0.44±0.01	0.45±0.05	0.49±0.02
Spleen	0.22±0.09	0.22±0.07	0.17±0.06	0.23±0.05

Data are expressed as mean±SEM, n=12.

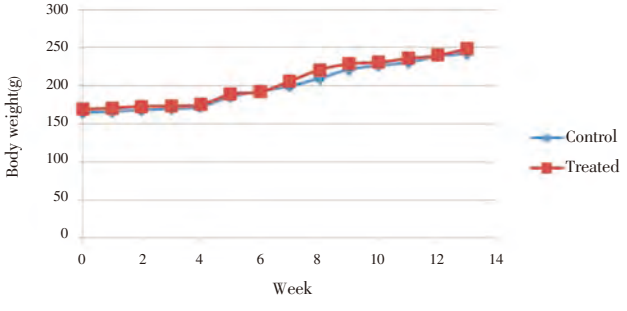


Figure 1. Body weight in treated and control female group during the 90-day safety assessment.

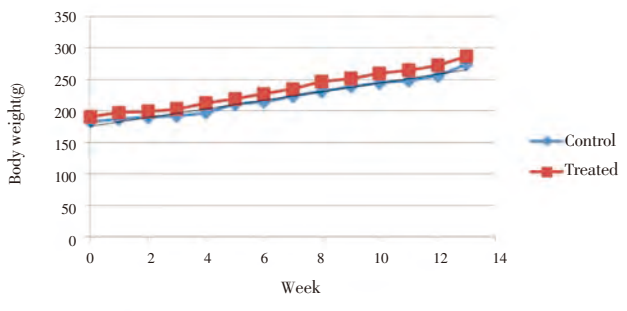
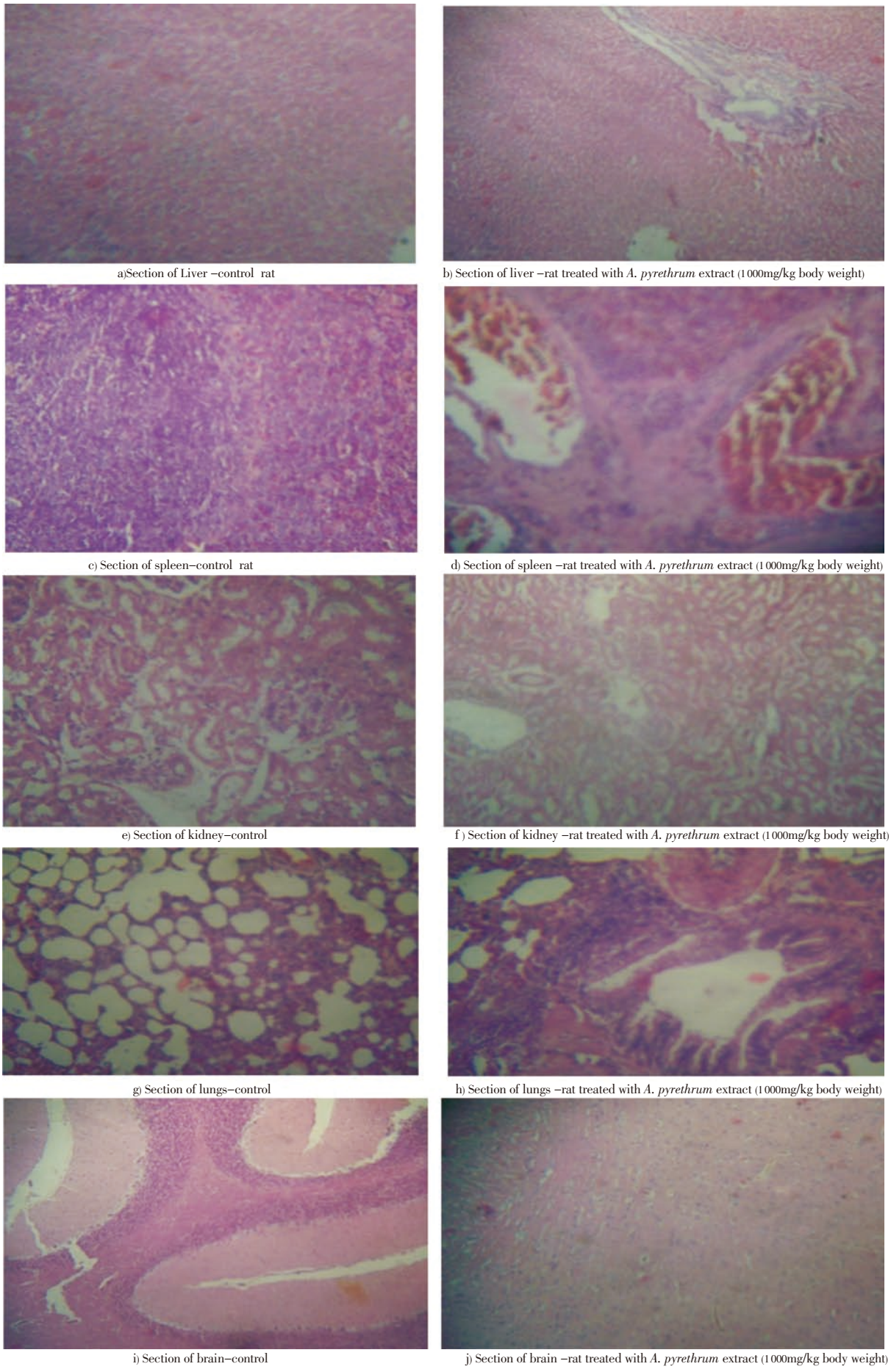


Figure 2. Body weight in treated and control male group during the 90-day safety assessment.



a)Section of Liver –control rat

b) Section of liver –rat treated with *A. pyrethrum* extract (1 000mg/kg body weight)

c) Section of spleen–control rat

d) Section of spleen –rat treated with *A. pyrethrum* extract (1 000mg/kg body weight)

e) Section of kidney–control

f) Section of kidney –rat treated with *A. pyrethrum* extract (1 000mg/kg body weight)

g) Section of lungs–control

h) Section of lungs –rat treated with *A. pyrethrum* extract (1 000mg/kg body weight)

i) Section of brain–control

j) Section of brain –rat treated with *A. pyrethrum* extract (1 000mg/kg body weight)

Figure 3. Histopathological observation of rat organs.

Table 2
Haematological parameters of rats in sub chronic toxicity of ethanolic extract of *A. pyrethrum*.

Haematological parameter	Control		*A. pyrethrum* (1 000 mg/kg)	
	Female	Male	Female	Male
Total R.B.C. count ($\times 10^6$/mm^3)	09.09±1.46	08.13±1.66	8.89±2.05	8.43±1.78
Total W.B.C. Count ($\times 10^3$/mm^3)	13.68±1.97	09.58±1.45	11.29±1.88	12.63±1.26
Haemoglobin (Hb) (g/dl)	15.82±1.94	13.79±1.27	17.62±0.72	16.12±1.33
Hematocrit (%)	42.54±1.36	44.95±1.49	40.12±3.06	41.27±2.47
Platelets ($\times 10^3$/mm^3)	652.34±12.34	961.75±16.64	843.35±15.67	893.74±15.35
Neutrophils (%)	17.79±2.03	20.94±3.11	10.06±2.75	14.12±2.72
Lymphocytes(%)	82.43±3.43	77.56±2.45	79.00±4.55	82.04±3.52
Eosinophil(%)	2.38±0.43	1.82±0.75	1.64±0.25	1.42±0,64
Monocyte(%)	3.10±0.13	0.00±0.00	1.50±0.03	0.00±0.00
Basophil (%)	0.00±0.00	1.01±0.01	0.00±0.00	0.00±0.00

Data are expressed as mean±SEM, n=12.

Table 3
Biochemical parameters of rats in sub chronic toxicity of ethanolic extract of *A. pyrethrum*.

Biochemical parameter	Control		*A. pyrethrum*(1 000 mg/kg)	
	Female	Male	Female	Male
Creatinine (mg/dl)	0.57±0.07	0.43±0.03	0.64±0.01	0.61±0.05
Urea (mg/dl)	17.14±1.52	20.75±1.31	15.17±1.45	18.12±2.53
Triglycerides (mg/dl)	52.83±4.92	47.25±7.34	54.21±9.03	49.24±5.32
Total Cholesterol (mg/dl)	49.33±2.03	43.00±2.46	58.25±3.95	52.17±3.76
Total protein (g/dl)	7.23±0.24	5.11±0.23	3.57±0.14	4.91±0.94
Albumin (g/dl)	3.64±0.05	4.29±0.03	4.27±0.19	3.25±0.12
AST (IU/L)	124.01±17.6	138.54±19.4	119.6±28.8	112.71±23.84
ALT (IU/L)	61.47±3.19	71.33±6.19	63.47±5.61	72.45±4.02
ALP (IU/L)	114.3±12.0	108.4±15.32	97.54±12.76	104.13±13.52
T. Bilirubin (mg/dl)	0.23±0.05	0.27±0.12	0.31±0.09	0.29±0.03

Data are expressed as mean±SEM, n=12.

uniform and were not significantly different. The extract had not show any reduction in the body weight which was an evidence for absence of toxicity.

Relative organ weight of rats treated with *A. pyrethrum* did not show any evidence of drug-related toxicity. There were no significant difference between control and *A. pyrethrum* extract treated groups in organ weight (Table 1).

The effect of *A. pyrethrum* extract administration on haematological parameters of experimental groups and control rats was presented (Table 2). The result indicated that all haematological parameters such as total red blood cell count, total white blood cell count, platelet count, haemoglobin, hematocrit and differential leukocyte count remained within the physiological range in both control and treated group during the experimental period. The data of biochemical parameters in treated and control rats were presented (Table 3). Sub chronic oral administration of *A. pyrethrum* extract did not show any significant changes in biochemical parameters such as creatinine, urea, triglycerides, total cholesterol, total protein, albumin, AST, ALT, ALP and total bilirubin when compared to control groups. There were no statistically significant difference in the hematological and serum biochemical parameters analyzed and are within normal limits.

The histological examination of the various organs was performed in both control and treated groups. All the sampling tissue sections were within the normal limits and revealed normal architecture on comparison with control group. No alterations were seen in the microscopic examination of internal organ and there were no degenerative or infiltrative lesion observed in the extract treated group. Histopathology of treated groups revealed following microscopic observation. Liver section showed normal architecture of hepatocytes, mild sinusoidal dilation and normal vessels. Section of spleen showed nomal white pulp, red pulp, few congested blood vessels and scattered haemosideris. Kidney section showed normal glomeruli, tubules, blood vessels and intestitium. Lungs section showed normal alveoli and septae. The bronchi and bronchioles showed normal lining with peribronchial collections and lymphocytes. Brain Section showed normal cerebellum, focal areas show normal choroid plexus.Pathological examination of tissues indicated that there were no detectable abnormalities (Figure 3).

4. Discussion

Herbal remedies requires an influential and deep evaluation of their efficacies and safety due to their growing use all over the world[23]. The results of the present study indicates that the ethanolic extract of *A. pyrethrum* should be regarded practically as non toxic. Results from the body weight of treated groups when compared to control rats suggest that at sub chronic administration of *A. pyrethrum* had no effect on the normal growth of rats. Change in body weight have been used as an indicator of adverse effect of drugs and chemicals[24]. The haematological system is the one of the most sensitive targets for toxic compounds and important index of physiological and pathological states in men and animals[25]. In the present study, all the haematological parameters remained under the reference

range for the rats in both drug treated and control groups. A similar absence of toxic effects was observed in biochemical parameters. There were no significant effect on the levels of AST, ALT, ALP, bilirubin, urea and creatinine, which are good indicators of liver and kidney functions. This is further confirmed by histological assessment of these organs. There were no treatment related biological significance on adverse effect of *A. pyrethrum* extract on the biochemical parameters in rats. However the values were within the normal laboratory range. Results of histological analysis of internal organs revealed that there were no treatment related histopathological findings. All the findings were consistent with normal background lesions in clinically normal rats of age and strain used in this study. Based on the results no observed adverse effect was concluded to groups treated with *A. pyrethrum* at dose of 1 000mg/kg for 90 days. In conclusion, the sub chronic oral administration of *A. pyrethrum* extract at a dose of 1 000mg/kg has not produced any significant alteration in the hematological, biochemical parameters and histological observation in albino wistar rats. This study provides valuable data on toxicity profile of *A. pyrethrum* roots, which could stand as assurance for medicinal use of this plant for long-term treatment.

Conflict of interest statement

We declare that we have no conflict of interest.

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