Original Article

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Quick Response Code:

Website: https://www.jdrasccras.com/

DOI: 10.4103/jdras.jdras_122_22

Safety assessment of Libirite capsule (a polyherbal formulation) in experimental animals (Sprague Dawley rats and Swiss albino mice)

Vasant E. Narke[1], Sanjay U. Nipanikar[2], Ujwala V. Khisti[1], Sachin A. Upasani[2]

Abstract

BACKGROUND: Libirite capsule (LC) is a polyherbal formulation, developed to treat erectile dysfunction and loss of libido.

OBJECTIVE: Acute oral toxicity studies in Swiss Albino mice and Sprague Dawley rats, and repeated dose subchronic 90-day toxicity study were performed to assess the safety of LC.

MATERIALS AND METHODS: In an acute study, LC was orally administered at 2000 mg/kg to the animals as per organization for economic cooperation and development-423 guidelines. In a repeated dose oral toxicity study, LC was administered through oral gavage in a dose of 250, 500, and 1000 mg/kg for 90 days and compared with control groups as per organization for economic cooperation and development-408 guidelines. Posttreatment changes in food consumption, body weight, and biochemical, hematological, and laboratory parameters were observed. No significant changes in the histopathological examination were observed in any group.

RESULTS: LC did not produce any adverse or mortality events in animals during acute studies. In a 90-day toxicity study, rats exhibited no toxicity symptoms or death. No significant changes were found in hematological and biochemical parameters. No significant alteration was seen in organ and body weight. Microscopic findings were incidental and identical for control and treated animals at 1000 mg/kg. LC did not produce any histopathological changes in target organs. No change in the recovery group was observed when compared with the control group animals, which indicated a complete reversal.

CONCLUSIONS: Median lethal dose$_{50}$ of LC was observed to be more than 2000 mg/kg. No observed adverse effect level of LC was considered 1000 mg/kg.

Keywords:

Acute, libido, Libirite capsule, erectile dysfunction, polyherbal, subchronic, toxicity

[1]Department of Toxicology, Bibwewadi, Pune, ToxIndia, [2]Medical Services Department, Ari Healthcare Pvt. Ltd., Pune, Maharashtra, India

Address for correspondence: Dr. Sanjay U. Nipanikar, Ari Healthcare Pvt. Ltd., Office at No., 107, 1st Floor, S.No.1, World Trade Center, Tower one, Opp. EON SEZ, Kharadi, Pune 411014, Maharashtra, India. E-mail: sanjay.n@arihealthcare.in

Submitted : 04-08-2022
Revised : 13-03-2023
Accepted : 09-05-2023
Published : XX-XX-XXXX

Introduction

Erectile dysfunction (ED) or male impotence is an inappropriate penile erection sufficiently required for sexual activity.[1] Various studies reported that around 15%–20% of men described some sexual problems.[2,3] About 80% of cases of ED have an organic etiological factor behind them, which was once considered psychogenic.[2-4] Androgen deficiency, atherosclerosis, hypertension, diabetes mellitus, hyperlipidemia, prostate issues, cardiac disorders, spinal cord injuries, penis anatomical deformity, social and psychological reasons, stress, and depression can cause male impotency.[3,4]

The management of male sexual impotence includes, but is not limited to, oral phosphodiesterase type 5 inhibitor drugs such as sildenafil citrate and intracorporeal agents with vasodilatory effects.[5] However, prolonged usage of many of these drugs can cause major side effects, including

How to cite this article: Narke VE, Nipanikar SU, Khisti UV, Upasani SA. Safety assessment of Libirite capsule (a polyherbal formulation) in experimental animals (Sprague Dawley rats and Swiss albino mice). J Drug Res Ayurvedic Sci 2023;XX:XX-XX.

cardiovascular complications.[6-8] Many health agencies worldwide have recommended utilizing traditional plants to manage various disorders, because they are nontoxic with lesser side effects. Around the world, traditional herbal remedies are used to treat sexual disorders with potent aphrodisiac activity.[7-16]

Libirite capsule (LC) is a polyherbal formulation developed by Ari Healthcare Pvt. Ltd., Pune, India, for managing mild-to-moderate cases of ED and loss of libido. LC contains extracts of *Tribulus terrestris* L., *Withania somnifera* Dunal., *Asparagus adscendens* Roxb., *Mucuna pruriens* Baker.,*Piper betle* L., *Myristica fragrans* Houtt., *Syzygium aromaticum* (L.) Merr & M Perry, and powder of *Crocus sativus* L. Almost all the ingredients used in LC possess aphrodisiac (libido enhancer), antistress, and antidepressant properties.[17-28] These herbs help relax the penile tissue and, thus, maintain an erection. Also, these herbs help in alleviating psychological elements involved in sexual dysfunction.[24-28] Though the utilization of traditional medicine has passed the test of time, it can produce various toxic adverse effects in new and higher dosage forms. Thus, there is a necessity for the evaluation of toxicity of herbal formulations and raw materials.[7-28] Hence, this study evaluated the acute oral toxicity and repeated dose oral toxicity of LC in experimental animals.

Material and Methods

Study design

The method was designed, and studies were performed in a stepwise manner. Acute toxicity studies and subchronic 90-day toxicity studies were conducted after the approval of the Institutional Animal Ethics Committee (IAEC) meeting on January 23, 2016, with project number TI/101003. Experiments complied with the organization for economic cooperation and development (OECD) (1998) principles of good laboratory practice. LC's acute oral toxicity studies were performed in compliance with the OECD-423, Section-4 guidelines; the repeated dose 90-day oral toxicity study of LC was performed as per OECD-408 guidelines.

Animals

Sprague Dawley (SD) rats of age from 9 to 11 weeks and Swiss Albino (SA) mice of age 7–9 weeks and female were selected for two separate acute toxicity studies (OECD-423 guidelines). SD rats of both sex of age from 7 to 9 weeks were selected for a 90-days oral toxicity study (OECD-408 guidelines). Animals were bred and tended at the animal house of ToxIndia, Pune, India. Female animals were nonpregnant and nulliparous. All animals were sustained and adapted under standard household conditions, viz. temperature was 22 ± 3°C, relative humidity was between 30% and 70% with 12–15 air changes per hour and 12-h light and 12-h dark cycle. Animals were selected randomly after the veterinary examination. All animals were fasted overnight before treatment. Food was offered 3 and 1 h after dosing in rats and mice, respectively. All the animals were provided clean water (passed through reverse osmosis water filtration and UV ray system) and Nutrimix brand pelleted standard rat and mice feed (M/s: Nutrivet Life Sciences, Pune, India) *ad libitum*.

All the experiments were conducted after approval with approval number TI/101003 dated January 23, 2016, of the IAEC meeting and as per the Committee for Control and Supervision on Experiments on Animals, India, for the care and ethical use of experimental animals and as per standard operating procedures currently in force at ToxIndia, Pune, India.

Preparation of herbal formulation

LC (test Item) was manufactured and provided by Ari Healthcare Pvt Ltd. The ingredients of LC were as follows: hydroalcoholic extracts of *Gokshura* (*Tribulus terrestris* L.) fruit 140 mg, *Ashvagandha* (*Withania somnifera* Dunal.) root 120 mg, *Shveta Musali* (*Asparagus adscendens* Roxb.) root 100 mg; aqueous extract of *Kapikacchu* (*Mucuna pruriens* Baker.) seed 90 mg; hydroalcoholic extracts of *Nagavalli* (*Piper betle* L.) leaf 40 mg, *Jatiphala* (*Myristica fragrans* Houtt.) fruit 20 mg, *Lavanga* (*Syzygium aromaticum* L. Merr. & M.Perry) flower bud 15 mg, and powder of *Kesara* (*Crocus sativus* L.) stigma 5 mg. Each hydroalcoholic extract contained approximately 40% and 60% of alcohol and water, respectively.

Drug dosages

The test item (received from the sponsor) was in capsule form. The powder was removed from the capsule and used as a test item for dose preparation. The drug dosage was prepared with the suitable amount of the test item (2 g) into a mortar for acute oral toxicity study, adding water and further mixing into a paste, and then shifting the paste to a vessel. Before dosing, 10 mL water was added for an appropriate 200 mg/mL volume of the test item. Animals were treated in sequence at 24-h intervals. However, the dosing intervals were determined by assessing the toxic sign's duration, onset, and severity. According to historical data, the initial dosing level of 2000 mg/kg was selected from one of the four dosing levels, that is, 5, 50, 300, and 2000 mg/kg.

The dose levels for subchronic (90 days) oral toxicity were established upon the findings of the dose-ranging study. The dose of 1000 mg/kg of body weight was selected as the highest dose. Of these dose levels, the lowest dose was 250 mg/kg. The intermediate 500 mg/kg dose has been geometrically placed between the two dose levels.

The excess formulation was disposed of following the appropriate regulatory requirements and information supplied by the sponsor. Food was suspended overnight

before the feed of the test item. Animals were allowed to access the water *ad libitum*.

Study procedure

Standard procedures were set for safe handling of the test drugs. Eye and skin shield equipment were used while dosing animals. The sponsor/manufacturer supplied information regarding the safety of the test item. Test drug substance was classified according to the Globally Harmonized System (GHS) for the classification of chemicals and predefined dosage method.

Acute oral toxicity study in SD rats and SA mice

Stepwise assessment of the toxicity of LC was done following the treatment of animals through oral gavage. Three female SD rats and three female SA mice were used per step. According to historical data of the test item, the initial dosing level of 2000 mg/kg was selected from one of the four dosing levels, that is, 5, 50, 300, and 2000 mg/kg. Food was suspended overnight before the feed of the test item. Animals were allowed to access the water *ad libitum* in both acute toxicity studies.

In step 1, animals were dosed with 2000 mg/kg of body weight. The treated animals were observed for clinical signs, toxicity, and mortality signs at 30 min, 1 h, 2 h, 3 h, 4 h, and 6 h, and after that once a day for 14 consecutive days. Additional parameters that display toxicity signals viz. changes in the appearance of the skin, mucus membrane, and eyes; changes in the behavior or activity of animals; or any systemic changes were observed (if necessary). Body weights of animals were recorded before dosing and on a weekly basis in acute toxicity studies. All animals were subjected to gross necropsy with internal and external observations of organs and cavities. No abnormal changes (if any) in targeted organs were noted during histopathological examination. After observation of clinical signs and mortality at step 1, the same dose was administered at step 2 on three more female animals (SD rats and SA mice) in both acute studies, respectively. All animals were observed for adverse signs and mortality for 14 days.

Ninety-days repeated dose oral toxicity study in SD rats

Based upon the dose-ranging study results, 10 female and 10 male SD rats were fed with LC through oral gavage on a daily basis with doses of 0 (control), 250, 500, and 1000 mg/kg of body weight till 90 days. Further, animals were euthanized and evaluated for toxicity findings. Simultaneously, control group animals were fed with distilled water at 10 mL/kg. Satellite group animals receiving distilled water at 10 mL/kg and LC at 1000 mg/kg levels were observed for 28 days and 90-day exposure to assess reversibility, diligence, or late toxicity incidences.

Initially, animals were examined for clinical signs. Toxicity and mortality signs were examined on a daily basis and after that during exposure and reversal periods. Weekly recording of body weight and food consumption was done. Hematological and biochemical investigations and urinalysis were done at the termination of the study. Animals were euthanized and evaluated for toxicity findings. Histopathological analysis was done on organ tissues of control and high-dose level group animals as mentioned in the trial protocol.

Statistics

Changes in body weights, organ weights, food consumption and their ratios, biochemical and hematological parameters, and urine analysis parameters were assessed. Dunnet as a post hoc test and analysis of variance (one-way) test were used to analyze and compare the different treatment groups with the control group. Student *t* test was used to compare high-dose recovery and control recovery groups. All statistical parameters were assessed at a 95% confidence level ($P < 0.05$).

Results

Acute oral toxicity study

In step 1, LC at 2000 mg/kg dose did not induce any toxicity signs throughout the study. No mortality observations were

Table 1: Body weight (g) summary of acute oral toxicity study in Sprague Dawley rats

Step	Dose (mg/kg)	Animal ID (rats)	Day 0	Day 7	% gain	Day 14	% gain
1	2000	1010/R001	177	189	6.78	203	14.69
		1010/R002	168	177	5.36	188	11.90
		1010/R003	181	191	5.52	199	9.94
		Mean	175.33	185.67	5.89	196.67	12.18
		±SD	±6.66	±7.57	-	±7.77	-
		N	3	3	-	3	-
2	2000	1010/R004	165	174	5.45	186	12.73
		1010/R005	184	195	5.98	203	10.33
		1010/R006	173	187	8.09	197	13.87
		Mean	174.00	185.33	6.51	195.33	12.31
		±SD	±9.54	±10.60	-	±8.62	-
		N	3	3	-	3	-

SD: standard deviation

found in SD rats and SA mice during the 14-day trial period in acute toxicity studies. During the 14-day posttreatment analysis period, the LC group was not shown any adverse gain in body weights of treated animals [Tables 1 and 2]. LC group was not shown any pathological alterations in the organs of animals during the necropsy [Table 3]. In step 2, similar results were observed in animals.

Table 2: Body weight (g) summary of acute oral toxicity study in Swiss albino mice

Step	Dose (mg/kg)	Animal ID (mice)	Day 0	Day 7	% gain	Day 14	% gain
1	2000	1010/M001	30.3	33.7	11.22	36.1	19.14
		1010/M002	28.7	31.3	9.06	34.5	20.21
		1010/M003	32.4	35.3	8.95	38.4	18.52
		Mean	30.47	33.43	9.74	36.33	19.29
		±SD	±1.86	±2.01	-	±1.96	-
		N	3	3	-	3	-
2	2000	1010/M004	29.9	32.5	8.70	35.4	18.39
		1010/M005	31.6	35.3	11.71	38.9	23.10
		1010/M006	28.8	31.5	9.38	34.1	18.40
		Mean	30.10	33.10	9.93	36.13	19.97
		±SD	±1.41	±1.97	-	±2.48	-
		N	3	3	-	3	-

SD: standard deviation

Table 3: Individual animal fate and necropsy findings in acute oral toxicity study

Female rats		Dose: 2000 mg/kg					
Sr. No (%).	List of tissues	Animal numbers					
		G1	G2	G1	G2	G1	G2
		1010/R001	1010/R004	1010/R002	1010/R005	1010/R003	1010/R006
		Euthanized on day 15		Euthanized on day 15		Euthanized on day 15	
1	Brain	NAD	NAD	NAD	NAD	NAD	NAD
2	Spinal cord	NAD	NAD	NAD	NAD	NAD	NAD
3	Pituitary	NAD	NAD	NAD	NAD	NAD	NAD
4	Thyroid	NAD	NAD	NAD	NAD	NAD	NAD
5	Parathyroid	NAD	NAD	NAD	NAD	NAD	NAD
6	Thymus	NAD	NAD	NAD	NAD	NAD	NAD
7	Salivary glands	NAD	NAD	NAD	NAD	NAD	NAD
8	Esophagus	NAD	NAD	NAD	NAD	NAD	NAD
9	Stomach	NAD	NAD	NAD	NAD	NAD	NAD
10	Small intestines	NAD	NAD	NAD	NAD	NAD	NAD
11	Large intestines	NAD	NAD	NAD	NAD	NAD	NAD
12	Liver	NAD	NAD	NAD	NAD	NAD	NAD
13	Pancreas	NAD	NAD	NAD	NAD	NAD	NAD
14	Kidneys	NAD	NAD	NAD	NAD	NAD	NAD
15	Adrenals	NAD	NAD	NAD	NAD	NAD	NAD
16	Spleen	NAD	NAD	NAD	NAD	NAD	NAD
17	Heart	NAD	NAD	NAD	NAD	NAD	NAD
18	Trachea	NAD	NAD	NAD	NAD	NAD	NAD
19	Lungs	NAD	NAD	NAD	NAD	NAD	NAD
20	Aorta	NAD	NAD	NAD	NAD	NAD	NAD
21	Ovaries	NAD	NAD	NAD	NAD	NAD	NAD
22	Uterus	NAD	NAD	NAD	NAD	NAD	NAD
23	Mammary gland	NAD	NAD	NAD	NAD	NAD	NAD
24	Urinary bladder	NAD	NAD	NAD	NAD	NAD	NAD
25	Lymph nodes	NAD	NAD	NAD	NAD	NAD	NAD
26	Peripheral nerve	NAD	NAD	NAD	NAD	NAD	NAD
27	Bone	NAD	NAD	NAD	NAD	NAD	NAD
28	Skin	NAD	NAD	NAD	NAD	NAD	NAD
29	Eyes	NAD		NAD		NAD	

NAD: no abnormalities detected

Ninety-day repeated dose oral toxicity study

Clinical and mortality signs

There were no mortality incidences among the male and female rats in the LC group up to 1000 mg/kg dose levels. Rats fed with LC up to 1000 mg/kg dose showed no notable abnormal signs in either sex. Accidental diarrhea and urination were noted in a few animals of all groups, including the vehicle control group. It was clear that accidental diarrhea and urination were not because of drug consumption, as the weight and other vital signs of all the animals were within normal limits till the completion of the study.

Feeding and body weights

LC group has not shown any substantial and remarkable change in mean body weights of animals treated at and up to 1000 mg/kg dose levels [Tables 4 and 5]. In food consumption analysis, animals fed with LC at and up to 1000 mg/kg doses were compared with control group animals. Ocular findings were normal in control as well as high-dose groups.

Laboratory investigations

Mean values of hematological analysis such as Hb%, total erythrocyte counts, packed cell volume, leucocyte counts, erythrocytes indices, platelet count, and clotting time in animals fed with LC at and up to the level of 1000 mg/kg, and reversal group (1000 mg/kg) were not shown any change at the end of the study (day 90) and at reversal period (day 118). Also, male and female rats treated with LC at and up to the dose of 1000 mg/kg were not exhibited any substantial change in biochemical parameters at the termination of the study [Tables 6 and 7]. Parameters evaluated during the urine analysis showed no remarkable treatment effect up to 1000 mg/kg. The reversal group exhibited normal values compared with the control and the parameters evaluated during the urine analysis.

Necropsy

After necropsy, mean values of absolute and relative organ weights viz. kidneys, adrenal glands, liver, spleen, brain, testes/ovaries, uterus, thymus, and heart of animals treated with LC at and up to 1000 mg/kg were comparable to respective control groups at treatment termination and reversal periods [Tables 8 and 9]. LC group animals did not show any substantial pathological alterations in any organ or tissues at and up to 1000 mg/kg and in the reversal group [Table 10].

Parameters evaluated during the urine analysis did not exhibit any remarkable treatment effect up to the 1000 mg/kg.

Histopathological findings

All microscopic changes noticed during histopathological analysis seemed incidental and identical for the control

Table 4: Mean body weight (g) of male Sprague Dawley rats in 90-day oral toxicity study

Group and dose (mg/kg)		Weeks																	
		0 (%)	1 (%)	2 (%)	3 (%)	4 (%)	5 (%)	6 (%)	7 (%)	8 (%)	9 (%)	10 (%)	11 (%)	12 (%)	13 (%)	14 (%)	15 (%)	16 (%)	17 (%)
G1	Mean (%) ± SD	182.60±9.61	193.80±8.79	205.50±9.62	217.70±9.70	229.50±8.92	241.10±8.60	252.50±8.63	264.10±7.40	275.80±7.58	287.30±6.93	299.20±6.65	310.70±8.29	321.40±8.50	333.10±7.34	-	-	-	-
0 (%)	*N*	10	10	10	10	10	10	10	10	10	10	10	10	10	10	-	-	-	-
G2 (R)	Mean ± SD	182.20±9.88	193.20±9.63	205.40±8.88	215.80±9.63	227.00±9.70	240.00±.781	251.40±8.96	263.40±8.82	276.40±8.79	288.80±8.17	299.40±9.13	310.20±9.20	320.60±8.26	331.80±9.36	342.60±8.56	353.40±8.05	364.40±9.21	375.60±8.56
0	*N*	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
G3	Mean ± SD	180.30±9.14	191.80±9.60	204.30±9.02	216.30±9.42	228.40±8.60	241.00±9.43	253.60±9.49	263.90±9.31	274.40±9.69	287.90±9.54	299.00±7.96	310.20±8.27	321.20±6.97	332.60±7.44	-	-	-	-
250	*N*	10	10	10	10	10	10	10	10	10	10	10	10	10	10	-	-	-	-
G4	Mean ± SD	182.50±9.90	193.30±9.39	203.60±8.97	214.30±9.46	226.10±9.62	239.00±9.81	251.40±9.89	261.70±9.73	273.90±9.43	285.30±9.03	298.30±8.87	308.80±8.24	318.30±8.55	328.60±8.07	-	-	-	-
500	*N*	10	10	10	10	10	10	10	10	10	10	10	10	10	10	-	-	-	-
G5	Mean ± SD	180.10±9.54	192.80±9.70	204.40±8.45	215.10±8.03	227.00±9.30	239.60±7.95	250.40±8.09	261.80±9.02	272.60±9.35	283.90±9.11	296.40±8.75	306.60±8.58	317.50±9.45	328.80±9.61	-	-	-	-
1000	*N*	10	10	10	10	10	10	10	10	10	10	10	10	10	10	-	-	-	-
G6 (R)	Mean ± SD	179.20±8.93	191.00±8.75	203.00±9.25	215.40±9.81	227.00±8.40	237.00±9.03	248.00±8.63	259.40±8.82	271.20±8.84	283.80±8.44	295.80±8.87	306.40±8.62	317.20±8.04	326.80±7.66	339.40±6.66	351.40±6.07	363.00±6.32	373.00±5.48
1000	*N*	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5

SD: standard deviation

Table 5: Mean body weight (g) of female Sprague Dawley rats in 90-day oral toxicity study

Group and dose (mg/kg)		Weeks																	
		0 (%)	1 (%)	2 (%)	3 (%)	4 (%)	5 (%)	6 (%)	7 (%)	8 (%)	9 (%)	10 (%)	11 (%)	12 (%)	13 (%)	14 (%)	15 (%)	16 (%)	17 (%)
G1	Mean (%) ± SD	176.50±7.47	186.40±7.01	196.10±7.48	206.20±7.97	216.10±7.34	226.80±7.91	237.10±7.89	246.20±8.32	256.50±8.95	266.60±8.92	276.40±8.38	287.00±8.51	297.80±9.05	308.10±8.85	-	-	-	-
0 (%)	*N*	10	10	10	10	10	10	10	10	10	10	10	10	10	10	-	-	-	-
G2 (R)	Mean ± SD	175.40±9.96	185.80±8.67	197.20±8.84	206.40±9.66	217.20±9.31	227.00±9.22	237.60±9.56	248.60±9.32	258.80±8.29	269.20±6.94	278.60±8.29	289.40±8.38	298.80±9.42	310.40±8.38	320.60±9.96	330.20±8.67	339.20±8.07	349.00±6.82
0	*N*	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
G3	Mean ± SD	174.10±9.46	184.70±9.21	194.50±8.62	205.40±8.11	216.20±9.40	226.30±9.79	236.10±9.22	246.00±9.17	256.00±8.97	264.70±9.36	274.80±8.39	284.50±7.79	294.80±7.50	305.10±7.32	-	-	-	-
250	*N*	10	10	10	10	10	10	10	10	10	10	10	10	10	10	-	-	-	-
G4	Mean ± SD	176.20±9.55	185.70±9.51	196.90±9.68	206.60±9.01	215.50±8.41	224.60±9.74	235.00±9.33	244.40±8.73	253.80±9.11	263.60±7.71	273.70±8.37	285.00±8.99	294.80±8.60	305.30±9.14	-	-	-	-
500	*N*	10	10	10	10	10	10	10	10	10	10	10	10	10	10	-	-	-	-
G5	Mean ± SD	174.70±7.86	184.30±8.53	195.10±9.01	205.20±8.55	214.90±8.91	225.10±10.02	235.50±9.95	245.20±9.87	254.10±8.89	263.10±8.27	273.00±8.67	284.10±8.40	294.70±8.18	304.40±8.60	-	-	-	-
1000	*N*	10	10	10	10	10	10	10	10	10	10	10	10	10	10	-	-	-	-
G6 (R)	Mean ± SD	175.20±10.18	186.00±7.71	195.80±9.20	205.60±7.33	214.80±6.91	225.80±7.69	235.80±7.98	245.20±8.79	255.40±8.62	265.40±9.15	274.60±7.47	284.60±7.09	293.60±5.94	303.80±5.07	313.00±9.87	325.60±9.56	338.20±8.56	350.00±8.86
1000	*N*	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5

SD: standard deviation

Table 6: Mean hematology values between the groups (day 90)

Group and dose (mg/kg)		Hb (g/dL) (%)	PCV (%)	Total RBC (×10⁶/mm³)	RBC indices			Total WBC (×10³/mm³)	Differential WBC (%)				Plate (×10³/mm³	CT (s)
					MCH (pg) (%)	MCV (fL)	MCHC (g/dL)		N (%)	L (%)	E (%)	M (%)		
Male rats														
G1	Mean ± SD	14.70±1.56	39.83±3.33	8.44±0.85	17.56±2.41	47.59±5.75	37.24±5.98	11.53±1.31	27.80±3.46	68.60±2.76	2.00±1.05	1.60±0.97	847.00±56.12	101.30±5.70
0	*N*	10	10	10	10	10	10	10	10	10	10	10	10	10
G3	Mean ± SD	14.05±1.19	40.45±2.42	8.48±1.01	16.87±3.11	48.33±6.64	34.91±4.19	11.19±1.42	28.40±3.17	67.80±3.94	2.30±1.16	1.50±0.97	853.80±50.41	104.00±6.62
250	*N*	10	10	10	10	10	10	10	10	10	10	10	10	10
G4	Mean ± SD	13.71±1.47	39.93±3.28	8.34±0.90	16.66±2.83	48.29±5.93	34.56±4.66	11.55±1.53	26.40±3.89	69.70±3.77	2.20±1.03	1.70±1.06	851.60±60.73	104.30±6.70
500	*N*	10	10	10	10	10	10	10	10	10	10	10	10	10
G5	Mean ± SD	13.89±0.98	40.15±2.69	8.55±0.81	16.36±1.78	47.23±4.44	34.80±4.06	11.41±1.52	27.10±4.20	68.90±4.82	2.40±1.17	1.60±0.97	848.40±59.76	103.40±5.56
1000	*N*	10	10	10	10	10	10	10	10	10	10	10	10	10
Female rats														
G1	Mean ± SD	13.69±1.05	40.12±2.33	8.55±0.79	16.10±1.50	47.24±4.67	34.23±3.28	11.21±1.59	28.60±3.75	68.00±3.83	2.10±0.99	1.30±0.95	847.80±60.77	102.50±6.59
0	*N*	10	10	10	10	10	10	10	10	10	10	10	10	10
G3	Mean ± SD	13.43±0.91	39.71±2.70	8.75±0.76	15.47±1.80	45.75±5.61	33.90±2.43	11.20±1.43	27.00±3.62	69.20±3.52	2.00±1.25	1.80±1.03	854.20±60.15	105.10±6.64
250	*N*	10	10	10	10	10	10	10	10	10	10	10	10	10
G4	Mean ± SD	14.04±1.13	40.15±2.41	8.39±0.69	16.85±2.02	48.07±4.09	35.05±3.20	11.95±1.48	28.90±3.00	67.30±3.47	2.20±1.23	1.60±1.17	848.80±59.65	105.70±5.96
500	*N*	10	10	10	10	10	10	10	10	10	10	10	10	10
G5	Mean ± SD	13.68±0.98	40.32±2.68	8.60±0.66	15.99±1.63	47.09±4.12	34.02±2.80	11.47±1.48	26.80±3.39	69.80±3.43	2.00±1.05	1.40±0.97	842.80±63.22	104.60±5.19
1000	*N*	10	10	10	10	10	10	10	10	10	10	10	10	10

CT: clotting time, E: eosinophils, Hb: haemoglobin, L: lymphocytes, M: monocytes, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration, N: neutrophils, PCV: packed cell volume, RBC: red blood corpuscles SD: standard deviation, WBC: white blood cells

Table 7: Mean hematology values between the groups (day 118)

Group and dose (mg/kg)		Hb (g/dL)	PCV (%)	Total RBC (10^6/mm^3)	RBC indices			Total WBC ($\times10^3$/mm^3)	Differential WBC (%)				Plate ($\times10^3$/mm^3)	CT (s)
					MCH (pg)	MCV (fL)	MCHC (g/dL)		N (%)	L (%)	E (%)	M (%)		
Male rats														
G2 (R)	Mean ± SD	13.64 ± 1.42	40.02 ± 2.35	8.38 ± 0.63	16.26 ± 0.88	47.82 ± 1.77	34.08 ± 2.95	11.14 ± 1.39	29.00 ± 2.92	67.20 ± 2.68	2.00 ± 1.22	1.80 ± 0.84	856.60 ± 62.55	95.60 ± 6.23
0	*N*	5	5	5	5	5	5	5	5	5	5	5	5	5
Male rats														
G6 (R)	Mean ± SD	14.04 ± 1.04	39.18 ± 2.43	9.16 ± 0.64	15.35 ± 1.04	42.90 ± 3.30	35.94 ± 3.44	11.38 ± 1.37	28.40 ± 4.93	67.60 ± 3.65	2.40 ± 1.14	1.60 ± 1.34	851.60 ± 69.18	96.80 ± 6.94
1000	*N*	5	5	5	5	5	5	5	5	5	5	5	5	5
Female rats														
G2 (R)	Mean ± SD	13.46 ± 1.13	38.74 ± 2.86	8.56 ± 0.64	15.81 ± 1.89	45.31 ± 2.24	34.87 ± 3.66	10.94 ± 1.29	27.00 ± 3.08	69.00 ± 3.67	2.40 ± 1.14	1.60 ± 0.89	835.00 ± 48.11	96.40 ± 8.02
0	*N*	5	5	5	5	5	5	5	5	5	5	5	5	5
Female rats														
G6 (R)	Mean ± SD	12.60 ± 1.54	38.70 ± 3.98	8.46 ± 1.17	15.01 ± 1.85	45.96 ± 2.48	32.60 ± 2.86	11.30 ± 1.54	28.40 ± 3.78	67.60 ± 3.78	2.60 ± 1.14	1.40 ± 1.14	848.80 ± 64.94	97.80 ± 6.42
1000	*N*	5	5	5	5	5	5	5	5	5	5	5	5	5

CT: clotting time, E: eosinophils, Hb: haemoglobin, L: lymphocytes, M: monocytes, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration, N: neutrophils, PCV: packed cell volume, RBC: red blood corpuscles SD: standard deviation, WBC: white blood cells

Table 8: Mean relative organ weight in male rats between the groups

Group and dose (mg/kg)		Adrenal	Testes	Heart	Kidneys	Liver	Epididymides	Thymus	Spleen	Brain
G1	Mean ± SD	0.021 ± 0.003	0.854 ± 0.059	0.540 ± 0.036	0.877 ± 0.044	4.637 ± 0.379	0.508 ± 0.041	0.224 ± 0.027	0.474 ± 0.035	0.803 ± 0.041
0	*N*	10	10	10	10	10	10	10	10	10
G2 (R)	Mean ± SD	0.018 ± 0.003	0.757 ± 0.053	0.451 ± 0.037	0.771 ± 0.041	4.103 ± 0.469	0.406 ± 0.025	0.207 ± 0.026	0.405 ± 0.052	0.712 ± 0.038
0	*N*	5	5	5	5	5	5	5	5	5
G3	Mean ± SD	0.021 ± 0.003	0.851 ± 0.064	0.538 ± 0.047	0.862 ± 0.050	4.576 ± 0.325	0.496 ± 0.043	0.236 ± 0.024	0.490 ± 0.044	0.796 ± 0.049
250	*N*	10	10	10	10	10	10	10	10	10
G4	Mean ± SD	0.022 ± 0.003	0.852 ± 0.072	0.550 ± 0.045	0.874 ± 0.059	4.561 ± 0.488	0.495 ± 0.057	0.223 ± 0.031	0.476 ± 0.034	0.807 ± 0.056
500	*N*	10	10	10	10	10	10	10	10	10
G5	Mean ± SD	0.021 ± 0.003	0.854 ± 0.1010	0.539 ± 0.048	0.859 ± 0.049	4.689 ± 0.362	0.509 ± 0.052	0.228 ± 0.022	0.484 ± 0.037	0.812 ± 0.050
1000	*N*	10	10	10	10	10	10	10	10	10
G6 (R)	Mean ± SD	0.019 ± 0.002	0.780 ± 0.058	0.441 ± 0.034	0.788 ± 0.052	4.124 ± 0.469	0.425 ± 0.063	0.204 ± 0.018	0.398 ± 0.033	0.713 ± 0.035
1000	*N*	5	5	5	5	5	5	5	5	5

SD: standard deviation

Table 9: Mean relative organ weight in female rats between the groups

Group and dose (mg/kg)		Adrenal	Ovaries	Heart	Kidneys	Liver	uterus	Thymus	Spleen	Brain
G1	Mean ± SD	0.022±0.003	0.033±0.003	0.572±0.054	0.892±0.060	4.481±0.381	0.486±0.043	0.218±0.035	0.483±0.037	0.815±0.049
0	*N*	10	10	10	10	10	10	10	10	10
G2 (R)	Mean ± SD	0.022±0.002	0.032±0.008	0.458±0.045	0.796±0.056	3.483±0.257	0.442±0.055	0.202±0.033	0.444±0.044	0.758±0.066
0	*N*	5	5	5	5	5	5	5	5	5
G3	Mean ± SD	0.023±0.003	0.033±0.004	0.590±0.061	0.906±0.072	4.486±0.430	0.489±0.038	0.217±0.037	0.484±0.044	0.830±0.046
250	*N*	10	10	10	10	10	10	10	10	10
G4	Mean ± SD	0.022±0.003	0.032±0.002	0.568±0.064	0.909±0.055	4.636±0.310	0.490±0.026	0.207±0.037	0.499±0.048	0.826±0.063
500	*N*	10	10	10	10	10	10	10	10	10
G5	Mean ± SD	0.022±0.004	0.033±0.003	0.579±0.060	0.889±0.043	4.512±0.524	0.490±0.040	0.215±0.028	0.505±0.037	0.838±0.052
1000	N	10	10	10	10	10	10	10	10	10
G6 (R)	Mean ± SD	0.021±0.002	0.028±0.003	0.483±0.024	0.820±0.048	3.432±0.403	0.471±0.049	0.203±0.025	0.436±0.059	0.716±0.057
1000	*N*	5	5	5	5	5	5	5	5	5

SD: standard deviation

Table 10: Summary of necropsy findings in 90-day oral toxicity study

Group and dose (mg/kg)					
G1	G2 (R)	G3	G4	G5	G6 (R)
0	0	250	500	1000	1000
Male rats					
Incidence (number of animals with findings/initial number of animals)					
0/10	0/5	0/10	0/10	0/10	0/5
Female rats					
Incidence (number of animals with findings/initial number of animals)					
0/10	0/5	0/10	0/10	0/10	0/5

Table 11: Summary of histopathological findings in 90-days oral toxicity study

Group and dose (mg/kg)	
G1	G5
0	1000
Male rats	
Incidence (number of animals with findings/initial number of animals)	
0/10	0/10
Female rats	
Incidence (number of animals with findings/initial number of animals)	
0/10	0/10

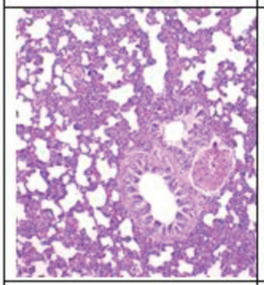
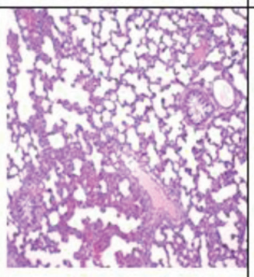
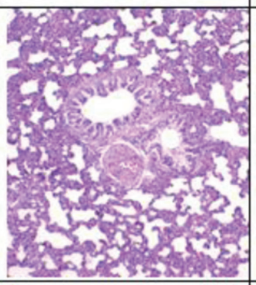
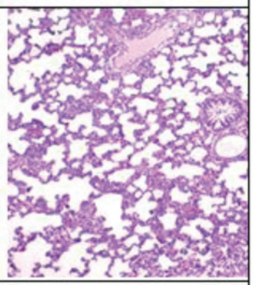
and the treated animals at and up to 1000 mg/kg. No histopathological changes were observed in rats fed with LC at and up to 1000 mg/kg doses under conditions as mentioned earlier [Table 11 and Figures 1–9].

No observed adverse effect level

As per the study results, the test item LC did not show any adverse event on overall body growth and animal health during the biochemical, hematological, neurological, and behavioral analysis of animals at and up to the dose level of 1000 mg/kg. It reveals that the LC did not show a significant toxicological change in any animal till 90 days up to the dose level of 1000 mg/kg body weight. Thus, after oral administration of LC for 90 days in rats, no observed adverse effect level (NOAEL) of LC was found to be 1000 mg/kg of body weight. A summary of adverse events is presented in Table 12.

Discussion

LC is a combination of standardized extracts of seven herbs and genuine Saffron powder for treating sexual disorders, including mild-to-moderate ED and loss of libido. Almost all the ingredients used in LC possess aphrodisiac (libido enhancer), antistress, and antidepressant properties. A few ingredients of LC also help release nitric oxide from the nerve endings of the penis, which, in turn, helps relax the penile tissue and, thus, maintains an erection.[17-28] Also, in various research studies, these herbs are shown to help alleviate various elements involved in sexual dysfunction.[17-28]

Control- M; Lungs; Animal No: 1010/R061	**Control- F; Lungs;** Animal No: 1010/R075	**Control (R)- M; Lungs;** Animal No: 1010/R075	**Control (R)- F; Lungs;** Animal No: 1010/R086
			
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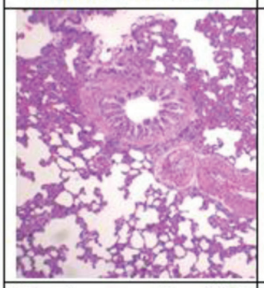
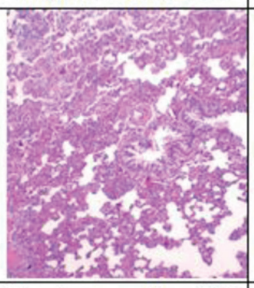
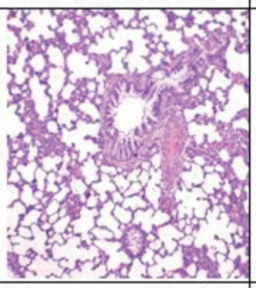
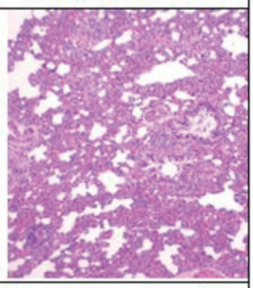
High Dose-M; Lungs; Animal No: 1010/R131	**High Dose-F; Lungs;** Animal No: 1010/R145	**High Dose(R)-M; Lungs;** Animal No: 1010/R145	**High Dose(R)-F;** Lungs; Animal No: 1010/R156
			
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Magnification: 10X, Stain: H & E Stain M: Male F: Female R: Reversal			

Figure 1: Histopathological representation of bronchial epithelium and alveoli

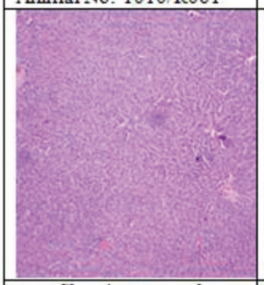
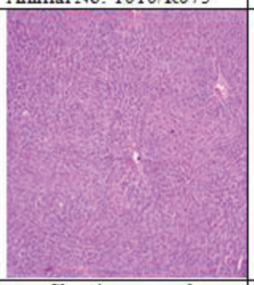
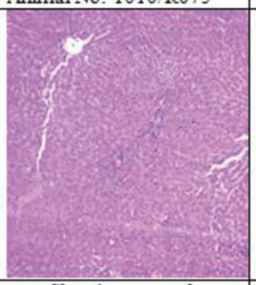
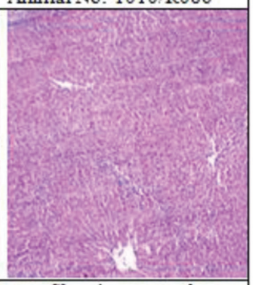
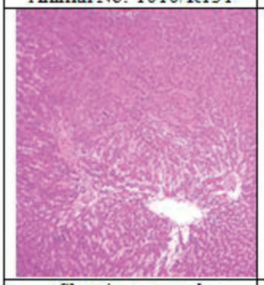
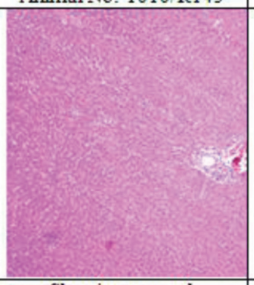
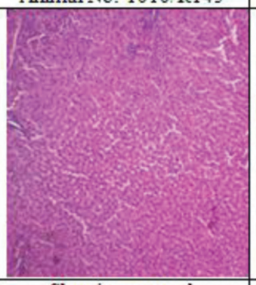
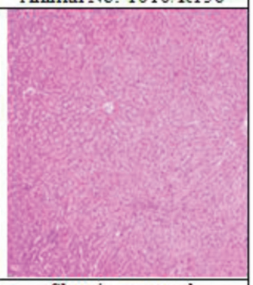
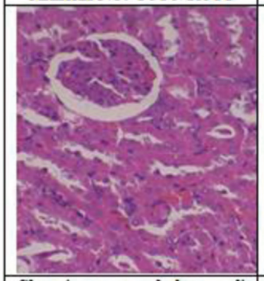
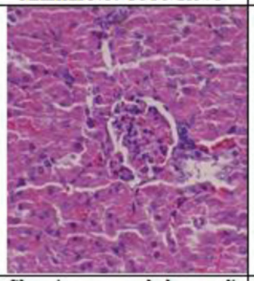
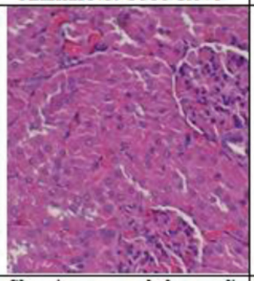
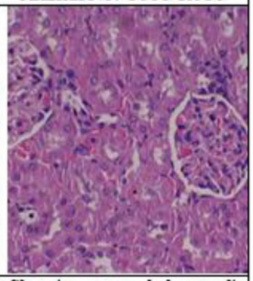
Control- M; Liver; Animal No: 1010/R061	**Control- F; Liver;** Animal No: 1010/R075	**Control (R)- M; Liver;** Animal No: 1010/R075	**Control (R)- F; Liver;** Animal No: 1010/R086
			
Showing normal centrilobular area and hepatic parenchyma	Showing normal centrilobular area and hepatic parenchyma	Showing normal centrilobular area and hepatic parenchyma	Showing normal centrilobular area and hepatic parenchyma
High Dose-M; Liver; Animal No: 1010/R131	**High Dose-F- Liver;** Animal No: 1010/R145	**High Dose(R)-M; Liver;** Animal No: 1010/R145	**High Dose(R)-F; Liver;** Animal No: 1010/R156
			
Showing normal centrilobular area and hepatic parenchyma	Showing normal centrilobular area and hepatic parenchyma	Showing normal centrilobular area and hepatic parenchyma	Showing normal centrilobular area and hepatic parenchyma
Magnification: 10X, Stain: H & E Stain M: Male F: Female R: Reversal			

Figure 2: Histopathological representation of centrilobular area and hepatic parenchyma

Control- M; Kidney; Animal No: 1010/R061	**Control- F; Kidney;** Animal No: 1010/R075	**Control (R)- M; Kidney;** Animal No: 1010/R075	**Control (R)- F; Kidney;** Animal No: 1010/R086
			
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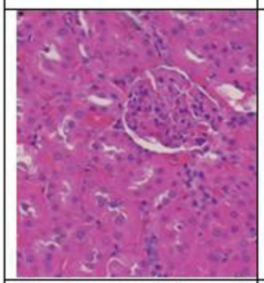
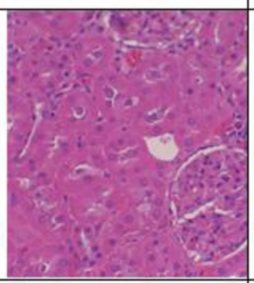
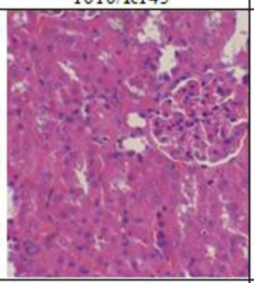
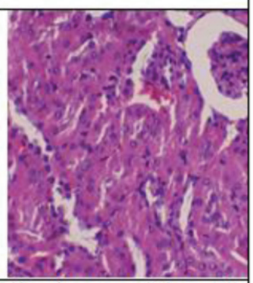
High Dose-M; Kidney; Animal No: 1010/R131	**High Dose-F- Kidney;** Animal No: 1010/R145	**High Dose(R)-M; Kidney;** Animal No: 1010/R145	**High Dose(R)-F; Kidney;** Animal No: 1010/R156
			
Showing normal glomeruli and renal tubules.	Showing normal glomeruli and renal tubules.	Showing normal glomeruli and renal tubules.	Showing normal glomeruli and renal tubules.
Magnification: 10X, Stain: H & E Stain M: Male F: Female R: Reversal			

Figure 3: Histopathological representation of glomeruli and renal tubules

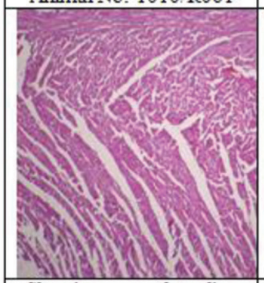
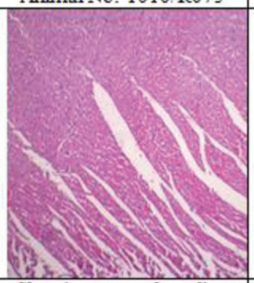
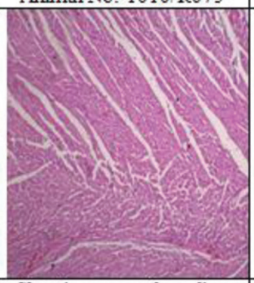
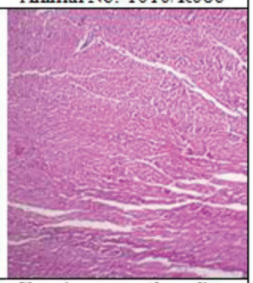
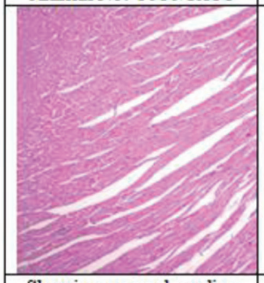
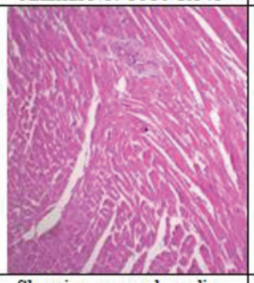
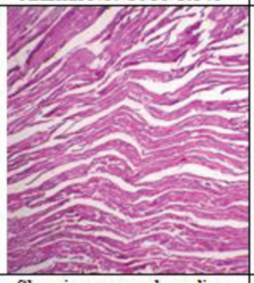
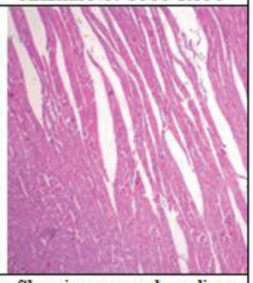
Control- M; Heart; Animal No: 1010/R061	**Control- F; Heart;** Animal No: 1010/R075	**Control (R)- M; Heart;** Animal No: 1010/R075	**Control (R)- F; Heart;** Animal No: 1010/R086
			
Showing normal cardiac muscles and myocytes	Showing normal cardiac muscles and myocytes	Showing normal cardiac muscles and myocytes	Showing normal cardiac muscles and myocytes
High Dose-M; Heart; Animal No: 1010/R131	**High Dose-F- Heart;** Animal No: 1010/R145	**High Dose(R)-M; Heart;** Animal No: 1010/R145	**High Dose(R)-F; Heart;** Animal No: 1010/R156
			
Showing normal cardiac muscles and myocytes	Showing normal cardiac muscles and myocytes	Showing normal cardiac muscles and myocytes	Showing normal cardiac muscles and myocytes
Magnification: 10X, Stain: H & E Stain M: Male F: Female R: Reversal			

Figure 4: Histopathological representation of cardiac muscles and myocytes

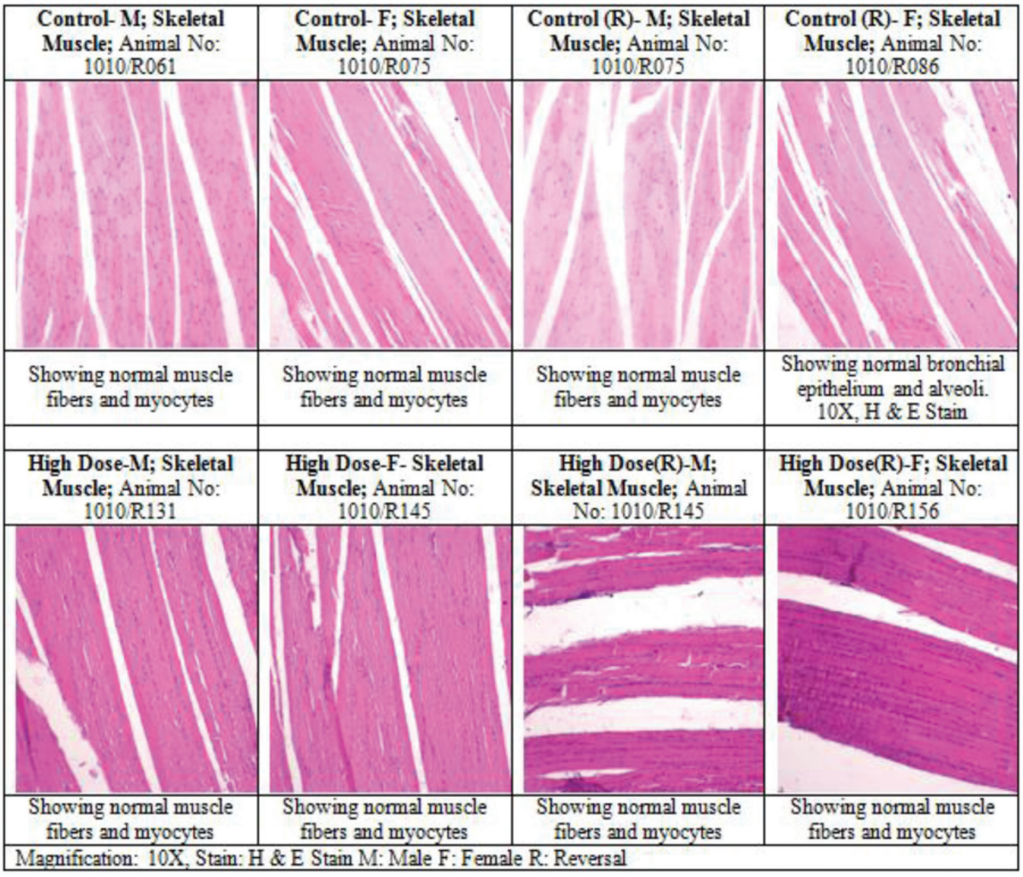


Figure 5: Histopathological representation of skeletal muscle fibers and myocytes

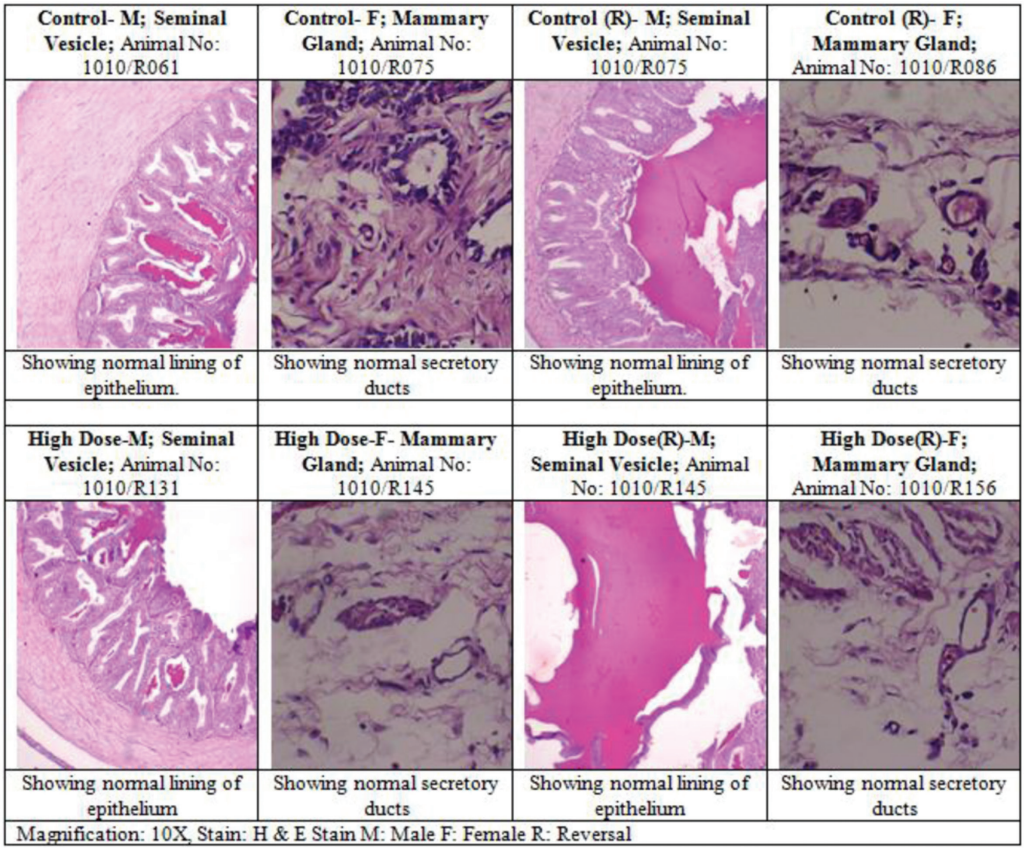


Figure 6: Histopathological representation of seminal vesicle and mammary gland

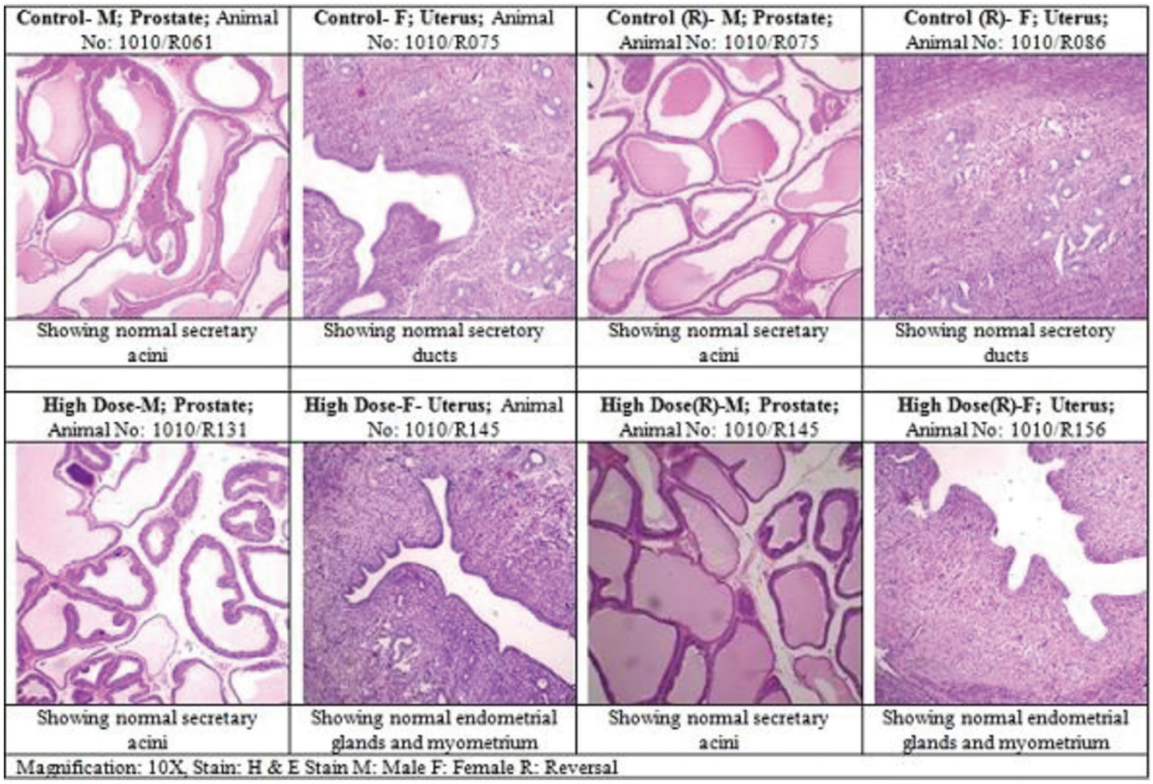


Figure 7: Histopathological representation of the uterus and prostate gland

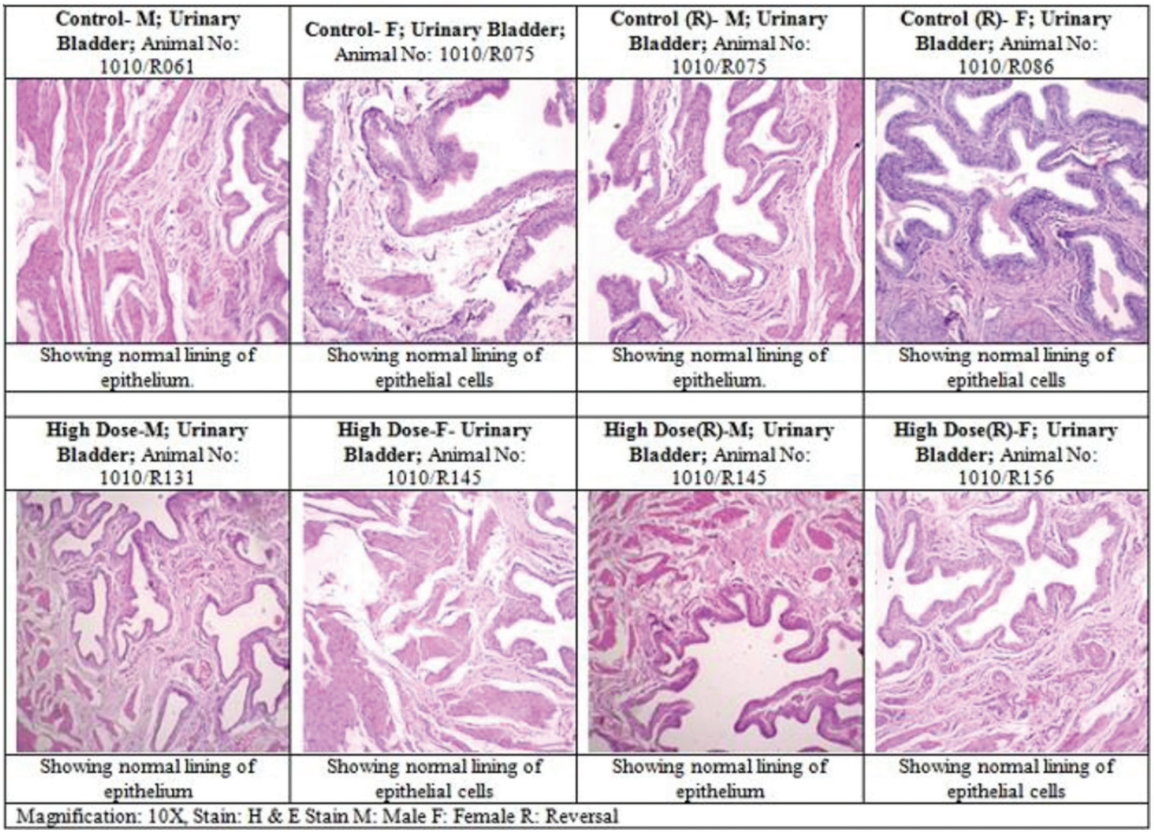


Figure 8: Histopathological representation of urinary bladder

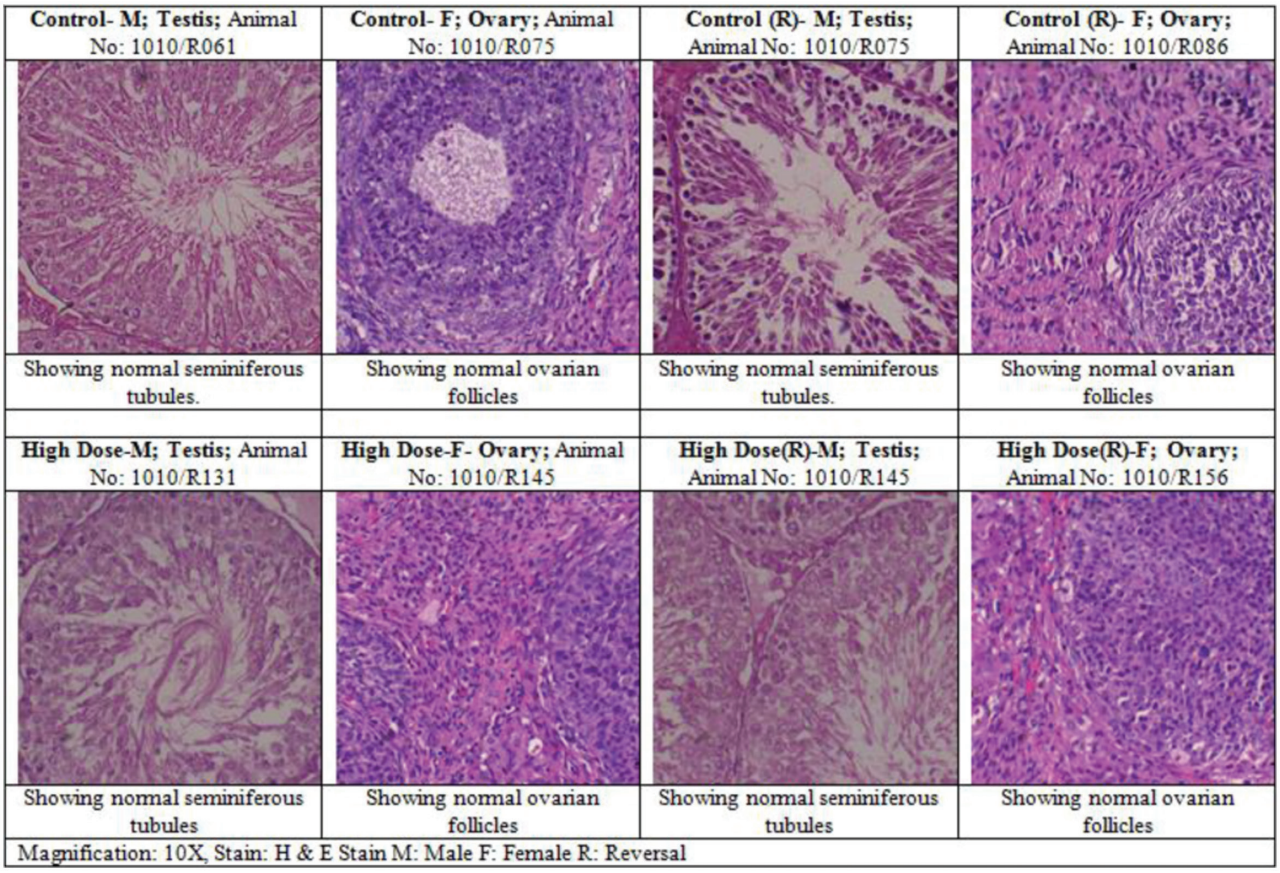


Figure 9: Histopathological representation of ovary and testis

Table 12: Summary of clinical signs (from days 1 to 90)

Clinical finding	Group and dose (mg/kg)					
	G1	G2 (R)	G3	G4	G5	G6 (R)
	0	0	250	500	1000	1000
	Incidence (number of animals with findings/initial number of animals)					
Male rats						
NAD	7/10	5/5	9/10	10/10	8/10	4/5
Diarrhea	2/10	0/5	1/10	0/10	1/10	1/5
Urination	1/10	0/5	0/10	0/10	1/10	0/5
Female rats						
NAD	10/10	2/5	9/10	10/10	9/10	5/5
Diarrhea	0/10	1/5	0/10	0/10	1/10	0/5
Urination	0/10	1/5	1/10	0/10	0/10	0/5

For thousands of years, traditional Ayurvedic medicines have been used safely and effectively in managing various disease ailments. However, herbs used in traditional medicines are emerging with new extractive materials and advanced dosage forms. As per the demand of the scientific era, it is crucial to conduct toxicity studies to optimize the safety of plant-based medicines in consideration of their historical applications on humans. Hence, the present studies were designed to evaluate the safety of LC in experimental animals.[7-16]

In assessing and evaluating a test substance's toxicity, determining the acute toxic effect by the oral route is one of the initial steps. Toxicity analysis is helpful for providing information on acute health hazards likely to arise from human overdoses. Acute toxicity data can serve as a base for the establishment of a safe dosing range for repeated administration and can provide basic information on side effects or toxic action of drug substances. Also, the conduct of acute toxicity studies in two rodent species (mice and rats) provides valid safety confirmation.[7-16]

In the principle of acute toxicity study, lethal dose$_{50}$ calculation shows a proportion of death of the animals with expected lethality dose of the test drug. lethal dose$_{50}$ value can be determined only when at least two doses result in mortality higher than 0% and lower than 100%. The predefined dosages of test substances (any) in acute findings improve the opportunity for laboratory-to-laboratory reporting consistency and repeatability.[7-16]

The repeated dose 90-day oral toxicity study provides evidences of potential health hazards, which can arise from repeated dosing exposure of test drug over a limited period. The data that originate in this type of study provides information on the detrimental effects of the drug on targeted organ tissues, the reversibility of toxic activity, and calculations for NOAEL. The findings of repeated dose subchronic oral toxicity studies can be used in selecting dose levels for chronic toxicity studies.[7-16]

In acute toxicity studies, LC at 2000 mg/kg dose level did not induce any sign of toxicity in experimental animals (rats and mice) throughout the studies. These observations indicated the nontoxicity of LC up to 2000 mg/kg as a single dose. Also, the lethal dose$_{50}$ cutoff value in experimental animals was 5000 mg/kg.

In the 90-day toxicity study, LC treatment group animals at and up to 1000 mg/kg were not shown any death or abnormal sign compared with control group animals. This observation indicates the absence of toxicity of the LC up to this level in the study. In addition, the treated rats' diets were well accepted, suggesting that the test item was not possibly causing any alteration in the food metabolism of animals. In microscopic findings, the animals of the treatment groups did not show any alteration in tissues compared with the control group. The statistical analysis revealed an insignificant difference in the weights of the adrenals, kidneys, testes/ovaries, liver, and heart at and up to 1000 mg/kg compared with concurrent controls.

Test item treatment did not show significant variation in hematology and clinical chemistry parameters at and up to 1000mg/kg dose level. Urinalysis did not reveal any substantial treatment-related toxicity. The incidence of necropsy findings was not dose-dependent and, hence, considered for toxicological assessment, which was confirmed on histopathological results at and up to 1000 mg/kg. Thus, in current toxicity studies, the nonappearance of drug-related toxicity signs is an indication of the harmless nature of the test drug (LC) over long-term exposure.

Conclusion

The study results established that the LC was nontoxic, up to 2000 mg/kg when administered as a single oral dose in SD rats and SA mice. Thus, LC can be classified as GHS "unclassified" or "category 5" for labeling requirement requisite for oral toxicity. NOAEL of LC can be considered 1000 mg/kg in experimental animals under the study conditions and dosages employed.

Financial support and sponsorship

Ari Healthcare Pvt. Ltd.

Conflicts of interest

There are no conflicts of interest.

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Hindi synopsis

इस अभ्यास का उद्देश प्राणीयों में लिबीराईट कैप्सुल की तत्कालीन खुराक से एवं लगातार ९० दिन के खुराक से होनेवाली विषाक्तता के अध्ययन के अनुसार किये जाने वाली सुरक्षीतता की जांच करना था. अलग-अलग गुटों में शामील सभी प्राणीयों में लिबीराईट कैप्सुल यह दवाई तत्कालीन खुराक के तौरपर २००० मिग्राम/किग्राम एवं लगातार ९० दिन के खुराक के तौरपर २५०, ५००, १००० मिग्राम/किग्राम मुंह के द्वारा खाने के लिये दि गयी थी. अध्ययन के आखिर में सभी प्राणीयों का वजन, खाने की मात्रा, रक्त और अवयव प्रयोग शालेय निदानोंके के परिणामों के लिये जांचे गये. प्राणीयों के अवयवों की सूक्ष्म जांच भी कि गयी थी. तत्कालीन खुराक की विषाक्तता के अध्ययन में एवं लगातार ९० दिन के खुराक से होनेवाली विषाक्तता के अध्ययन में, लिबीराईट कैप्सुल के सेवन से सभी प्राणीयों में विषाक्तता या जीवित हानी के कोई परिणाम नही मिले थे. अध्ययन के आखिर में सभी प्राणीयों के वजन में, खाने की मात्रा में, रक्त और अवयवों में लिबीराईट कैप्सुल इस दवाई के प्रयोग से विषाक्तता के कोई परिणाम नही मिले थे. आखिर में इस अभ्यास द्वारा यह निष्कर्ष निकलता था की लिबीराईट कैप्सुल १००० मिग्राम/किग्राम तक भी खुराक के तौरपर इस्तेमाल के लिये सुरक्षीत है और लिबीराईट कैप्सुल की जानलेवा खुराक २००० मिग्राम/किग्राम के उपर थी .