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Toxicological Evaluation of *Vaisvanara churna* an Ayurvedic Formulation

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Abstract

Vaisvanara churna an ayurvedic formulation was screened for its toxicity profile. The study was carried out based on CCRAS Protocols. For the acute oral toxicity study, Group I served as vehicle control, *Vaisvanara churna* at a single oral dose of 6500 mg/kg b.w., was administered to 5 male and 5 female Swiss albino mice (Group II) and observed for 14 consecutive days for clinical signs, body weight and mortality. For 90 days repeated dose toxicity study eighty animals (40 male and 40 female) were distributed into four groups. Group I served as vehicle control. Groups II, III, and IV were administered with 450, 2250 and 4500 mg/kg b.w., respectively for 90 days orally. During this period, feed intake, body weight, signs of toxicity, haematological and biochemical parameters were recorded at periodical intervals. After 90 days animals were sacrificed for necropsy, vital organs were weighed and preserved for histopathological analysis. In an acute toxicity study, a single dose of *Vaisvanara churna* at 6500 mg/kg b.w., caused no mortality in animals, suggesting that the median lethal dose is greater than 6500 mg/kg b.w. In 90-day, repeated dose toxicity study, administration of *Vaisvanara churna* for 90 days, up to 4500 mg/kg b.w., caused no significant changes in body weights or organ weights of rats in the treated groups when compared with the control group. In addition, haematological and biochemical parameters were within the physiological limits. Histopathological analysis revealed no signs of degeneration. These results showed that *Vaisvanara churna* is apparently safe as indicated therapeutically for long-term administration for chronic ailments.

Keywords: Acute Toxicity, Chronic Toxicity, Median Lethal Dose, NOAEL, Therapeutic Dose, *Vaisvanara churna*

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1. Introduction

Toxicity studies are done to evaluate either the short or long-term toxic effects of a drug or chemical on animals. Most of the natural products have solid scientific evidence with regard to their biological activities. There is a common perception that all the traditional herbal medicines and their formulations were devoid of toxicity[1]. In the process of healing a particular disease, the traditional healers prepare the formulations by their recipes and dispense them to the patients[2]. To create scientific evidence for their safety profile and increase their consistent usage, there is a necessity for documentation of research work to prove their non-toxic outcomes. Therefore, the traditional use of any medicinal formulation for medicinal purposes should ensure the safety of the formulation. There is a need to raise concern about the potential toxic effects resulting from the short-term and long-term use of such medicinal formulations[3]. To increase confidence in the safety of humans, it is crucial to assess the toxic potential of the formulation.

Vaisvanara churna, an Ayurvedic polyherbal formulation contains four components from herbal plant sources and one mineral component. The herbal materials are *Terminalia chebula* (12 parts), *Zingiber officinalis* (5 parts), *Carum roxburghiyanum* (3 parts), *Carum copticum* (2 parts) and a mineral part *Saindhava lavana* (2 parts). This drug has been claimed for various therapeutic effects that are used to treat rheumatoid arthritis (Amavata), constipation (Vibandha) and other disease conditions[4]. The therapeutic assessment of this Ayurvedic formulation and the safety reports are still unexamined and unreported. In this modern age, scientific evidence is very much essential for better acceptability of a therapeutic agent along with the history of the traditional use of that medicine[5]. So, this study aims to ascertain the safety profile of *Vaisvanara churna*; a valuable Ayurvedic formulation commonly used for various medicinal purposes.

2. Materials and Methods

2.1 Drugs, Chemicals and Reagents

The raw drugs were procured from local market of Chennai, and the polyherbal formulation was prepared in-house as per the Ayurvedic Formulary of India[6]. Commercially available reagents of analytical grade were used for the study.

2.2 Experimental Animals

The experiments were conducted on Swiss albino mice and Wistar rats obtained from the King Institute of Preventive Medicine, Guindy, Chennai. For acute toxicity total of 20 (10 male and 10 female) and 90 days repeated dose oral toxicity study, a total of 80 animals (40 Male + 40 Female), of 8 to 9 weeks of age were selected. They were housed under standard conditions. The body weight variation of the animals selected for the study on the day of randomization was taken care not to exceed ±20% of the mean body weight of each sex. This study was performed as per the CCRAS protocols[7] and confirmed to the care and use of laboratory animals guidelines, Committee for Control and Supervision of Experiments on Animals (CPCSEA), after duly approved by the Institutional Animal Ethics Committee with approval no. of IAEC/CSMDRIAS/04/2014 for acute toxicity study and IAEC/CSMDRIAS/05/2014 for 90 days repeated dose oral toxicity study.

2.3 Acute Toxicity

The oral acute toxicity test was done in male and female albino mice. The animals were randomly distributed into two groups (Groups I and II). Each group consisted of 5 male and 5 female animals each. The control group received lukewarm water 10 ml/kg b.w., and treatment group received *Vaisvanara churna* 6500 mg/kg b.w., suspended in 10 ml lukewarm water.

Animals were deprived of food and water 8 hrs prior to the drug administration. The mice were observed at 0, 30, 60 and 120 min after treatment. The animals were observed for morbidity and mortality once a day for up to 14 days. The percentage change in body weight at weekly interval with respect to day 1 was recorded. The toxicological effect was assessed on the basis of mortality, which was expressed as Maximum Lethal Dose[8].

2.4 90 Days Repeated Dose Oral Toxicity Study

Eighty wistar rats of 40 male and 40 female were divided into four groups of 10 male and 10 female in each group. Group I served as Vehicle control, Groups II, III and IV were assigned for drug treatment of low, mid and high dose groups. The vehicle control used in this study is lukewarm water which is the anupana (terminology similar to vehicle in Ayurveda) for *Vaisvanara churna* for Amavata treatment. Immediately before administration

the drug was mixed with vehicle at constant temperature of 40˚C on hot plate under constant stirring (250 rpm).

The control group received the vehicle (lukewarm water 10 ml/kg b.w.), and the others received increasing oral doses of *Vaisvanara churna* (450, 2250, 4500 mg/kg b.w.,) suspended in 10 ml of lukewarm water. The doses were selected based on the converted rat doses from human therapeutic dose as low dose (therapeutic dose), mid dose (5 times Therapeutic dose) and high dose (10 times Therapeutic dose). These doses were selected as per the traditional claims in Ayurveda. Except dose, all other parameters in the study are in par with the OECD guidelines 408[9]. The test item *Vaisvanara churna* and vehicle were administered to the animals once daily up to 90 days by oral (gavage) route using appropriate graduated disposable syringe.

Animals were observed daily for clinical signs and behavioural pattern. Feed intake and body weight were monitored weekly once. Hematology, plasma biochemistry and urine analysis were carried out at 30 days interval up to 90 days. Before each blood collection for hematology and biochemistry analysis, feed was withheld overnight, and blood samples were collected from the rat retro orbital plexus under mild isoflurane anaesthesia. For hematology parameters, blood was collected in K2-EDTA coated tubes and were determined by using an automatic Digital Cell Counter (MS9-3) Melet Schloesing Laboratories, France. For clinical biochemistry parameters blood was collected in plain tubes containing heparin (20µl/ml blood; 5,000 IU heparin/ml saline) and were centrifuged at 3500 rpm for 10 min and estimated using an automated Clinical Chemistry Analyzer (XL 640) Transasia Bio Medicals Ltd, India. Urine collection was carried out as a pooled sample of 3 animals/group in metabolic cages (Techni plast) for 16 hrs. Feed was withheld during this period. To avoid microbial contamination small quantity of thymol was added to the urine collecting tubes. Urine analysis of samples were performed by using Uro-dipcheck 300 automated urine analyzer, Erba Mannheim, Germany.

After 90th day blood collection, animals were sacrificed for gross necropsy and histopathology. Vital organs such as heart, lungs, liver, kidneys, spleen, adrenals, testes, brain, ovaries with oviducts, thymus, uterus (with cervix), epididymis were carefully dissected, washed with normal physiological buffer (Phosphate buffered saline pH 7.4) and weighed. Selected organs were preserved in 10% neutral formal saline for histo pathology analysis.

2.5 Statistical Analysis

Numerical data are presented as mean ± S.D. Differences between groups were analyzed by Student t test to determine the value of significance in acute toxicity study and One way Analysis of Variance (ANOVA) followed by Dunnett's multiple comparisons test for 90 days repeated dose oral toxicity study. Minimum criteria for statistical significance were considered at $p < 0.05$ for all comparisons.

3. Results

3.1 Acute Toxicity Study

No mortality and morbidity or any signs of behavioural changes or toxicity were observed throughout the 14-day period after single oral administration of *Vaisvanara churna* up to the dose level of 6500 mg/kg b.w. Morphological characteristics (fur, skin, eyes, and nose) appeared normal. There was no significant change in body weight between the control and treatment groups. The change in body weights were represented in Table 1.

Table 1. Body weight (g) change in male and female mice during 14 days acute toxicity study

Treatment	Male		Female	
	Group I (Vehicle 10 ml/kg b.w.)	**Group II (*Vaisvanara churna* 6500mg/kg b.w.)**	**Group I (Vehicle 10 ml/kg b.w.)**	**Group II (*Vaisvanara churna* 6500mg/kg b.w.)**
Day 1	32.9 ± 1.65	33.0 ± 2.16	26.6 ± 2.10	26.6 ± 1.64
Day 7	35.0 ± 2.29 *(4.7)*	31.1 ± 2.32 *(-5.7)*	27.5 ± 2.55 *(3.2)*	27.3 ± 1.82 *(2.6)*
Day 14	36.7 ± 2.25 *(9.4)*	33.6 ± 3.09 *(1.8)*	26.9 ± 2.10 *(1.0)*	26.5 ± 2.00 *(-0.1)*

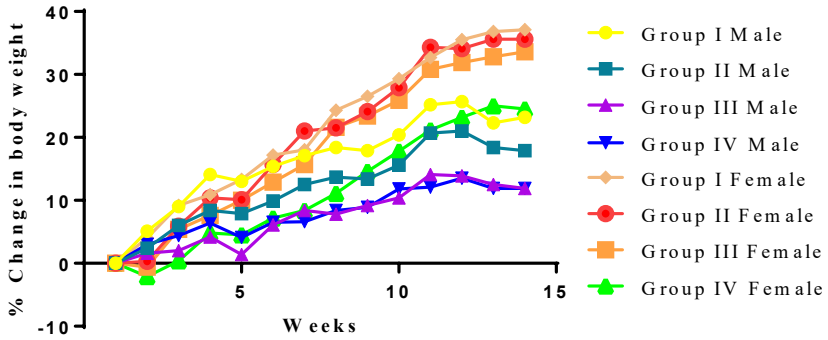
Values are mean ± SD of respective group of animals, n = 5. Values given in parenthesis are the percentage change in body weight with respect to Day 1.

Values are non-significant in comparison with control group (Group I) student. T test used to determine the value of significance.

3.2 90 Days Repeated Dose Oral Toxicity Study

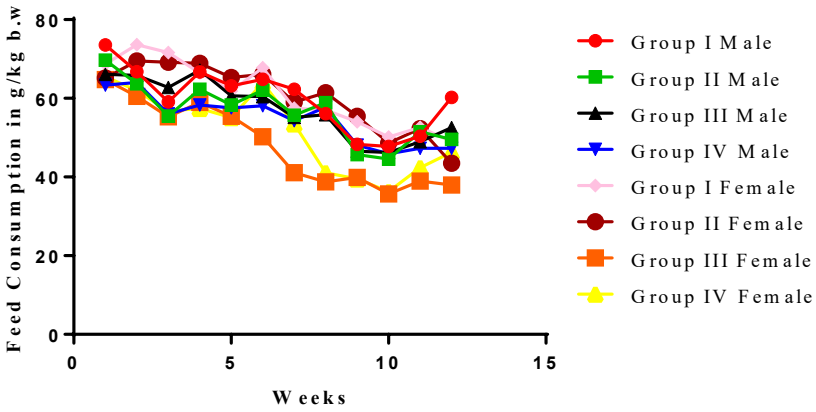
3.2.1 Mortality, Body Weight and Feed Consumption

The animals were healthy with no differences being noted with respect to the control group. No significant changes were observed in the body weight of the treated groups as compared to control (Figure 1) and no mortality was observed during the experimental procedures. No significant change in feed consumption of the treated groups as compared to the control group (Figure 2).



Group I - Vehicle Control (10 ml/kg)
Group II - Low dose group (450 mg/kg)
Group III - Mid dose group (2250 mg/kg)
Group IV - High dose group (4500 mg/kg)

Figure 1. Percentage change in body weight of rats



Group I - Vehicle Control (10 ml/kg)
Group II - Low dose group (450 mg/kg)
Group III - Mid dose group (2250 mg/kg)
Group IV - High dose group (4500 mg/kg)

Figure 2. Feed consumption in rats

3.2.2 Haematology

Results of the haematological parameters viz., Total White Blood Cell count (WBC), Red Blood Cell count (RBC), Haemoglobin concentration (HGB), Platelet count (PLT), Differential Leucocytes Count (DLC) were given in Table 2. No significant difference was observed in haematological parameters of *Vaisvanara churna* treated animals, when compared with control animals on 90 days repeated dose oral toxicity study of *Vaisvanara churna*.

3.2.3 Biochemical Parameters

The results of Biochemical profile viz, Cholesterol (mg/dl), Triglycerides (mg/dl) AST (U/L), ALT (U/L), ALP (U/L), were given in Table 3. No significant difference was observed in biochemical parameters of *Vaisvanara churna* treated animals when compared with control groups on 90 days repeated dose oral toxicity study of *Vaisvanara churna.*

Table 2. Haematological observations of 90 days repeated dose oral toxicity study of *Vaisvanara churna* in male and female rats

Parameters	Units	Group I		Group II		Group III		Group IV	
		Male	Female	Male	Female	Male	Female	Male	Female
30 Days									
RBC	**(10^6/mm^3)**	8.13 ± 0.26	7.60 ± 0.81	8.07 ± 0.21	7.56 ± 0.53	7.96 ± 0.34	7.43 ± 0.41	7.78 ± 0.46	8.21 ± 0.96
WBC	**(10^3/mm^3)**	14.74±3.52	9.82±1.82	12.73±1.22	11.38±2.43	14.41±2.01	13.54±2.61	13.76±3.70	12.19±1.94
Hb	**(g/dl)**	15.14 ± 0.46	14.50 ± 1.59	14.81 ± 0.42	14.67 ± 1.03	14.64 ± 0.64	14.00 ± 0.50	14.52 ± 0.88	15.57 ± 1.88
Lymphocyte	**(%)**	76.42 ± 3.86	75.74 ± 4.59	75.79 ± 3.72	77.65 ± 5.13	78.20 ± 4.48	79.77 ± 1.94	76.19 ± 3.81	72.20 ± 2.21
Monocyte	**(%)**	6.77 ± 0.92	6.47 ± 1.83	7.06 ± 0.79	6.53 ± 0.93	7.31 ± 1.24	6.81 ± 0.87	7.21 ± 1.70	6.30 ± 1.29
N/gr	**(%)**	16.80 ± 3.91	17.79 ± 4.08	17.16 ± 3.38	15.81 ± 4.77	14.50 ± 3.39	13.40 ± 1.75	16.60 ± 4.48	21.51 ± 2.07
Platelet count	**(10^5/mm^3)**	7.75 ±0.58	5.76±1.43	8.08 ± 0.90	6.23±0.77	8.00 ± 1.24	6.97±0.77	7.97 ± 1.63	6.47±0.75
60 Days									
RBC	**(10^6/mm^3)**	7.13 ± 0.26	6.13 ± 0.26	9.07 ± 0.21	9.07 ± 0.21	8.96 ± 0.34	8.96 ± 0.34	6.78 ± 0.46	7.78 ± 0.46
WBC	**(10^3/mm^3)**	7.82±1.52	18.74±3.52	13.38±2.43	13.73±1.22	12.54±2.61	15.41±2.01	11.19±1.94	13.76±3.70
Hb	**(g/dl)**	15.14 ± 0.46	13.14 ± 0.46	15.81 ± 0.42	12.81 ± 0.42	14.64 ± 0.64	14.64 ± 0.64	14.52 ± 0.88	14.52 ± 0.88
Lymphocyte	**(%)**	78.74 ± 4.59	79.42 ± 3.86	75.65 ± 5.13	75.79 ± 3.72	81.77 ± 1.54	78.20 ± 4.48	76.20 ± 12.21	77.19 ± 3.81
Monocyte	**(%)**	5.47 ± 1.83	2.77 ± 0.92	8.53 ± 0.93	10.06 ± 0.79	7.81 ± 0.87	8.31 ± 1.24	4.30 ± 1.29	9.21 ± 1.70
N/gr	**(%)**	13.79 ± 4.08	16.80 ± 3.91	15.21 ± 4.77	15.16 ± 3.38	13.40 ± 1.55	14.50 ± 3.39	21.51 ± 1.07	16.60 ± 4.48
Platelet count	**(10^5/mm^3)**	7.95 ± 0.58	6.75 ± 0.58	7.08 ± 0. 90	8.08 ± 0.90	9.0 ± 1.24	9.00 ± 1.24	8.97 ± 1.63	7.97 ± 1.63
90 Days									
RBC	**(10^6/mm^3)**	6.13±0.16	7.13 ± 0.26	9.07 ± 0.21	9.07 ± 0.21	8.96 ± 0.34	7.16 ± 0.34	7.78 ± 0.46	8.78 ± 0.46
WBC	**(10^3/mm^3)**	12.34±3.52	13.74±3.52	10.73±1.22	12.73±1.22	14.41±2.01	16.41±2.01	13.76±3.70	13.76±3.70
Hb	**(g/dl)**	13.14 ± 0.46	13.14 ± 0.46	12.81 ± 0.42	13.81 ± 0.42	14.64 ± 0.64	14.04 ± 0.64	14.52 ± 0.88	16.52 ± 0.88
Lymphocyte	**(%)**	79.42 ± 3.86	78.42 ± 3.86	75.79 ± 3.72	75.79 ± 3.72	78.20 ± 4.48	78.20 ± 4.48	77.19 ± 3.81	76.19 ± 3.81
Monocyte	**(%)**	2.77 ± 0.92	8.77 ± 0.92	10.06 ± 0.79	7.06 ± 0.79	8.31 ± 1.24	7.01 ± 1.24	9.21 ± 1.70	6.21 ± 1.70
N/gr	**(%)**	16.80 ± 3.91	17.80 ± 3.91	15.16 ± 3.38	17.16 ± 3.38	14.50 ± 3.39	14.50 ± 3.39	16.60 ± 4.48	16.60 ± 4.48
Platelet count	**(10^5/mm^3)**	6.75 ± 0.58	8.56 ± 0.48	8.08 ±0.90	7.28 ± 0.90	9.0 ± 1.24	7.56 ± 1.24	7.97± 1.63	7.49 ± 1.63

Values are mean ± SD of respective group of animals, n = 20. Values are non-significant in comparison with control group (Group I) and fall within the normal physiological range.

One way Analysis of Variance (ANOVA) followed by Dunnett's multiple comparisons test was used for comparison.

Table 3. Plasma Biochemical Profile of 90 days repeated dose oral toxicity study of *Vaisvanara churna* in male and female rats

Parameters	Units	Group I		Group II		Group III		Group IV	
		Male	Female	Male	Female	Male	Female	Male	Female
30 Days									
ALT	**(U/L)**	86.10 ± 5.07	90.00 ± 9.09	86.00 ± 7.11	95.00 ± 11.31	86.75 ± 8.46	98.14 ± 14.69	81.75 ± 27.49	102.56 ± 15.23
AST	**(U/L)**	105.60 ± 16.15	103.70 ± 13.12	104.67 ± 12.70	113.10 ± 22.66	104.50 ± 12.80	112.29 ± 18.86	109.22 ± 16.24	124.56 ± 20.36
ALP	**(U/L)**	492.10 ±191.72	355.10 ± 85.91	520.67 ± 115.39	384.40 ±135.29	446.88 ±139.15	365.57 ± 89.77	420.78 ± 116.93	350.33 ± 100.04
Cholesterol	**(mg/dl)**	55.00 ± 8.31	64.10 ± 7.62	55.67 ± 6.63	66.50 ± 13.48	57.88 ± 3.52	72.00 ± 6.22	57.56 ± 4.42	64.11 ± 10.15
Triglycerides	**(mg/dl)**	84.30 ± 15.92	122.10 ± 37.15	83.67 ± 16.09	116.70 ± 32.01	93.75 ± 27.01	112.43 ± 40.48	82.56 ± 28.57	119.33 ± 39.29
60 Days									
ALT	**(U/L)**	96.52 ± 11.08	103.88 ± 18.35	102.26 ± 13.13	102.64 ± 10.96	104.80 ± 11.00	98.27 ± 9.03	89.04 ± 10.80	95.81 ± 14.10
AST	**(U/L)**	96.59 ± 13.00	104.49 ± 11.33	104.29 ± 16.87	103.20 ± 12.62	102.49 ± 15.33	103.67 ± 16.69	99.96 ± 11.42	112.93 ± 22.27
ALP	**(U/L)**	268.50 ± 60.94	209.50 ± 41.78	295.00 ± 79.83	242.50 ± 84.92	263.44 ± 53.89	198.57± 38.91	260.89 ± 35.69	253.14± 49.13
Cholesterol	**(mg/dl)**	44.10 ± 8.29	48.70 ± 5.81	43.33 ± 4.87	56.50 ± 11.58	42.67 ± 4.97	55.29 ± 5.19	42.33 ± 6.52	52.57 ± 11.49
Triglycerides	**(mg/dl)**	66.80 ± 19.83	99.30 ± 20.39	63.56 ± 12.20	99.40 ± 26.31	61.88 ± 10.30	116.29 ± 28.09	70.67 ± 17.23	87.71 ± 26.42
90 Days									
ALT	**(U/L)**	92.77 ± 14.82	105.09 ± 17.39	108.06 ± 30.45	100.78 ± 11.94	91.57 ± 10.31	93.43 ± 8.35	93.99 ± 3.96	92.43 ± 9.65
AST	**(U/L)**	96.72 ± 15.78	99.32 ± 15.52	98.97 ± 16.16	84.13 ± 15.17	95.50 ± 23.25	86.20 ± 13.75	97.00 ± 9.25	93.66 ± 12.97
ALP	**(U/L)**	226.80 ± 50.00	184.40 ± 24.38	206.00 ± 59.82	183.60 ± 68.08	232.44 ± 49.72	200.86 ± 37.32	257.56 ± 33.49	192.43 ± 40.83
Cholesterol	**(mg/dl)**	45.00 ± 6.65	51.20 ± 6.32	48.00 ± 5.96	57.40 ± 9.24	47.56 ± 5.88	59.43 ± 7.93	46.89 ± 3.44	55.00 ± 8.70
Triglycerides	**(mg/dl)**	81.40 ± 18.12	86.80 ± 19.53	84.33 ± 17.66	85.10 ± 18.40	81.33 ± 20.48	77.00 ± 17.24	65.78 ± 14.21	71.71 ± 27.48

Values are mean ± SD of respective group of animals, n = 20. Values are non-significant in comparison with control group (Group I) and fall within the normal physiological range

One way Analysis of Variance (ANOVA) followed by Dunnett's multiple comparisons test was used for comparison.

3.2.4 Absolute and Relative Organ Weight

Vaisvanara churna treated animals did not produce any significant change in organ weight (absolute and relative with respect to body weight and brain weight) of adrenals, liver, testes, brain, ovaries with oviducts, thymus, heart, uterus (with cervix), kidneys, spleen, epididymis when compared with control animals.

3.2.5 Urine Analysis

Urine analysis and microscopic examination of urine sediments of *Vaisvanara churna* treated animals did not exhibit any significant change when compared with control animals.

3.2.6 Histopathology

No abnormalities were detected in histopathological examinations of the tissues during microscopic examination of internal organs in tissues of control and test animals. *Vaisvanara churna* did not affect the histology of vital organs, viz., lungs, heart, spleen and liver (Figure 3).

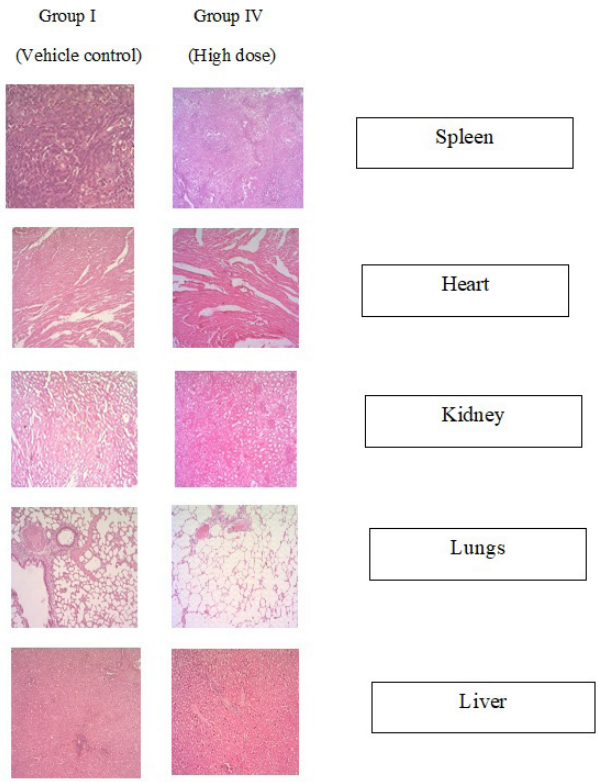


Figure 3. Histopathology of *Vaisvanara churna* on 90 days repeated dose oral toxicity study (10 x 10 X magnification).

4. Discussion

In the acute toxicity study, there was no mortality up to a maximum dose of 6500 mg/kg b.w., of *Vaisvanara churna* per oral administration. There was no significant change in the body weight of treated animals when compared with the control. The changes in the body weight have been used as an indicator of adverse effect[10]. In 90-day repeated dose, oral toxicity study showed no remarkable changes in animal behaviour, body weight, feed consumption, and organ weight at all dose levels in treated animals as compared to the control group. It can be inferred that *Vaisvanara churna* is nontoxic at all the administered dose levels. Based on the data analysis of animal blood parameters it can be observed that the facts can be translated for humans as well since changes in the haematological system have a predictive value for human toxicity[11]. However, the study showed that there are no significant changes in the haematological parameters between the control and treated groups. Transaminases

(AST and ALT) and ALPs are generally used as indices for liver and kidney damage, respectively[12,13]. No significant change was found in plasma levels of AST, ALT, and ALP enzymes. *Vaisvanara churna* did not provoke any detrimental effect on the liver and kidneys. After statistical interpretation of the results of the haemaotological and biochemical analysis showed that the values are within the normal physiological range, and it is observed that there is no drug-induced toxic effects. The non-toxicity of *Vaisvanara churna* on specific organs was further confirmed by histopathological assessment. Histopathological examination of selected vital organs (heart, lung, liver, kidney, and spleen) from both treated and control animals showed normal architecture, suggesting no microscopic changes were observed at all dose levels of *Vaisvanara churna*.

5. Conclusion

These results showed that *Vaisvanara churna* in acute toxicity study at the dose of 6500 mg/kg b.w. administered orally was considered to be safe, and 90 days repeated oral dose toxicity study up to 4500 mg/kg b.w., did not cause any adverse effects as indicated therapeutically for long term administration for specific chronic ailments. This article documented the safety profile of ayurvedic polyherbal formulation Vaisvanara churna for its intended long term use in specific ailments.

6. Acknowledgement

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