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Study of the safety profile of raw (*aśodhita)* and cow's urine processed (*gomūtra śodhita)* tubers of *Gloriosa superba* L. (*Lāngali*) in albino rats

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Lāngali (*Gloriosa superba* L.) is a drug in *upaviṣa varga* (semi-poisonous drug) which has to undergo *śodhana* procedure before its therapeutic use. The present study was planned to assess the role of *śodhana* of *Lāngali* tuber by *gomūtra* on its safety aspects in experimental animals, which is recommended by Ayurvedic Formulary of India. The therapeutic dose of *Lāngali* as per API is 125 to 250 mg/day. For the present study the higher dose i.e. 250 mg was selected. Wistar strain albino rats were used for the study. The animals were divided into seven groups and each group contained 12 animals (six male and six female). The study was done for 90 days followed by 30 days (total 120 days) recovery study. Various haematological, biochemical parameters and histopathology of the organs were studied. The study showed that *śodhana* process attenuates the toxicity producing potential of raw *Lāngali.* When processed with *gomūtra*, *lāngali* root produces good spermatogenic effect while raw/ *aśodhita lāngali* hampers spermatogenesis at 10 times dose level. So it can be concluded that both raw and *śodhita* Langali found relatively safe up to 5 times therapeutic dose level.

Keywords: *Gloriosa superba* L., G*omūtra*, *Lāngali*, S*odhana,* Toxicity, U*paviṣa*.

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Introduction

Therapeutic safety is much essential than efficacy, for any drug, used either to prevent or cure any disease. It becomes more necessary when the drug is known for its adverse effects. In Ayurvedic system of medicine, '*śodhana*' (processing) procedures have been adopted to ensure both safety and therapeutic efficacy of certain *viṣa* (poisonous) drugs[1]. According to *Ayurveda, viṣa* (poison) when used with proper *yukti* (justification) can be good medicine, in the same way, a medicine, if not used in a proper way, can act as a *viṣa* (poison)[2]. To ensure safety and efficacy of the drugs, the word *Yukti,* can be considered as a selection of proper dose, duration, dosage form, after purification with different media etc. It is therefore essential to evaluate the safe margin of therapeutic effective poisonous drugs used in the recommended dose and prolonged duration.

Ayurvedic pharmacopoeia recommends *Lāngali,* a drug among *upaviṣa* (less poisonous) group of drugs, in various disease conditions, both internally or externally, such as *kuṣṭha* (skin disease)*, arśa* (piles)*, jwara* (pyrexia), *karnaroga* (ear diseases), *indralupta* (alopecia) either as a single drug or part of compound formulations and also used as *rasāyana* in low doses[3]. It recommends the use of this drug, like other poisonous drugs*,* after *śodhana*[4]. *Lāngali* contains colchicine (ranging from 0.15 to 0.56%), an active constituent, which is reported for its toxic effects[5]. The toxicity of colchicines is dose-dependent. The high fatality rate was reported after acute ingestions exceeding 0.5 mg/kg. The lowest reported lethal doses of oral colchicine are 7-26 mg[6]. Some other chemical constituents are gloriosine, N-formyl desacetylcolchicine, monomethyl γ-resorcylate and colchicoside[7]. It is considered that, by the process of *śodhana*, harmful elements from the *dravya* are pacified and it is made compatible with the human body[8]. Ayurvedic Formulary of India recommends *śodhana* of *Lāngali* with *gomūtra*[9] but in routine practice, it is used in raw condition only. Animal experimentation is the only way for the assessment of risk factors. A recent review of the literature shows that neither raw nor *śodhita Lāngali* has been evaluated for their toxic effects in an animal study[10].

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Hence, to assess the toxic effect, if any, chronic toxicity for 90 consecutive days of *aśodhita* (Raw) *Lāngali* tuber and *gomūtra śodhita Lāngali* was evaluated experimentally on therapeutic dose as well as five times and ten times higher than the therapeutic dose. Further, to observe the recovery of the experimental animals from toxicity after a certain interval of toxicity study is one of the recommendations of the World Health Organization (WHO) guidelines for conducting toxicity study[5]. Therefore after prolonged exposure to the higher dose of *Lāngali*, if the animals recovered from the toxic effect or not, should also be observed. Hence, the present study was planned to assess the role of *śodhana* on *Lāngali* root on its safety aspects in experimental animals.

Materials and Methods

Test drugs

Gloriosa superba L. was identified as *Lāngali* in the field by observing its reported taxonomical and morphological characters[11-14]. Fresh and mature tubers were collected from Salem district of Tamil Nadu, 78.14° East altitude and 11.67° North longitude, in the month of December 2010. A sample herbarium has been preserved in Pharmacognosy Laboratory of the Institute (Herbarium no. Phm/6060). *Śodhana* was performed in the department of Dravyaguna using *gomūtra* as media[15]. For the purpose of *śodhana*, raw tubers (250 g) were cut into pieces, dipped in 750 mL *gomūtra* collected from the local cowshed and allowed to stand for overnight. Then they were taken out the next morning, washed with lukewarm water and dried under sunlight. Dried *śodhita* sample was powdered, stored in an airtight glass container and labelled as GS. The raw (*Aśodhita*) *Lāngali* was also powdered and kept labelled as AS.

Animals

Wistar strain albino rats of either sex, weighing 210±60 g were used for the study. The animals were obtained from the animal house (Registration No.548/2002/CPCSEA) attached to I.P.G.T.&R.A., Gujarat Ayurved University, Jamnagar. Six animals were housed in each cage. They were housed at 22±3°C with constant humidity 50-70%, on a 12 h day and night cycle. They were fed with Amrut brand rat pellet feed supplied by Pranav Agro Industries and with tap water ad libitum. The experimental protocol was approved (dated 13/09/2011) by the Institutional Animals Ethics Committee (IAEC/09/11/01) as per OECD 452 guideline.

Dose

The therapeutic dose of *Lāngali* as per API is 125 to 250 mg/day. For the present study, the higher dose i.e. 250 mg was selected. The rat dose was calculated by extrapolating the human dose to animal dose based on the body surface area ratio. The dose fixation was done on the basis of body surface area ratio using the table of Paget and Barnes (1964) as follows[16].

Human dose × body surface area ratio convertibility factor
Dose for rats: Human dose×0.018
= 250 mg×0.018
=4.5 mg/200g body weight of rat
=22.5 mg/kg/day of rat

For the animal experiment, the test drugs were made into fine suspension with the help of carboxy methyl cellulose (q.s.) procured from Thomas Baker company as a suspending agent, in vehicle (deionized water) in a suitable concentration and administered by oral route with a gastric catheter.

Study protocol for chronic toxicity (main study)

Twelve animals were taken in each group for the present study and divided into seven groups. Details of grouping, naming and dose are presented in Table 1.

Bodyweight was noted down before the commencement of the study and afterwards every 7th day and also before sacrifice along with general behaviour pattern by exposing each animal to open arena. On the 91st day, 6 rats from each group were sacrificed; blood was collected by puncturing supra-

Table 1 — Details of grouping and sex of animals in chronic toxicity study

Cage number	Groups	Dose (mg/kg)	Sex	Duration (Days)
1	Control	--	Male	90
2	Control	--	Female	90
3	AS TED*	22.50	Male	90
4	AS TED*	22.50	Female	90
5	AS TED × 05	112.50	Male	90
6	AS TED × 05	112.50	Female	90
7	AS TED × 10	225	Male	90
8	AS TED × 10	225	Female	90
9	GS TED*	22.50	Male	90
10	GS TED*	22.50	Female	90
11	GS TED × 05	112.50	Male	90
12	GS TED × 05	112.50	Female	90
13	GS TED × 10	225	Male	90
14	GS TED × 10	225	Female	90

*TED – Therapeutically equivalent dose; AS – *Aśodhita*, GS – *Gomūtra śodhita*

orbital plexus for haematological and biochemical tests. All vital organs were collected, cleaned, weighed and the histopathological study was carried out.

Study protocol for chronic toxicity (recovery study)

Six rats, an equal number of male and female from each treated groups were kept for recovery study. During this recovery period (30 days after the main study), no drug was administered, and only normal diet and drinking water were given during the recovery period. This schedule was continued for 30 days after treatment of 90 days in the main study. Gross behaviour was observed throughout the period of study. On the 121st day, rats were sacrificed.

Morphological and histopathological studies

Various organs i.e. liver, heart, lung, spleen, brain, pituitary, thymus, trachea, stomach, intestine, kidney, testis, prostate, uterus, seminal vesicles, skin, and uterus were removed and dissected carefully. The organs were transferred to 10% formaldehyde solution for preservation. The sections were cut, taken on a slide and were stained by serially placing them in xylol, acetone, 95% alcohol, running water, hematoxylin to stain the cytoplasm of the cells and eosin to stain the nuclei, and mounted by using diphenlypthalein xylene (DPX), a coverslip was placed. They were studied under the binocular research Carl-Zeiss microscope (Germany) at various magnifications to note down the changes in the microscopic features of the tissues studied.

Statistical analysis

All the values were expressed as mean±SEM (standard error of mean). Statistical analysis was done by applying student 't' test. ANOVA followed by Dunnett's multiple 't' test was applied where results of 't' test were found significant and data was recorded. A level of P <0.05 was considered as statistically significant level of significance was noted and interpreted accordingly.

Results and Discussion

No mortality was observed during the chronic toxicity study. No significant behavioural changes were observed. Food intake was also not affected by the chronic administration of both raw and *śodhita Lāngali* at all the dose levels. Analysis of the results of the chronic toxicity study showed some similarities and dissimilarities between *śodhita Lāngali* and *aśodhita Lāngali* administered groups.

The body weight gain pattern was comparable in both the sample administered groups. This indicates that chronic administration of either form of test drugs has no adverse effect on the nutrition assimilation status of the animals. Normal progressive body weight gain was observed in normal control as well as all treated group (Table 2). However, percentage increase in body weight was higher in AS (23.75%), AS TED 5 (31.06%), AS TED 10 (24.45%); as well as GS (24.21%), GS TED 5 (31.65%), GS TED 10 (27.09%) treated group as compared to control group (12.52%).

Effect on ponderal changes on various organs

There are no statistically significant changes in the relative weight of organs like heart, liver, seminal vesicle, prostrate and uterus except decrease (P <0.05)

Table 2 — Effect of AS and GS on body weight of albino rats

	Body weight (g)						
	Experimental phase					Recovery phase	
Groups	0 day	30th day	60th day	90th day	% change (0 to 90 days)	120th day	% change (0 to 120 days)
Control	211.00 ±14.57	227.25 ±14.58	228.57 ±18.95	237.43 ±20.63	12.52↑	--	-
AS TED	200.67 ±8.67	205.00 ±6.19	222.67 ±6.82	248.33 ±17.94	23.75↑*	--	-
AS TED×05	173.27 ±6.42	189.27 ±7.06	208.91 ±5.03	227.09 ±9.69	31.06↑*	231.20 ±11.62	33.43↑*
AS TED×10	173.27 ±7.33	180.73 ±9.49	182.72 ±10.08	215.64 ± 11.89	24.45↑*	234.40 ±21.17	35.28↑*
GS TED	191.33 ±12.40	186.67 ±10.22	206.00 ±12.29	237.67 ±20.99	24.21↑	--	-
GS TED×05	165.45 ±8.63	181.09 ±8.86	199.64 ±8.44	217.82 ±12.17	31.65↑*	220.40 ±15.34	33.21↑*
GS TED×10	174.17 ±5.23	183.67 ±4.79	193.17 ±7.75	221.36 ±7.34	27.09↑*	255.67 ±19.56	46.79↑*

Data: Mean±SEM, ↑-Increase, ↓ - Decrease, *P <0.05 when comaperd to initial values (Paired 't' test)

was observed in thymus weight in GSTED×10 treated group and uterus weight in AS TED and GS TED groups. A significant increase was observed in spleen weight in GS TED group while, thymus in AS TED×10 as well as GS TED×10 treated groups (Table 3).

It may be said that some of the active principles in the drug may be helpful in the increase in body weight of the animals. It is observed that aqueous, chloroform and alcohol extract of *G. superba* resulted in an increase in body weight and organ weight at the dose of 500 mg/kg body weight in a dose-dependent manner while weights of the testis, seminal vesicles and prostate were found to increase significantly in all these three extracts[17]. The result may be due to the *Rasayana* property of *Lāngali* which is mentioned in texts[18].

Increase in the relative weight of spleen may be indicative of mild stimulation in the activity of the organ. Another possible reason for weight increase can be pathological changes like oedema formation etc. However, such features were not seen in histopathological examination of spleen hence pathological changes can be ruled out. Kidney weight was found to be significantly decreased in AS group which was not seen in GS TED, this indicates interference with kidney functioning by *aśodhita* sample which may be decreased after *śodhana*. However, the histopathological study does not reveal any changes in the cytoarchitecture of the kidney in both groups. Significant decrease in the weight of uterus in the two groups can be attributed to uterine contraction activity of the drug. However, in the higher dose group, no significant change in the weight was observed.

The weight of the testis was found to be increased in AS TED×5 group in comparison no such change could be observed in *śodhita* therapeutic dose group. This may be indicative of inflammatory or other changes since a marked decrease in spermatogenesis was observed in the testis of *aśodhita Lāngali* treated groups. Colchicine also resulted in a dose-related decrease in testis weight 2 and 8 weeks after injection[19]. Reports of a toxic effect of colchicine in sperm production in man and laboratory animals have appeared and have led to recommendations for its cautious use in males in the reproductive age group[20].

Effect on Haematological parameters (Table 4)

WBC count was decreased in the ASTED group at 90 days while it was normal in GSTED group at all the dose levels. Statistically significant decrease in Hb% was found 90th day at ASTED×5 dose level while in ASTED and ASTED×10 groups showed a statistically non-significant decrease in Hb%. It was found to be increased in GSTED and GSTED×5 group which was statistically non-significant. In GS×10 group Hb% was found to be decreased. This means all the AS groups showed a decrease in Hb% significantly or insignificantly, however, it was found

Table 3 — Effect of AS and GS on various organs of albino rats

Relative weight (mg/100g)	Control group	Aśodhita (AS)			Gomūtra śodhita (GS)		
		TED	TED×5	TED×10	TED	TED×5	TED×10
Thymus	133.70± 6.78	140.29± 10.79	158.98± 10.17	156.53± 7.28*↑	169.85± 14.74	156.71± 8.51	156.03± 3.43*↑
Heart	271.30± 2.72	265.82± 10.43	304.37± 10.09	294.37± 11.51	331.35± 24.92	313.21± 12.72	287.15± 4.01
Liver	2809.50± 188.06	2625.15± 240.97	2859.93± 126.13	3063.93± 260.08	3036.77± 130.03	3022.10± 96.58	2914.86± 82.30
Spleen	168.60± 14.73	136.73± 3.08	186.10± 7.79	216.38± 11.63	217.47± 9.80*↑	173.13± 5.70	191.89± 13.47
Kidney	678.50± 37.47	541.43± 34.41*↓	690.55± 18.14	657.66± 22.39	763.81± 33.41	698.99± 21.32	638.58± 20.71
Testis	893.20± 94.79	964.26± 67.92	1180.22± 25.24#↑	889.24± 167.87	1103.47± 232.73	814.73± 223.63	1143.98± 283.24
S. vesicle	402.80± 24.47	290.87± 30.45	507.86± 34.50	501.93± 26.80	528.13± 96.64	479.36± 33.80	584.37± 53.26
Prostate	135.30± 1.92	144.47± 28.09	149.33± 8.70	176.12± 16.92	169.56± 28.91	135.03± 11.32	162.96± 32.59
Uterus	239.50± 18.7	144.05± 19.20*↓	283.95± 81.57	293.89± 31.60	153.34± 12.55*↓	301.12± 32.30	219.98± 3.76

Data: Mean±SEM, ↑-Increase, ↓ - Decrease, *P <0.05 when compared with control group (Unpaired 't' test), #P <0.05 when compared with control group (ANOVA followed by Dunnett's multiple 't' test)

Table 4 — Effect of AS and GS on haematological parameters of albino rats

Parameters	Control group	AS			GS		
		TED	TED×5	TED×10	TED	TED×5	TED×10
TWBC (10^3/Cumm)	9985.71 ± 1222.86	8050 ± 1148.26	8520.00 ± 946.90	7080.00 ± 726.76*↓	7766.67 ± 571.94	8900.00 ± 879.27	7650.00 ± 509.53
Neutrophil (%)	23.00± 0.87	22.17±4.50	27.90±3.16	29.80 ±2.24*↑	14.50 ±1.33*↓	27.60±2.03	26.00±1.65
Lymphocyte (%)	73.14±0.94	73.50±4.77	67.70±3.27	65.40 ±2.45*↓	81.50 ±1.57#↑	67.90±2.22	69.33±1.84
Eosinophils (%)	2.14±0.14	2.33±0.21	2.30±0.15	2.70±0.21	2.17±0.17	2.30±0.15	2.50±0.15
Monocyte (%)	1.92±0.19	2.00±0.26	2.10±0.23	2.10±0.18	1.83±0.31	2.20±0.20	2.17±0.17
Haemoglobin (gm%)	15.16±0.26	14.93±0.18	14.14±0.22*↓	14.66±0.19	15.42±0.19	15.37±0.22	15.14±0.16
PCV (%)	47.61 ±0.94	48.57 ±0.58	45.53 ±0.83	46.65 ±0.68	49.62 ±0.49	48.98 ±0.7	48.06 ±0.54
TRBC ($10e^6$/µL)	8.42±0.18	8.64±0.17	8.04±0.21	8.25±0.19	8.66±0.08	8.60±0.14	8.51±0.12
Platelet ($10e^3$/µL)	1175.57 ±46.61	1182.67±36.83	1076.00 ±95.60	1161.10 ±44.81	1182.33±41.71	1161.70 ±31.21	1220.25±55.87
MCV (fL)	56.59 ±0.42	56.23±0.46	56.82±0.85	56.62 ±0.59	57.30 ±0.66	56.98±0.48	56.48±0.31
MCH (pg)	18.00 ±0.19	17.30±0.17*↓	17.63±0.28	17.87 ±0.30	17.83±0.28	17.89±0.26	17.78±0.16
MCHC (mg/dL)	31.84 ±0.24	30.75±0.17#↓	31.08±0.14*↓	31.43 ±0.28	31.07±0.26	31.38±0.20	31.52±0.19

Data: Mean±SEM, ↑-Increase, ↓ - Decrease, *P <0.05 when compared with control group (Unpaired 't' test), #P <0.05 when compared with control group (ANOVA followed by Dunnett's multiple 't' test)

decreased only in GS×10 group which was non-significant which was highest dose level while same as that of control in GS and GS×5 groups. The decreased Hb% may be due to alkaloids like colchicine present in the drug which is known to reduce Hb%[21]. MCH was found decreased in AS TED group while it was not altered at other dose levels. MCHC was also found decreased at all the dose levels except for the GS TED group which may be due to reduced toxicity by *gomūtra*. MCHC is reported to be decreased in iron deficiency anaemia[22] hence, there may be a certain component in the drug which is responsible for decreased iron concentration in the blood while it was found normal in the *śodhita* samples which may be due to removal of that particular component through *śodhana*. Other parameters were also observed altered to a certain extent.

Further, analysis of the haematological parameters shows that neutrophil percentage significantly increased and RBC count significantly decreased, in one or the other AS treated groups at some point during their administration these changes were not seen in GS sample administered groups. This indicates that *śodhana* process removes or reduces certain principles which have the tendency to cause changes in the haematological parameters mainly myelosuppression. The monocytes were found to be decreased in both groups.

Biochemical parameters (Table 5)

Blood sugar was decreased on 90th day at only AS×10 dose level. It shows that the drug may be altering with the glucose metabolism in the body at the higher dose. The effect may be due to the content colchicine which is responsible for the decreased absorption of sugar from the intestine that may lead to decreased blood glucose level[23]. However, this effect may not be considered as toxic as the decrease was within normal limits.

Triglycerides were found decreased in AS group at higher dose levels while it was not observed in GS groups which shows that GS TED is safe as compared to *aśodhita Lāngali*. Lowered triglycerides, cholesterol as well as HDL may also be due to the alkaloid colchicine which is responsible for the decrease in lipoproteins[24].

Alkaline phosphatase was found increased after 90 days in all the groups. An elevation in activities of alkaline phosphatase can be found in diseases of the bone, liver and in pregnancy. In the absence of bone disease and pregnancy, elevated alkaline phosphatase levels generally reflect hepatobiliary disease. The greatest elevation (3 to 10 times normal) occurs in biliary tract obstruction. Slight to moderate increase is seen in parenchymal diseases such as in hepatitis and cirrhosis and in metastatic liver disease[25]. This increase in the alkaline phosphatase may be correlated to the alkaloid colchicine which is responsible for its increased activity[26]. Since histopathological slides do not show the cirrhotic changes in the liver this may indicate stimulation of the liver functions.

Fifteen biochemical parameters were monitored during the study. In AS given groups (at three

Table 5 — Effect of *Aśodhita* and *Gomūtra śodhita Lāngali* on serum biochemical parameters of albino rats

Parameters	Control group	AS			GS		
		TED	TED×5	TED×10	TED	TED×5	TED×10
FBS (mg/dL)	96.28±3.65	101.00±1.21	81.80±5.07	79.00±4.49#	106.50±4.75	86.20±5	86.00±3.06
Cholesterol (mg/dL)	53.86±3.39	101.00±1.21	46.50±3.81	44.30±2.97	106.50±4.75	49.80±2.89	51.67±3.27
Trigliceride (mg/dL)	129.71 ±10.69	117.67±20.54	98.30±6.68*	100.70±5.74*	109.50±13.51	116.50 ±11.81	101.33±9.42
HDL (mg/dL)	34.71±2.22	30.17±3.53	29.10±1.91	26.90±2.54*	28.33±1.82	31.50±1.29	33.58±2.63
Urea (mg/dL)	63.86±1.89	61.17±3.09	53.40±1.66#	60.80±2.89	54.00±5.68	58.80±2.62	58.67±1.32*
Creatinine (mg/dL)	0.64±0.04	0.63±0.02	0.67±0.02	0.64±0.03	0.60±0.03	0.67±0.02	0.62±0.03
SGPT (IU/L)	37.71±3.24	54.17±3.12*	43.90±3.33	45.20±2.79	65.50±11.69#	44.10±2.86	37.50±2.13
SGOT (IU/L)	156.86 ±12.25	140.17±5.18	162.70 ±11.26	155.40±9.68	153.17±13.29	159.20 ±13.62	138.92±8.46
Total protein (g/dL)	7.59±0.09	7.88±0.07*	7.22±0.16	7.48±0.16	8.05±0.03*	7.69±0.11	7.87±0.11
Albumin (g/dL)	4.29±0.09	3.95±0.06*	5.31±0.54	3.69±0.11*	3.85±0.07*	5.94±0.65	4.01±0.07*
Globulin (gm/dL)	3.30±0.09	3.93±0.11*	3.76±0.10*	3.79±0.23	4.20±0.09#	3.70±0.13*	3.86±0.11#
ALP (IU/L)	104.57 ±16.17	300.17±27.18#	192.10 ±18.43#	207.90 ±20.14#	279.17±27.68#	198.70 ±21.87#	154.75 ±15.44*
T. Billirubin (mg/dL)	0.43 ±0.03	0.57 ±0.07	0.39 ±0.05	0.48 ±0.03	0.50±0.06	0.34 ±0.04	0.41 ±0.03
D. billirubin (mg/dL)	0.10±0.00	0.15±0.02*	0.14±0.02	0.12±0.01	0.15±0.02*	0.10±0.00	0.11±0.01
Uric acid (mg/dL)	0.84±0.08	0.80±0.13	0.75±0.07	0.65±0.05*	0.90±0.12	0.84±0.08	0.87±0.08

Data: Mean±SEM, ↑-Increase, ↓ - Decrease, *P <0.05 when compared with control group (Unpaired 't' test), # P <0.05 when compared with control group (ANOVA followed by Dunnett's multiple 't' test)

dose levels) eleven parameters were found to be significantly altered. In the lower dose group, AS TED group – six parameters were found to be significantly altered. The same number of parameters was found to be altered in GS TED group. At AS 5 TED and AS 10 TED, nine parameters were found to be altered while six parameters were found to be altered in GS group receiving the same doses. Four parameters; SGPT activity, Total protein, direct bilirubin, and serum glucose level were almost normal. This clearly shows that *śodhana* process is effective in reducing the toxic potential of the *aśodhita Lāngali*.

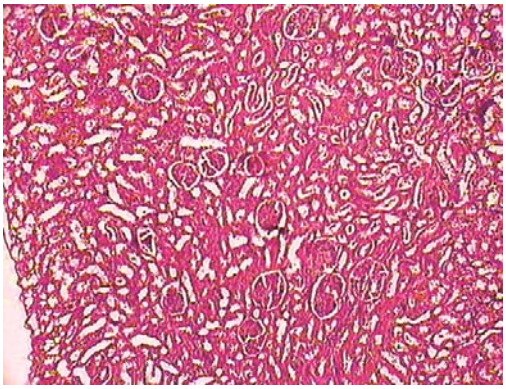

Plate 1 — Kidney (AS 10 group) showing haemorrhagic streaks

Histopathological study

Histopathological study shows that among 16 organs studied, significant pathological changes were observed in kidney, liver and testis (Plate 1-5). *Aśodhita Lāngali* (AS) group only one kidney section was observed with epithelial cell disruption and haemorrhage patch. However, sections of testis exhibited a marked decrease in spermatogenesis and necrotic changes in the spermatids. Increase in the proportion of interstitial cells was seen in most of the testis section in this group. In *Gomūtra shodhtia Lāngali* (GS) group central vein congestion and increase Kupffer cell was observed in one section of the liver only. Two sections of kidneys exhibited normal profile whereas hemorrhagic streaks of moderate-intensity were seen in the other two sections. However, such haemorrhagic changes were observed in the control group also and hence, may not be considered pathologic. Degenerative changes were not observed in the testis sections from GS administered groups. A decrease in spermatogenesis in the AS treated group and good spermatogenesis in GS treated group shows that *śodhana* process with *gomūtra* may have removed the toxic principle[11] which may be responsible for the degenerative changes observed in the testis.

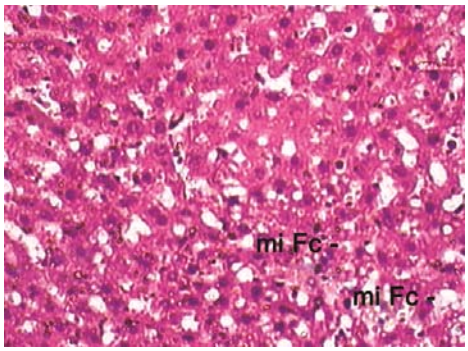

Plate 2 — GS5 Liver showing central vein congestion and increased kupffer cells (miFc-micro fatty changes)

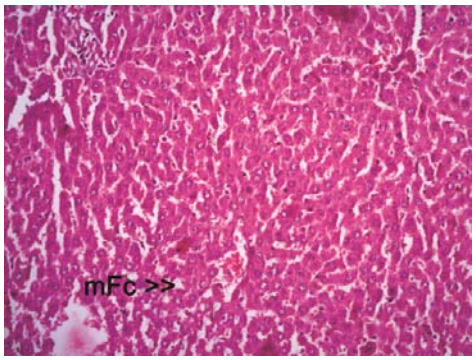

Plate 3 — GS 10 Liver with central vein congestion (mFc-mild fatty changes)

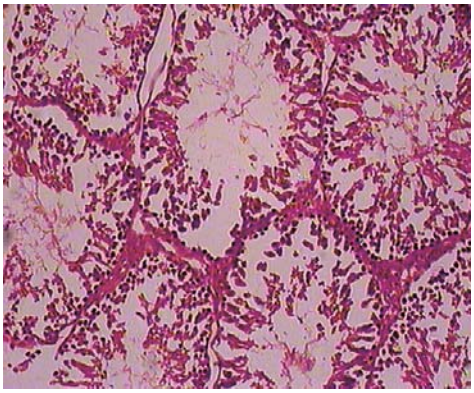

Plate 4 — AS10 Testis showing decreased spermatogenesis

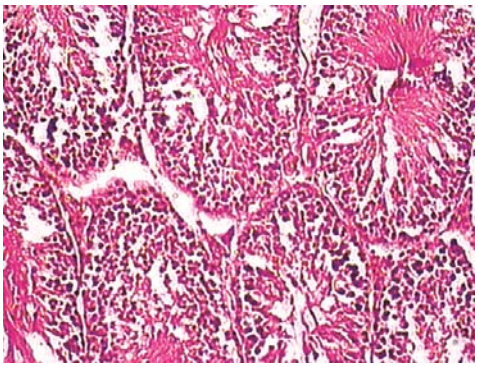

Plate 5 — Testis GS10 showing good spermatogenesis

Conclusion

No mortality and significant behavioural changes were observed during the chronic toxicity study. No significant behavioural changes were observed. The results indicate that *śodhana* process removes or reduces certain principles which have the tendency to cause changes in some haematological and biochemical parameters. Raw *Lāngali*, at ten times dose level, showed significant alterations in some haematological and biochemical parameters. Raw *Lāngali*, at ten times dose level also showed decreased spermatogenesis whereas GS *Lāngali* showed moderate to good spermatogenesis. So it can be concluded that both raw and *śodhita Lāngali* found relatively safe up to five times therapeutic dose level.

Conflict of interest

The authors declare that there is no conflict of interest.

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