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Pre Clinical Safety Assessment of AYUSH Carctol S through Acute and Sub Chronic Oral Toxicity Study: Safety Assessment of AYUSH Carctol S

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Abstract

AYUSH Carctol S is a coded *Ayurvedic* compound formulation developed for treatment of cancer. The safety of the test drug was not established in the experimental animals yet, hence the present study was highlighted the same through acute and sub-chronic toxicity studies. The acute oral toxicity study of the test drug was performed in swiss albino mice with a dose of 2600 mg/kg. Similarly, the sub-chronic oral toxicity study was carried out in wistar rats with three different test doses *i.e.,* 180 mg/kg, 900 mg/kg, and 1800 mg/kg. Cage side observations, feed intake, body weight, gross morphology, and necropsy findings of the animals of test drug-treated animals were normal and no mortality was reported in both either study. During the sub-chronic toxicity study, the hematological and biochemical parameters of AYUSH Carctol S-treated animals were found to be non-significant when compared to the control animals. Further, the histopathological analysis of major organs of AYUSH Carctol S 1800 mg/kg treated animals showed no major lesions and treatment-related changes. The test drug was considered to be nontoxic during the acute and sub-chronic oral toxicity studies. The LD_{50} of AYUSH Carctol S estimated during the acute toxicity was found as 2600 mg/kg and the sub-chronic safety dose *i.e.,* NOAEL is up to 1800 mg/kg.

Keywords: Acute Toxicity, AYUSH Carctol S, LD_{50}, NOAEL, Subchronic Oral Toxicity

1. Introduction

The Indian *Ayurvedic* traditional medicinal system is accepted globally primarily due to its holistic therapeutic approach, fewer adverse effects, and better therapeutic outcome[1]. *Ayurveda* focuses individualization of therapy concerning bio-identity, socio-economic status, and biochemical and physiological conditions, which are associated with a particular type of disease. Despite these facts, *Ayurveda* requires further exploration in

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terms of modern scientific approaches for addressing various healthcare issues[2]. There are numerous amount of drugs and chemicals are used worldwide in all the systems of medicine for the treatment of diseases[3]. However, the substances that were used for the long term may associated with chronic toxicity in the living system. Similarly, acute toxicity is associated with a high quantity of drug consumption capable of producing immediate toxic effects. The adverse effects may be mild or severe, depending on the characteristics of the drug[4]. In this context preclinical toxicity studies on *Ayurvedic* formulations may provide vital scientific information regarding their safety. Establishing the safety profile of *Ayurvedic* and herbal formulations will not only minimize the risk of adverse outcomes but also maximizes the clinical benefits[5]. Nonclinical assessment of drug toxicity typically consists of definitive animal toxicology investigations. Animal toxicity studies are required to be carried out to evaluate the potential unwanted pharma co-dynamic effects of drugs on anatomic functions concerning susceptibility within the therapeutic limit[6]. The assessment of the Lethal Dose (LD_{50}) has now been used as a major parameter in measuring acute toxicity[7]. Similarly, NOAEL (No Observed Adverse Effect Level) is a key parameter for determining the long-term toxicity in experimental animals[8]. AYUSH-carctol is a coded *Ayurvedic* formulation formulated by Nandanlal Tiwari Pharmaceutcials, Jaipur for the treatment of Cancer, it was further developed by CCRAS. Safety of this compound formulation was not established preclinically hence the current study focused on the same.The study was conducted as per the guidelines prescribed by CCRAS (Central Council for Research in Ayurvedic Sciences).

2. Materials and Methods

2.1 Animal Welfare

The study was approved by IAEC (Proposal No. 72 Pharma /SCRI/2009/ dated 05-12-2009). Animals were maintained as per the CPCSEA guidelines.

2.2 Vehicle

Milk was used as a vehicle for the formulation preparation.

2.3 Details of Dose Calculation

Human dose of the test drug 2000 mg once a day. Based on the conversion factors for rats and mice, the therapeutic dose of mice was found to be 260 mg/kg in mice, 10 times of therapeutic dose 2600 mg/kg was selected as dose for acute toxicity testing in mice. Similarly the therapeutic dose of 180 mg/kg in rats was selected as low dose, 5 times higher (900 mg/kg) and 10 times higher (1800 mg/kg) were choosed as average and high dose respectively for repeated dose toxicity studies.

2.4 Acute Toxicity Study

2.4.1 Test System

Albino mice of 8 to 9 weeks old, males weighing 30-40 g and females weighing 25-35 g were used. Only nulliparous and non-pregnant females are used in the experiment. A total of 20 mice (10 males + 10 females) were selected based on their body weight and randomly distributed into 2 dose groups (Group I and II). Each group had 5 animals/sex. 2600 mg/kg (10 times higher than the therapeutic dose) was used as the test dose.

2.4.2 Observations

Mice were examined for clinical signs and mortality after dosing, at 5-10 min, 30-45 min, 1 (±10 min), 2 (±10 min), 4 (±10 min), 6 (±10 min), and 24 (±10 min) hr post dosing followed by once daily throughout the 14 Days.

2.4.3 Body Weight

The body weight of the animals was recorded on the day of receipt, randomization, and on treatment day 1 and post-treatment days 7 and 14.

2.5 Subchronic Oral Toxicity

2.5.1 Test System

A total of 80 wistar rats (40 male and 40 female) of 6 to 7 weeks of age, were received from Animal Breeding Station, Tanuvas, Chennai, Tamil Nadu. The body weight variation of the animals selected for the study on the day of randomization did not exceed ± 20 % of the mean body weight of each sex. Female rats are nulliporus and non pregnant. Based on the body weight and randomly distributed to 4 Groups (Group I, II, III, and IV). Each group consisted of 20 animals each.

Group I: Vehicle Control (VC) – Milk

Group II: Therapeutic Dose (TH) – 180 mg/kg

Group III: 5 times Therapeutic Dose (5 TD) – 900 mg/kg

Group IV:10 times Therapeutic Dose (10 TD) – 1800 mg/kg

The test item AYUSH carctrol and vehicle (Milk) was administered to the animals once daily for up to 90 days by oral (gavage) route using an appropriate graduated disposable syringe and rat gavaging cannula. The animals from Groups II, III, and IV received test items whereas the animals from Group I were dosed with vehicle control.

2.5.2 Cage Side Clinical Signs

All the animals were examined for cage-side clinical signs once daily except on days of detailed clinical signs observations *viz.* salivation, fur, lacrimation, *etc.*

2.5.3 Mortality

Mortality and morbidity were recorded twice daily.

2.5.4 Body Weight

The body weight of the animals was recorded on the day of receipt, randomized weekly thereafter, and a fasting body weight on day 90 for study animals.

2.5.5 Feed Consumption

Feed consumption was recorded once weekly throughout the study for study animals.

2.5.6 Hematology

The hematology parameters *viz.*, total White Blood Cell count (WBC), total Red Blood Cell count (RBC), Hemoglobin concentration (HGB), Hematocrit (HCT), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), Platelet ount (PLT) and Differential Leucocytes Count (DLC) were analyzed.

2.5.7 Clinical Chemistry

The clinical biochemistry parameters were estimated by an aurtomated clinical chemistry analyzer.

2.5.8 Urinalysis

Animals were shifted to metabolic cages for urine collection for 14 to 16 hours during weeks 4, 8, and 12 for the main study animals. Feed was withheld, but water was provided *ad libitum* to all the animals during the phase of urine collection. The urine parameters *viz.* color, specific gravity, glucose, bilirubin, ketones, occult blood, pH, protein, urobilinogen, nitrite, and leukocytes were estimated by uristik.

2.5.9 Necropsy

All surviving animals were euthanized after the study period using ether anesthesia followed by cervical dislocation. A complete necropsy was carried out on all animals. All the vital organs were collected, weighed from each animal preserved in 10% neutral buffered formalin, and subjected to histopathology.

2.5.10 Histopathology

The organs or tissues collected from the vehicle control (Group I), high dose (Group IV) of the main study animals and gross lesions were trimmed, processed, embedded in paraffin blocks, sectioned, and stained with hematoxylin and eosin. The histopathology examination was carried out on all organs and tissues collected for vehicle control (Group I) and high dose (Group IV) of main study animals of both the sexes and gross lesions. Histopathology evaluations will be not performed for Groups II and III of the main study if no treatment-related histopathology changes are noticed in high-dose group animals.

2.6 Statistical Analysis

Data was expressed as mean ±SD and analyzed through t- a t-test for acute toxicity studies, One-way ANOVA for 90 days toxicity by using graph pad prism 8.4.2 software. p <0. 05 was considered statistically significant.

3. Results

3.1 Acute Toxicity

No mortality and moribund stage was observed with treatment.

There was no significant change in body weight and feed intake of AYUSH Carctol S treated animals when compared to the control (p>0.05. (Figure 1, 2).

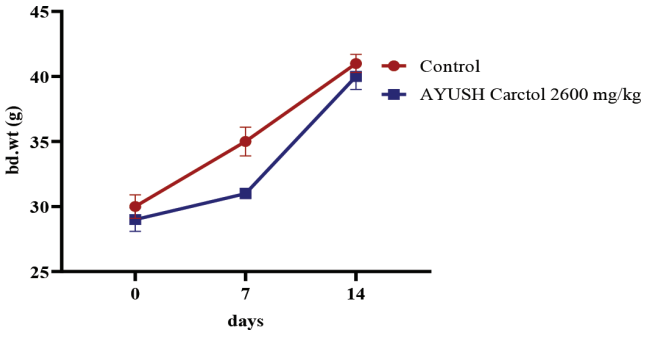


Figure 1. Body weight-acute toxicity.

3.2 Necropsy

After completion of 14 days, the overnight fasted animals were sacrificed on day 15th by Co2 asphyxiation. The gross necropsy findings were normal in AYUSH Carctol S treated animals and the organ weights of the same were not significantly changed as compared to the control group animals ($p>0.05$) (represented in Figure 3).

3.3 Subchronic Toxicity

The clinical signs of test drug-treated animals didn't show any alterations compared to the control and no treatment-related mortality was seen throughout the study period

Body weights of the AYUSH Carctol S treated animals didn't change significantly throughout the study period as compared to the vehicle control animals ($p>0.05$) and shown in Table 1.

The weekly feed intake of AYUSH Carctol S treated animals was found to be normal. The results of feed consumption didn't show any significant changes as compared to the vehicle control ($p>0.05$) and values in tabulated form are mentioned in Table 2.

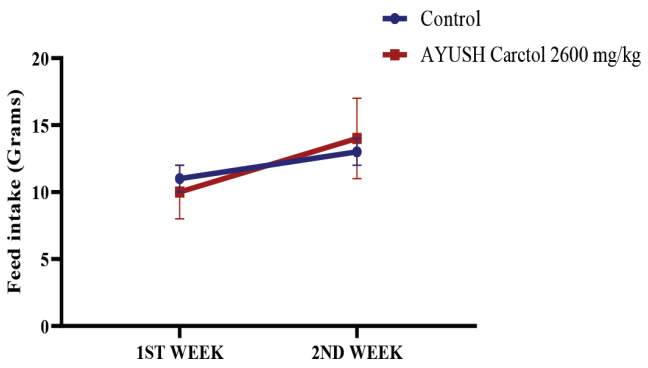


Figure 2. Feed consumption -acute toxicity.

3.4 Hematology

The hematological parameters such as RBC, WBC, platelet count as well as other indices were found to be within the range ($p>0.05$) and data is mentioned in Table 3.

3.5 Clinical Chemistry

All the serum biochemical parameters of carctrol-treated animals were not significantly different as compared to the control ($p>0.05$) and shown in Table 4.

3.6 Gross Necropsy and Relative Organ Weights

No gross lesions were found in the animals treated with the test drug. The organ weights did not show any significant difference when compared to the control ($p>0.05$) and mentioned in Table 5.

3.7 Urine Analysis

Urine analysis data of the AYUSH Carctol S treated animals did not show any noticeable changes as compared to the control and dtat is presented in Table 6.

3.8 Histopathology

The histological section of the adrenal, brain, eye, heart, ileum, intestine, kidney, liver, ovary, pancreas, sciatic nerve, spleen, stomach, and thymus in all treated groups showed normal architecture. No abnormalities are marked in the highest dose treated animals (Figure 4-6).

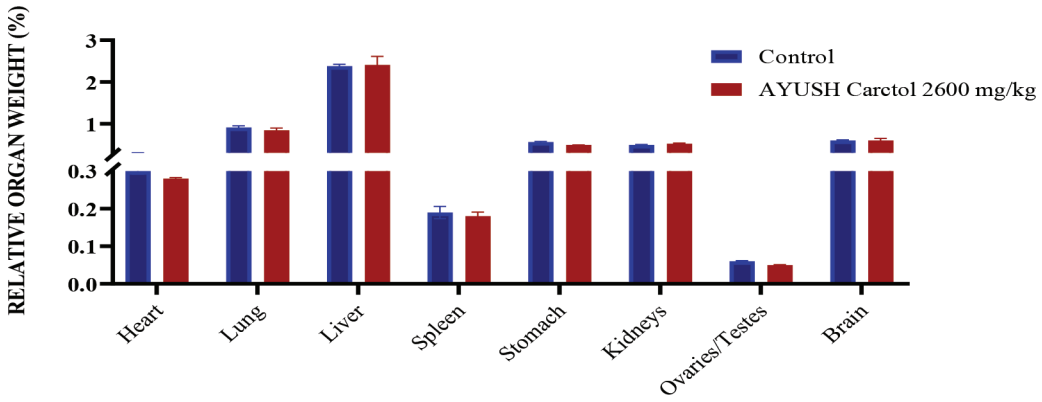


Figure 3. Relative organ weight-acute toxicity.

Table 1. Body weight in grams (weekly) of AYUSH Carctol S

Group	0th Day	1st week	2nd week	3rd week	4th week	5th week	6th week	7th week	8th week	9th week	10th week	11th week	12th week	13th week
Males														
Control	218± 18.73	225± 18.40	248± 18.13	267.5± 20.84	278.5± 17.16	282± 15.12	279± 6.14	283.5± 8.51	281.5± 10.81	289.5± 9.26	297± 7.88	303± 13.58	299.5± 39.04	313± 13.98
180 mg/ kg	160.5± 48.04 ns	163.5± 46.78 ns	184± 38.06 ns	197± 37.28 ns	201.5± 34.72 ns	202± 35.52 ns	209.5± 37.00 ns	221± 39.70 ns	228.5± 42.49 ns	237.5± 43.92 ns	234.5± 39.26 ns	252.5± 45.71 ns	269.5± 40.30 ns	279.5± 38.90 ns
900 mg/ kg	128.88± 29.34 ns	132.22± 28.95 ns	142.22± 29.90 ns	158.88± 31.10 ns	174.44± 30.04 ns	188.88± 25.46 ns	200± 27.27 ns	212.77± 26.93 ns	225.55± 35.74 ns	234.44± 36.69 ns	243.88± 46.08 ns	250.55± 37.86 ns	258.88± 35.06 ns	271.11± 33.79 ns
1800mg/ kg	184± 14.29 ns	189± 14.10 ns	201.5± 12.70 ns	211.5± 9.73 ns	226± 9.66 ns	239± 10.74 ns	247.5± 7.16 ns	244.5± 5.98 ns	238.5± 6.25 ns	229± 5.67 ns	231.5± 8.18 ns	221± 12.86 ns	218.5± 19.86 ns	226.5± 22.49 ns
Females														
Control	148.5± 22.85	151± 23.30	162.5± 24.74	171.5± 24.5	176.5± 20.55	184± 20.11	188± 17.51	189.5± 14.61	191.5± 16.16	198.5± 16.16	197± 13.98	202.5± 15.50	213± 18.28	222± 18.28
180 mg/ kg	135± 19.57 ns	140± 19.86 ns	150.5± 18.02 ns	160.5± 16.74 ns	163.5± 10.55 ns	170.5± 5.98 ns	175.5± 12.34 ns	179± 12.2 ns	184± 12.64 ns	188.5± 13.13 ns	194.5± 15.53 ns	196.5± 17.48 ns	196± 16.79 ns	201.5± 15.99 ns
900 mg/ kg	154.5± 29.0 ns	157± 25.62 ns	166± 21.7 ns	180± 16.66 ns	182.5± 10.34 ns	191± 5.67 ns	190± 12.47 ns	200± 12.47 ns	210± 17.63 ns	216.5± 21.47 ns	225± 25.05 ns	230.5± 31.83 ns	241.5± 32.32 ns	240.5± 31.04 ns
1800 mg/ kg	144.5± 27.53 ns	150.5± 24.99 ns	160.5± 14.42 ns	170± 10.54 ns	176± 10.48 ns	175.5± 9.84 ns	171.5± 9.44 ns	178± 8.23 ns	180± 4.71 ns	185± 7.45 ns	190.5± 6.43 ns	191.5± 8.31 ns	194± 12.42 ns	198± 18.73 ns

Data was expressed as Mean±SD, N=10, the level of significance was expressed as[ns]-non-significant

Table 2. Feed consumption in grams (weekly) of AYUSH Carctol S

Group	0th Day	1st week	2nd week	3rd week	4th week	5th week	6th week	7th week	8th week	9th week	10th week	11th week	12th week
Males													
Control	10.7± 3.52	13.3± 2.98	11.6± 2.41	14.6± 2.63	12± 2.49	9.8± 2.20	14± 3.23	12.5± 2.54	12.2± 2.52	11.8± 2.93	14.5± 3.80	12.8± 2.85	11.4± 1.71
180 mg/kg	9.8± 3.04^{ns}	10.4± 1.57^{ns}	12.7± 2.4^{ns}	11.9± 2.51^{ns}	12.1± 3.21^{ns}	10.8± 2.34^{ns}	13.6± 2.91^{ns}	12.4± 2.83^{ns}	10.8± 2.29^{ns}	12.6± 2.98^{ns}	13± 4.24^{ns}	13.5± 3.43^{ns}	13.3± 3.94^{ns}
900 mg/kg	10± 3.12^{ns}	9.8± 3.19^{ns}	14.77± 3.23^{ns}	14.44± 2.69^{ns}	15.44± 2.45^{ns}	10± 2.64^{ns}	12.55± 2.29^{ns}	9.11± 2.66^{ns}	10.88± 2.66^{ns}	11.55± 3.28^{ns}	12.66± 3.42^{ns}	16.6± 3.46^{ns}	12.11± 2.14^{ns}
1800 mg/kg	10.1± 1.28^{ns}	10.4± 2.83^{ns}	10.1± 3.10^{ns}	11.6± 2.63^{ns}	11.3± 3.05^{ns}	13.2± 3.52^{ns}	11.6± 3.13^{ns}	12.1± 2.42^{ns}	11.8± 3.32^{ns}	10.6± 2.54^{ns}	13± 3.19^{ns}	11.9± 2.84^{ns}	12.5± 2.46^{ns}
Females													
Control	10.6± 3.30	13.3± 2.40	12.4± 3.33	12.4± 3.06	13.3± 3.05	11.9± 3.66	13.5± 2.87	12.8± 3.91	9.6± 2.41	12± 3.01	12.8± 3.22	11± 2.49	12.5± 2.36
180 mg/kg	10.3± 3.40^{ns}	10.7± 3.83^{ns}	13± 2.49^{ns}	11.8± 2.69^{ns}	11.8± 3.76^{ns}	10.8± 2.85^{ns}	11.7± 2.40^{ns}	11.4± 3.40^{ns}	10.5± 3.37^{ns}	14.5± 3.10^{ns}	13.4± 2.36^{ns}	12± 2.30^{ns}	11.3± 2.11^{ns}
900 mg/kg	11.5± 2.87^{ns}	13.3± 2.90^{ns}	11.6± 2.22^{ns}	11.3± 1.63^{ns}	12.2± 2.69^{ns}	14.3± 3.83^{ns}	13.5± 4.64^{ns}	11.5± 2.46^{ns}	12± 2.86^{ns}	10.1± 2.18^{ns}	10.4± 2.18^{ns}	11.3± 3.12^{ns}	13.4± 3.27^{ns}
1800 mg/kg	9± 3.05^{ns}	12.2± 2.82^{ns}	13.4± 2.83^{ns}	13.2± 2.65^{ns}	16.3± 2.31^{ns}	10.9± 2.68^{ns}	12.6± 3.09^{ns}	12.4± 2.95^{ns}	12.8± 2.85^{ns}	12± 2.66^{ns}	11.1± 3.17^{ns}	13.3± 3.68^{ns}	15.3± 3.62^{ns}

Data was expressed as Mean±SD, N=10, the level of significance was expressed as^{ns}-non-significant

Table 3. Hematological parameters of AYUSH Carctol S

Group	WBC (10^3µl)	Lympho (10^3µl)	Mono (10^3µl)	Neutro (10^3µl)	RBC (10^6µl)	HB (g/dl)	MVC (fl)	MCH (pg)	MCHC (g/dl)	PLT (10^3µl)	PCT (10^3µl)
Males											
Control	9.7± 2.8	69.9± 5.5	2.3± 0.3	27.6± 5.4	7.9± 0.7	13.1± 1.4	48.9± 1.3	16.4± 0.7	33.7± 0.7	307± 54.5	0.18± 0.03
180 mg/kg	8.17± 2.97^{ns}	72.77± 4.29^{ns}	2.72± 0.55^{ns}	24.51± 4.05^{ns}	6.56± 1.79^{ns}	10.96± 3.33^{ns}	48.12± 1.55^{ns}	16.47± 0.81^{ns}	34.31± 0.87^{ns}	262.9± 64.74^{ns}	0.16± 0.03^{ns}
900 mg/kg	9.1± 2.53^{ns}	73.99± 4.67^{ns}	2.31± 0.22^{ns}	23.7± 4.71^{ns}	7.735± 0.67^{ns}	12.98± 0.79^{ns}	44.97± 14.12^{ns}	16.85± 0.38^{ns}	34.21± 0.50^{ns}	310.7± 39.35^{ns}	0.18± 0.03^{ns}
1800mg/kg	12.55± 2.95^{ns}	72.24± 4.90^{ns}	4.05± 0.65^{ns}	23.71± 4.46^{ns}	8.08± 1.03^{ns}	12.61± 1.62^{ns}	57.47± 8.73^{ns}	15.4± 0.69^{ns}	29.78± 11.16^{ns}	234.5± 97.65^{ns}	0.15± 0.06^{ns}
Females											
Control	4.44± 2.06	66.54± 8.66	2.67± 0.39	30.8± 8.93	5.733± 1.60	10.01± 2.90	51.36± 1.41	17.34± 0.38	33.9± 0.879	218.6± 68.58	0.122± 0.06
180 mg/kg	5.91± 0.95^{ns}	68.6± 6.03^{ns}	2.76± 0.33^{ns}	28.64± 5.78^{ns}	6.89± 0.53^{ns}	12.17± 1.09^{ns}	51.31± 0.90^{ns}	17.6± 0.56^{ns}	34.42± 0.70^{ns}	266.5± 44.46^{ns}	0.161± 0.02^{ns}
900 mg/kg	6.67± 2.05^{ns}	68.23± 7.57^{ns}	2.65± 0.49^{ns}	29.11± 7.55^{ns}	7.31± 1.21^{ns}	12.81± 1.90^{ns}	51.73± 0.97^{ns}	17.51± 0.49^{ns}	33.94± 0.72^{ns}	294.55± 85.61^{ns}	0.17± 0.05^{ns}
1800mg/kg	6.99± 1.51^{ns}	62.73± 8.19^{ns}	2.53± 0.40^{ns}	34.74± 8.03^{ns}	8.014± 1.72^{ns}	13.96± 2.97^{ns}	51.25± 1.06^{ns}	17.36± 0.42^{ns}	34± 0.69^{ns}	261.1± 71.8^{ns}	0.156± 0.04^{ns}

Data was expressed as Mean±SD, N=10, the Level of significance was expressed as^{ns}-Non-significant

Table 4. Biochemical parameters of AYUSH Carctol S

Group	Glucose (mg/dl)	Creatinine (mg/dl)	T. Protein (mg/dl)	Albumin (g/dl)	Bilirubin (g/dl)	Cholesterol (mg/dl)	TGL (mg/dl)	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)	Na (meq/l)	K (meq/l)	Cl (meq/l)	Ca (meq/l)
Males														
Control	90.6± 11.32	0.54± 0.11	7.09± 0.75	3.15± 0.25	0.329± 0.17	76.9± 19.09	111.2± 40.09	138.4± 16.46	69.6± 14.32	228.4± 26.59	142.9± 2.88	7.68± 0.70	109.7± 6.92	9.92± 1.60
180 mg/kg	77.5± 11.42	0.58± 0.06 ns	5.47± 0.49 ns	2.99± 1.19 ns	0.416± 0.14 ns	69± 10.11 ns	145.4± 27.18 ns	127.8± 17.27 ns	47.3± 8.16 ns	220.1± 77.57 ns	142.12± 1.50 ns	7.086± 0.35 ns	105.4± 2.01 ns	7.59± 2.40 ns
900 mg/kg	76± 9.26	0.71± 0.10 ns	6.75± 0.56 ns	3.07± 0.14 ns	0.57± 0.25 ns	62.8± 8.75 ns	131.11± 31.62 ns	120.22± 21.00 ns	57.88± 2.71 ns	196.33± 22.81 ns	143± 2.59 ns	10.91± 0.81 ns	104.55± 3.35 ns	10.12± 1.50 ns
1800 mg/kg	85.8± 15.73	0.64± 0.13 ns	6.59± 0.82 ns	3.13± 0.22 ns	0.418± 0.25 ns	68± 17.73 ns	140.8± 34.27 ns	122.1± 13.75 ns	57.2± 13.59 ns	192.6± 32.51 ns	142.4± 3.09 ns	8.94± 2.21 ns	107.8± 5.28 ns	8.94± 1.75 ns
Females														
Control	102.1± 12.06	0.57± 0.04	7.98± 0.70	3.56± 0.30	0.361± 0.04	74.1± 16.96	152.2± 48.57	132.4± 25.54	61.4± 7.10	195.7± 28.91	142.7± 3.05	6.9± 0.54	107.1± 2.76	10.22± 1.06
180 mg/kg	93.1± 12.69 ns	0.62± 0.04 ns	6.32± 1.24 ns	3.44± 0.20 ns	0.328± 0.23 ns	87.3± 13.39 ns	112.4± 37.12 ns	112.9± 14.47 ns	48.2± 8.02 ns	157.5± 45.32 ns	143.3± 1.63 ns	6.64± 0.44 ns	108.2± 2.20 ns	8.51± 2.26 ns
900 mg/kg	86.7± 12.94 ns	0.76± 0.09 ns	6.7± 0.39 ns	3.38± 0.31 ns	0.667± 0.27 ns	74.6± 12.40 ns	140.2± 46.13 ns	102± 32.26 ns	54.7± 4.19 ns	195.9± 32.70 ns	143.2± 1.47 ns	10.19± 0.83 ns	105.3± 4.34 ns	9.95± 1.97 ns
1800 mg/kg	73.5± 11.62 ns	0.65± 0.07 ns	7.32± 0.52 ns	3.05± 0.42 ns	0.409± 0.12 ns	91.3± 23.73 ns	94.3± 41.00 ns	123.8± 19.16 ns	50.5± 15.46 ns	212.8± 40.70 ns	144.3± 2.31 ns	7.83± 2.67 ns	108± 2.53 ns	9.08± 1.32 ns

Data was expressed as Mean±SD, N=10, the level of significance was expressed as[ns]-non-significant

Table 5. Organ weights (g) of AYUSH Carctol S

Dose mg/kg	heart	Liver	lung	spleen	kidneys	stomach	intestine	Testis/ovaries	Brain
Males									
Control	0.92 ± 0.13	7.84 ± 1.34	1.44 ± 0.26	0.53 ± 0.06	1.77 ± 0.12	2.14 ± 0.35	14.55 ± 1.68	2.56 ± 0.30	1.31 ± 0.25
AYUSH Carctrol 180 mg/kg	0.64 ± 0.05 ^{ns}	6.19 ± 0.66 ^{ns}	1.32 ± 0.31 ^{ns}	0.40 ± 0.04 ^{ns}	1.27 ± 0.15 ^{ns}	1.59 ± 0.30 ^{ns}	14.92 ± 1.38 ^{ns}	2.50 ± 0.31 ^{ns}	1.13 ± 0.11 ^{ns}
AYUSH Carctrol 900mg/kg	0.61 ± 0.08 ^{ns}	6.92 ± 0.60 ^{ns}	1.37 ± 0.17 ^{ns}	0.60 ± 0.13 ^{ns}	1.37 ± 0.17 ^{ns}	1.85 ± 0.14 ^{ns}	15.15 ± 1.78 ^{ns}	2.28 ± 0.20 ^{ns}	1.13 ± 0.15 ^{ns}
AYUSH Carctrol 1800 mg/kg	0.64 ± 0.10 ^{ns}	6.71 ± 0.79 ^{ns}	1.19 ± 0.12 ^{ns}	0.51 ± 0.19 ^{ns}	1.38 ± 0.12 ^{ns}	1.77 ± 0.25 ^{ns}	13.60 ± 1.14 ^{ns}	2.63 ± 0.24 ^{ns}	1.37 ± 0.32 ^{ns}
Females									
Control	0.55 ± 0.09	4.29 ± 0.62	0.96 ± 0.16	0.36 ± 0.08	1.09 ± 0.08	1.31 ± 0.13	10.65 ± 0.77	0.08 ± 0.02	1.18 ± 0.13
AYUSH Carctrol 180 mg/kg	0.50 ± 0.04 ^{ns}	5.04 ± 0.58 ^{ns}	1.11 ± 0.44 ^{ns}	0.38 ± 0.08 ^{ns}	1.06 ± 0.14 ^{ns}	1.26 ± 0.23 ^{ns}	11.14 ± 1.43 ^{ns}	0.09 ± 0.02 ^{ns}	1.17 ± 0.05 ^{ns}
AYUSH Carctrol 900mg/kg	0.55 ± 0.09 ^{ns}	5.16 ± 0.99 ^{ns}	1.13 ± 0.39 ^{ns}	0.44 ± 0.19 ^{ns}	1.03 ± 0.12 ^{ns}	1.26 ± 0.23 ^{ns}	11.51 ± 1.34 ^{ns}	1.19 ± 0.80 ^{ns}	1.07 ± 0.07 ^{ns}
AYUSH Carctrol 1800 mg/kg	0.55 ± 0.10 ^{ns}	5.99 ± 1.78 ^{ns}	1.11 ± 0.28 ^{ns}	0.54 ± 0.19 ^{ns}	1.11 ± 0.08 ^{ns}	1.53 ± 0.38 ^{ns}	10.88 ± 3.09 ^{ns}	1.67 ± 0.72 ^{ns}	1.36 ± 0.30 ^{ns}

Data was expressed as Mean±SD, N=10, the level of significance was expressed as^{ns}-non-significant

Table 6. Urine analysis (cumulative findings of male and female animals) of AYUSH Carctol S

Urine parameters	Control	AYUSH Carctol S		
	Vehicle	1800 mg/kg	900 mg/kg	180 mg/kg
Color	Pale to Dark yellow	Pale to Dark yellow	Pale to Dark yellow	Pale to Dark yellow
Specific gravity	1.000 to 1.006	1.000 to 1.007	1.003 to 1.005	1.000 to 1.009
Glucose	Negative	Negative	Negative	Negative
Bilirubin	Negative	Negative	Negative	Negative
Ketones	Negative	Negative	Negative	Negative
pH	6.9 to 7.4	6.9.0-8.1	7.2-8.2	6.6-7.6
Protein	Negative	Negative	Negative	Negative
Urobilinogen	1 (mg/dl)	0.8 (mg/dl)	0.9 (mg/dl)	1.0 (mg/dl)
Nitrite	Negative	Negative	Negative	Negative
Leukocytes	Negative	Negative	Negative	Negative
Hemoglobin	Negative	Negative	Negative	Negative

The sections of the lung showed normal structure in all treated animals whereas congestion hemorrhages, emphysema, and peribronchial mononuclear cell infiltration changes were observed in the highest dose treated animals and control group.

The sections of the testis showed normal structure in all treated animals, whereas mild interstitial oedema changes were observed in the treated group, and a mild reduction in spermatogenic activity was observed in the control group.

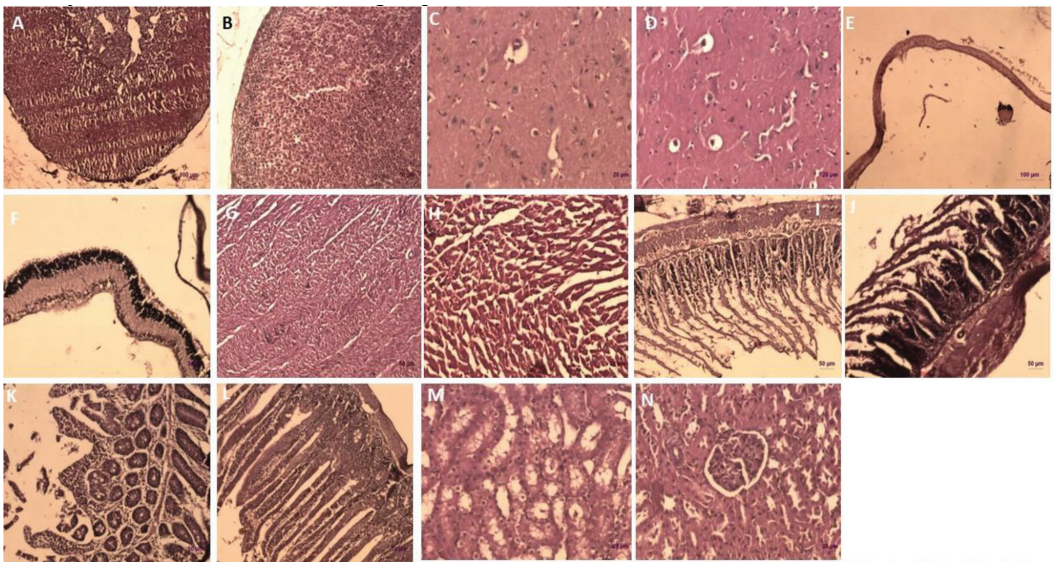


Figure 4. Histopathology of sub-chronic toxicity study (AYUSH Carctol S 1800 mg/kg).
(A) Control Adrenal, **(B)** Treatment Adrenal, **(C)** Control Brain, **(D)** Treatment Brain, **(E)** Control Eye, **(F)** Treatment Eye, **(G)** Control Heart, **(H)** Treatment Heart, **(I)** Control Ileum, **(J)** Treatment Ileum, **(K)** Control Intestine, **(L)** Treatment Intestine, **(M)** Control Kidney, **(N)** Treatment Kidney.

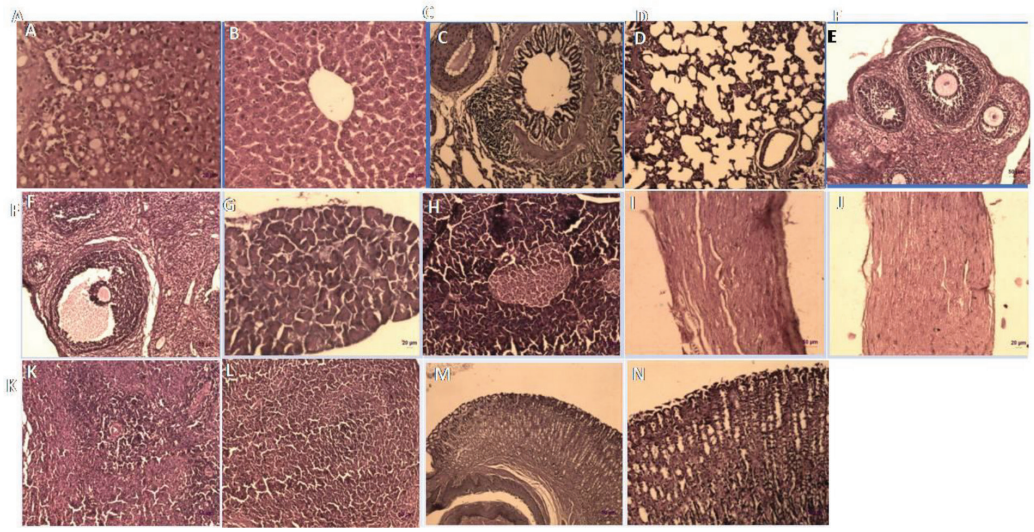


Figure 5. Histopathology of sub-chronic toxicity study (AYUSH Carctol S 1800 mg/kg).
(A) Control Adrenal, **(B)** Treatment Adrenal,
(C) Control Brain, **(D)** Treatment Brain, **(E)** Control Eye, **(F)** Treatment Eye, **(G)** Control Heart, **(H)** Treatment Heart, **(I)** Control Ileum, **(J)** Treatment Ileum, **(K)** Control Intestine, **(L)** Treatment Intestine, **(M)** Control Kidney, **(N)** Treatment Kidney.

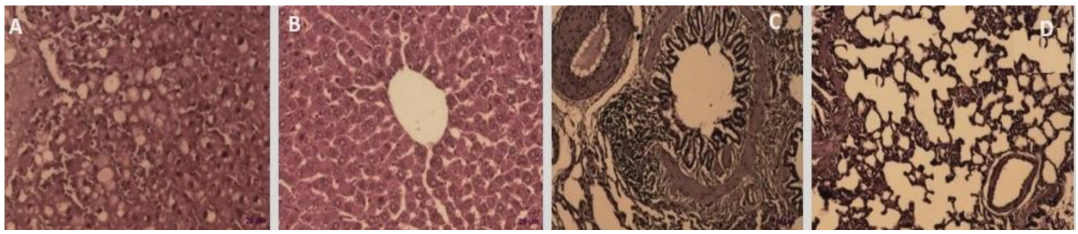


Figure 6. Histopathology of sub-chronic toxicity study (AYUSH Carctol S 1800 mg/kg).
(A) Control Testes, **(B)** Treatment Testes, **(C)** Control Thymus, **(D)** Treatment Thymus.

4. Discussion

The herbal products in the ancient system of medicine are regularly prescribed for the treatment of various which have sound safety profiles but few may associated with mild adverse effects[9]. However, continued use of plant-based medicines without validly reporting on their toxic profile may negatively affect human health[10]. Hence it is necessary to carry out a comprehensive safety analysis as per the regulatory guidelines[11]. To validate the safety of AYUSH Carctol S, the current study was undertaken to assess the toxicity through acute and subchronic toxicity. Parameters of general clinical signs, mortality, body weight, and feed intake are the main indices to evaluate the first signs of toxicity[12,13].

Body weight loss is more generally pronounced at the end of the study and it is often used to derive an endpoint for long-term risk associated with the test compound[12]. There were no observable signs of toxicity or mortality in AYUSH Carctol S treated animals, which demonstrates its safety. There were no significant changes in body weight and feed intake when compared to the vehicle-treated animals for the entire study period. Hematological and Bio-chemistry parameters indicate the toxic effects of drug substances[14]. Changes in the hematopoietic system are one of the important targets of toxic compounds and it is also a critical index of physiological and pathological status like anaemia15,16. In the present study administration of AYUSH Carctol S for 90 days produced no significant changes in blood parameters. The abnormal increase in liver enzymes such as SGOT, SGPT, and SAP are associated with liver damage. Hence it is meaningful to evaluate the safety of the liver by measuring these enzyme levels in blood[17]. AYUSH Carctol S treated animals exhibited no change in liver markers as compared to the vehicle control, which was also confirmed by the histology of the liver, showing no lesions. The functional status of the kidney was estimated by measuring serum creatinine, abnormal levels of this marker were associated with kidney failure[18]. During the present study, the creatinine levels were found to be normal, and no noticeable changes in histology of kidneys with test drug treatment. Even the general metabolic factors such as glucose, cholesterol, and triglyceride were found to be normal with test drug treatment. This establishes the safety of the test compound on general physiological status. The electrolyte status evaluation is one of the prime factors in toxicity studies, abnormal levels of electrolytes may associated with various disorders. AYUSH Carctol S treated animals exhibited no change in potassium, sodium, calcium and chloride levels, which are similar to control animals. Therefore, the assessment of histopathological alterations is considered as a basic test of safety assessment of tested material. The histopathological examination of vital organs in high-dose test drug-treated animals showed no lesions or damage. The findings of the current study confirm no treatment-related toxicity with AYUSH Carctol S treatment in experimental animals.

5. Conclusion

The carctol was found safe on acute oral administration and the LD_{50} was found to be greater than 2600 mg/kg bd.wt/p.o. Similarly, the long-term safety dose was up to 1800 mg/kg bd.wt/p.o

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Cinflicts of intrest: Nil

6. References

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