Toxicology International, *29(2) : 159-172; 2022. DOI: 10.18311/ti/2022/v29i2/28338*

Online ISSN : 0976-5131; Print ISSN : 0971-6580

Toxicology International

Contents available at: www.informaticsjournals.com/index.php/toxi



Assessment of the Genotoxic Potential of *Rasamanikya*, an Ayurvedic Arsenical Formulation in Albino Rats

Dipali Parekh[1]*, Om Pandey[1], Mukesh Nariya[2], Swapnil Chudhri[1] and B. J. Patgiri[1]

[1]Department of Rasa Shastra and Bhaisajya Kalpana, ITRA, Jamnagar – 361001, Gujarat, India; dipali178@gmail.com

[2]Pharmacology Department, ITRA, Jamnagar – 361001, Gujarat, India

ARTICLE INFO

KEYWORDS: Chromosomal Aberration, Genotoxicity, Orpiment, *Rasamanikya*, Safety

ARTICLE HISTORY

Received: 31-07-2021

Revised: 25-11-2021

Accepted: 16-12-2021

Abstract

Shodhita Haratala (processed orpiment) is a single ingredient of *Rasamanikya* (RM). Arsenicals are known for producing toxic effects in humans, if not processed as per classical text therefore, the present study was aim to generate data for the safety of RM in albino rats. In the present study, two samples of RM were prepared by using two different purification media of orpiment, i.e., juice of *Benincasa hispida* (Thunb.) Cong. and lime water. Extract of *Tinospora cordifolia* Linn. (GG) was used as an adjuvant. Both the samples of RM along with GG were administered to the albino rats for 60 consecutive days at the therapeutic dose, TED (90 mg/kg) and TED×5 (450 mg/kg) orally in albino rats. Sample of RM along with GG not having the potential to produce any mutagenic or genotoxicity effects in albino rats. Further, there is no significant difference found in the safety of the orpiment on changing of media for purification.

1. Introduction

Metals and minerals are therapeutically used in Ayurveda after certain processes such as *Shodhana* (purification and/or detoxification). These formulations are being safely used as integral part of Ayurveda therapeutics forages without any noticeable side effects. The process of *Shodhana* converts the raw metals or minerals into desired stable chemical compound form and imparts high therapeutic attributes so they can be safely administered by human beings. *Rasamanikya* is one such formulation, being used by Ayurveda physicians in the management of *Tvakavikara* (skin disorders), *Jwara* (fever), *Shwasa* (bronchitis)[1]. Though *Rasamanikya* is safely used in

*Author for correspondence

Ayurvedic therapeutics, arsenicals are generally reported as highly toxic and produce untoward effects on internal administration and external exposure[2]. Anticipating such untoward effects; seers of Ayurveda have explained meticulous handling procedures and administration modalities for all such metals and minerals including arsenicals. The role of these traditional pharmaceutical procedures in preparation of Ayurvedic formulations have been well-established by many scholars[3]. Ayurveda emphasizes on the administration of mineral formulations orally in specified quantities with great caution along with requisite *Anupana* (adjuvant) that is anticipated to play a key role in safety aspects of *Rasaushadhies*. *Anupana* facilitates drug administration, improves palatability and also reduces the toxic nature of the drug. The safety of *Rasamanikya* is also well established through acute and chronic toxicity studies[4,5]. Many studies have been carried out in recent past that evaluated the safety of metallic and mineral formulations. They concluded that metals or minerals when properly processed and transformed into medicines by strictly following the classical code of conduct as specified in ancient texts are devoid of ill effects even when administered at higher doses than the specified therapeutic doses[6,7]. Safety studies are must for the formulations like *Rasamanikya* which contain *Haratala* as an ingredient mentioned in Schedule E1 of Drugs and Cosmetics Rules 1945[8]. In addition to the existing toxicity studies, the Government of India expressed the need on conducting genotoxicity studies of different metal or mineral-based Ayurvedic drugs[9]. Safety of such formulations like *Tamra Bhasma*, *Hridayarnava Rasa*, *Swarna Makshika Bhasma* has been well evaluated at the genetic level through Sperm abnormality assay and Chromosomal Aberration (CA) assay which is one of commonly used methods to evaluate the genotoxic potential of drugs[10–12]. Till date, no study has been carried out for *Rasamanikya* in this direction, and there is a need to extend such toxicity studies up to the genetic level for those formulations containing arsenic as an integral component. Considering the significance of this aspect, the study was designed to evaluate possibilities of genotoxic potentials of *Rasamanikya* prepared with *Kushmanda Swarasa Shodhita Haratala* (arsenic trisulphide processed in fruit juice of *Benincasa hispida* (Thunb.) Cong.) and *Churnodaka Shodhita Haratala* (arsenic trisulphide processed in lime water)along with *Guduchi Ghana* (aqueous dry extract of *Tinospora cordifolia* Linn.) as an adjuvant using CA and sperm abnormality assay.

2. Materials and Methods

2.1 *Haratala Shodhana* (Processing of Arsenic Trisulphide)

Unprocessed *Haratala* was procured from the Pharmacy attached to the Institute. It was made into small pieces and bundled in a cotton cloth (*Pottali*). It was suspended in a stainless-steel vessel. Proper care was taken for not having the contact of the bottom and walls of the vessel with the *Pottali* assuring free movement. Quantity sufficient amount of *Kushmanda Swarasa* (fruit juice of *Benincasa hispida* (Thunb) Cong.) was added into the vessel to immerse the *Pottali* completely and subjected to heat at 90–95°C for three hours. Care was taken to immerse *Pottali* completely in *Kushmanda Swarasa* throughout the boiling process. At the end of three hours of boiling, *Haratala* was removed carefully from the *Pottali*, washed with hot water and dried to obtain *Shuddha* (processed) *Haratala*. It was labeled as KSH. Another sample of unprocessed *Haratala* was subjected to the same procedure of *Shodhana* by using *Churnodaka* as a media and labeled as CSH.

2.2 Preparation of *Rasamanikya*

KSH was powdered and sieved through 40#. It was filled in glass bottle having three layer of mud-smeared cotton cloth on it. It was subjected to heat in a Vertical Electrical Muffle Furnace (VEMF) at the temperature of 400°C till the complete melting of material and accumulation of product to the thin iron rod.

The bottle was removed after self-cooling and broken with proper care. The final product inside was taken out, triturated, weighed and stored in an airtight glass container. This product was labeled as KSHRM. The same procedure was followed for the preparation of *Rasamanikya* prepared by *Churnodaka Shodhita Haratala*. It was quoted as CSHRM.

2.3 Dose Fixation

The human therapeutic dose of *Rasamanikya* along with *Guduchi Ghana* (GG) is 500 mg (125 mg + 375 mg) twice a day. Rat dose was calculated by extrapolating the human dose to animal dose by referring table of Paget and Barnes (1964)[13]. It was found to be 90 mg/kg of the rat. Drug suspension was prepared in 7 ml distilled water, 1

ml honey and 2 ml *Ghrita* (cow's ghee) as vehicle per kg body weight of rats for administration of uniform volume of vehicle. The test drug was administered orally along with vehicle suspension with the help of an oral cannula for 60 consecutive days.

2.4 Experimental Animals

Male wistar albino rats weighing 200±20 g were obtained from the animal house attached to the Pharmacology Laboratory of the Institute. Animals were exposed to 12 h natural light and dark cycles under ideal laboratory conditions in terms of ambient temperature (23±3°C) and relative humidity (50–60 %). The animals were fed *ad libitum* with VRK brand rat pellet feed supplied by Keval Sales Corporation, Vadodara and RO water. Colchicine (SRL company Batch no. 1097948) and cyclophosphamide were purchased from the market. The experiment was carried out after obtaining permission from Institutional Animal Ethics Committee (IAEC/23/2018/02) and care of animals was taken as per the Committee for the Purpose of Control and Supervision of Experiments on Animals guidelines (CPCSE).

2.5 Experimental Design

The genotoxicity potential of both the drugs was assessed on chromosomal aberration, bone marrow smear cell morphology and sperm abnormality in albino rats[14]. Albino rats were divided into six groups, each consisting of six animals. Group I served as the Vehicle Control group (VC) received honey and *Ghrita* in distilled water. Group II served as positive control treated with two doses of Cyclophosphamide (CP) (40 mg/kg, *ip*) 48 and 24 h prior to termination of the study. Group III and IV were treated with test drug, KSHRM with *Guduchi Ghana* in the vehicle at TED (90 mg, *po*) and TED×5 (450 mg, *po*) levels respectively. Group V and VI were treated with test drug, CSHRM with *Guduchi Ghana* in the vehicle at TED (90 mg, *po*) and TED×5 (450 mg, *po*) respectively. Test drugs and vehicle were administered for 60 consecutive days. Gross behavior, body weight, food intake, water intake and any other symptoms were observed throughout the study period for assessment of toxicity or adverse effects of the drug, if any.

2.6 Chromosomal Aberration Assay and effects on Bone Marrow Cell Morphology[15]

On, 60th day, albino rats were kept overnight fasting before the dose of colchicine were injected. On 61st day, animals were injected with 4 mg/kg, *ip* colchicine's solution 90 min prior to sacrifice. Animals were sacrificed and bone marrow was collected from the femur bone. The bone marrow smear was prepared on the slide for observation of cell morphology.

For observation of chromosomal structure, bone marrow from all animals was kept into individual round bottom centrifuge tubes. Take 2 ml HBSS solution (Hanks' Balanced Salt Solution) (1 ml of hypotonic solution, 0.56 % potassium chloride) and flush bone marrow in it. Incubate it at 37 ^{0}C for 30 min. At the end of the hypotonic treatment, cells were centrifuged for 10 min at 1000 RPM (rate per min) and the supernatant part was discarded. Add 0.4 ml KCl in test tube with a pipette and kept for 10 min at room temperature. Then it was centrifuged for 10 min at 1000 RPM and the supernatant part was discarded. The cells were agitated with drop-wise addition of freshly prepared cold fixative in 2 ml quantity (Methanol: Glacial acetic acid=3:1). The content was kept for one-hour stabilization at room temperature. The supernatant part was discarded. Again, the cold fixative in 2 ml quantity (Methanol: Glacial acetic acid=3:1) was added and kept for 10 min. Then, it was centrifuged for 8 min at 1000 RPM and the supernatant part was discarded. The same procedure was repeated five times with 10 min of incubation time with a fixative. After the fifth centrifugation, the supernatant was mixed with fixative and slides were prepared. The slides were dried on hot plate at 40 ^{0}C. One more drop of fixative was added on the slides to see more reliable pictures of chromosomes and air-dried soon. Slides were stained after 3 h of preparation with 5 % Giemsa stain in phosphate buffer (pH 6.8) for 30 min. The chromosomes of 1000 cells in metaphase abnormalities were analyzed with a 100X oil immersion objective, using a trinocular microscope (Carl Zeiss, Germany). Metaphases with chromosomes and chromatid breaks, gaps, rings, stickiness, pulverization, centric fusion, deletion etc. were recorded.

2.7 Sperm Abnormality Study[16]

On 61st day, rats were sacrificed, cauda epididymis was removed and placed in a watch glass containing 1 ml phosphate buffered saline (pH 7.2), then rinsed and treated carefully to release the spermatozoa. The suspension was separated from the tissue fragments and filtered with the muslin cloth. 2 drops of Eosin Y solution

(10:1) was added to 1 ml suspension and kept for 30 min. Air dried smears were prepared on clean, grease-free glass slides and a uniform smear were made. About 1000 sperms per animal were examined at 400X magnification from each treatment and control group for the presence of sperm morphological abnormalities.

2.8 Statistical Analysis

The data was expressed as mean ± Standard Error of Mean (SEM) of each experimental group. Statistical comparisons were carried out by both unpaired Student's *t* test and one-way analysis of variance to compare the mean values of quantitative variables among the groups followed by Dunnett multiple *t* - test for unpaired data by using Sigma stat software (version 3.5, Systat Software Inc.). Significant difference level between groups was kept at $p<0.05$.

3. Result and Discussion

Any abnormal behavioural changes were not observed in treated groups during the course of genotoxicity

Table 1. Effect of test drugs on body weight of male albino rats during genotoxicity study

Groups	Body weight (g)				
	Initial	15th day	30th day	45th day	Final
Control	189.33±7.15	196.5±10.62	208.17±13.3*	209.67±13.9*	216.5±14.74**
CP	212.00±3.89	232.5±10.95	244.5±10.1**	242.7±9.98**	244.5±12.40*
KSHRM + GG	195.83±5.98	220.33±7.02*	224.33±7.17*	226.3±7.01**	235.83±8.62#
KSHRM + GG x 5	175.00±8.87	193.20±3.87*	224.40±3.84##	232.2±3.86##	228.7±4.88##
CSHRM + GG	197.00±83.0	245.83±3.02##	248.5±2.71##	251.67±3.46##	254.83±6.36##
CSHRM+ GG x5	173.83±4.32	172.33±5.97	205.83±6.16##	219.83±9.48##	247.83±10.37##

CP-Cyclophosphamide **KSHRM**-*Rasamanikya* prepared with *Kushmanda Swarasa Shodhita Haratala* **CSHRM**-*Rasamanikya* prepared with *Churnodaka Shodhita Haratala* **GG**-*Guduchi Ghana*

Data: Mean±SEM *P<0.05, **P<0.02, #P<0.01, ##P<0.001 when compared to initial body weight of rats (Paired 't' test)

Table 2. Effects of test drugs on chromosomal aberration in bone marrow of albino rats

Groups	Shape of chromosomal aberration					
	Chromatid		Chromosomal		Exchange	Pulverization and stickiness
	Gap	Break	Gap	Break		
Control	--	--	--	--	--	--
CP	++	++	++	++	++	++
KSHRM + GG	--	--	--	--	--	--
KSHRM + GG x 5	--	--	--	--	--	--
CSHRM + GG	--	--	--	--	--	--
CSHRM + GG x 5	--	--	--	--	--	--

CP-Cyclophosphamide **KSHRM**-*Rasamanikya* prepared with *Kushmanda Swarasa Shodhita Haratala* **CSHRM**-*Rasamanikya* prepared with *Churnodaka Shodhita Haratala* **GG**-*Guduchi Ghana*

Table 3. Effects of test drugs on chromosomal aberration in the bone marrow of albino rats

Groups	Shape of chromosomal aberration					
	Deletion	Fragmentation	Ring	Centric attenuation	Centric fusion	Ring
Control	--	--	--	--	--	--
CP	++	++	++	++	++	++
KSHRM + GG	--	--	--	--	--	--
KSHRM + GG x 5	--	--	--	--	--	--
CSHRM + GG	--	--	--	--	--	--
CSHRM + GG x 5	--	--	--	--	--	--

CP-Cyclophosphamide **KSHRM**-*Rasamanikya* prepared with *Kushmanda Swarasa Shodhita Haratala* **CSHRM**-*Rasamanikya* prepared with *Churnodaka Shodhita Haratala* **GG**-*Guduchi Ghana*

Table 4. Effects of test drugs on cytoarchitecture of head of sperm of albino rats

Groups	Shape of head of Sperms and abnormality				
	Normal shape	Hook less shape	Folded shape	Amorphous shape	Banana/ unusual shape
Control	994.0±0.68	4.50±1.02	0.0±0.0	0.0±0.0	0.0±0.0
CP	828.83±7.80[@]	67.167±2.66[@]	2.000±0.577[@]	24.83±1.90[@]	7.33±0.80[@]
KSHRM	993.5± 0.76	6.50±0.76	0.0±0.0	0.0±0.0	0.0±0.0
KSHRM×5	994.16±1.01	5.83±1.01	0.0±0.0	0.0±0.0	0.0±0.0
CSHRM	995.0±1.14	5.00±1.14	0.0±0.0	0.0±0.0	0.00±0.0
CSHRM×5	994.0±1.06	6.00±1.06	0.0±0.0	0.0±0.0	0.0±0.0

CP-Cyclophosphamide **KSHRM**-*Rasamanikya* prepared with *Kushmanda Swarasa Shodhita Haratala* **CSHRM**-*Rasamanikya* prepared with *Churnodaka Shodhita Haratala* **GG**-*Guduchi Ghana*

Data: Mean±SEM; [@]P<0.01, when compared to control group (ANOVA followed by Dunnett's multiple't' test)

Table 5. Effects of test drugs on cytoarchitecture of tail of sperm of albino rats

Groups	Dose (mg/kg)	Shape of tail of sperms and abnormality	
		Double tailed	Coiled tail
Control	--	0.0±0.0	1.33±0.61
CP	40.0	1.33±0.42[@]	70.0±5.24[@]
KSHRM	90.0	0.0±0.0	0.0±0.0
KSHRM×5	450.0	0.0±0.0	0.0±0.0
CSHRM	90.0	0.0±0.0	0.0±0.0
CSHRM×5	450.0	0.0±0.0	0.0±0.0

CP-Cyclophosphamide **KSHRM**-*Rasamanikya* prepared with *Kushmanda Swarasa Shodhita Haratala* **CSHRM**-*Rasamanikya* prepared with *Churnodaka Shodhita Haratala* **GG**-*Guduchi Ghana*

Data: Mean±SEM [@]P<0.01, when compared to control group (ANNOVA followed by Dunnett's multiple't' test)

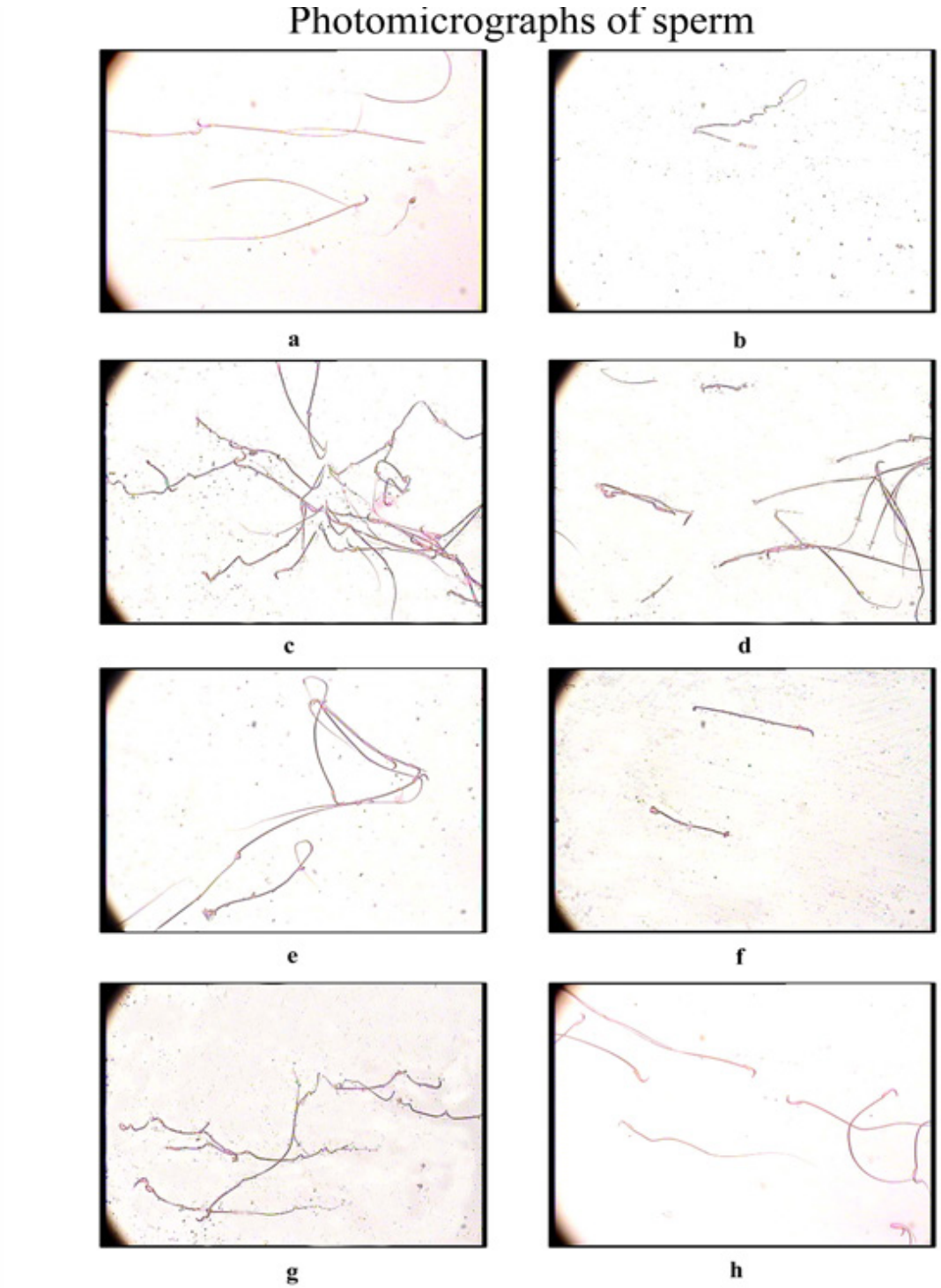


Figure 1. Photomicrographs of sperms of albino rats from cyclophosphamide control treated group (1×400 magnification). **(a)** Hookless and amorphous head sperm **(b)** Hook less head and coiled and abnormal tail sperm **(c)** Absolute abnormal sperms — Hook less and amorphous head; bent and asymmetrical neck; bent, coiled tail and abnormal tail **(d)** Banana shaped and hook less head sperm **(e)** Amorphous head and coiled tail sperm **(f)** Amorphous head and short tail sperm **(g)** Short, bent, coiled and abnormal tail **(h)** Double tail and split tail sperm.

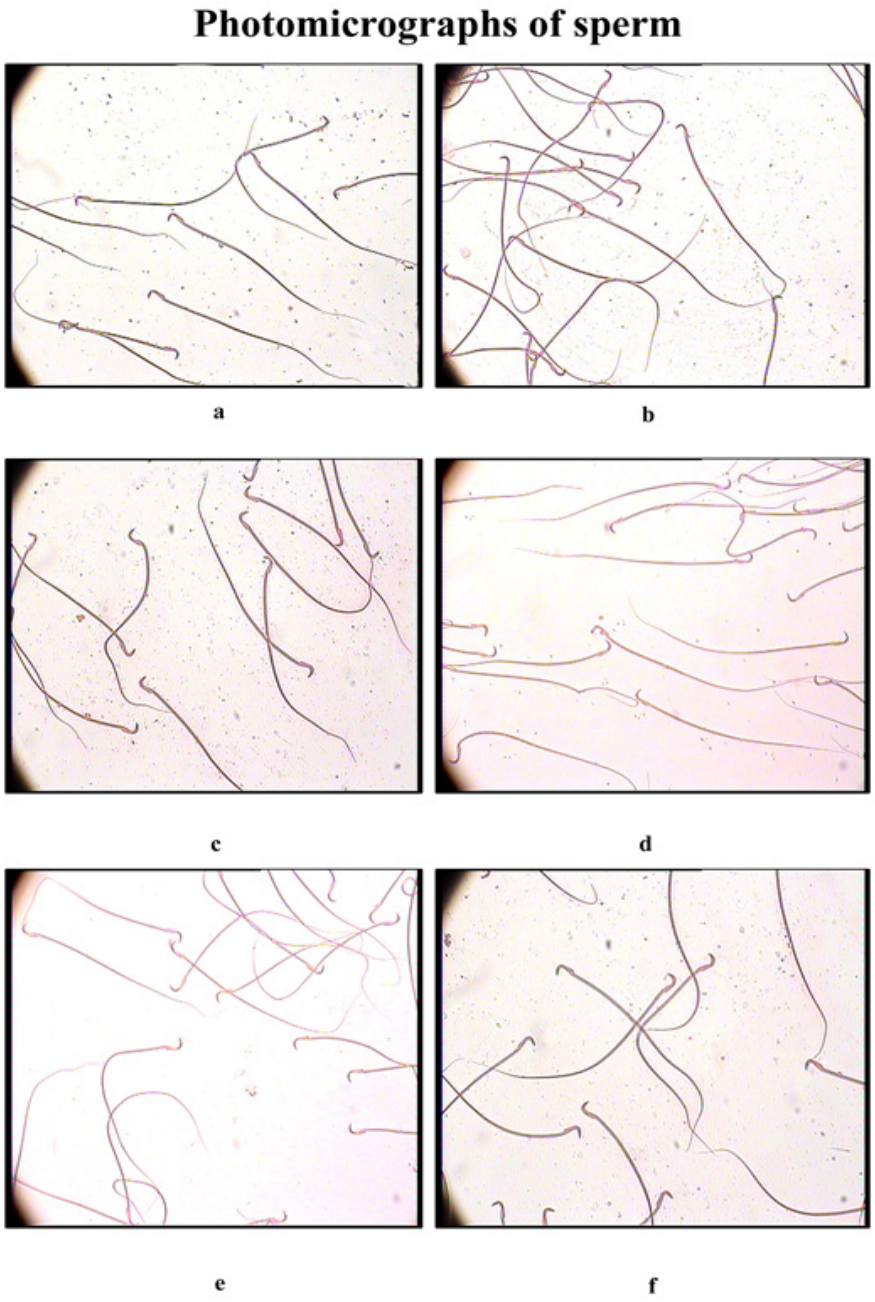


Figure 2. **(a)** to **(b)** Photomicrographs of sperms of albino rats from Normal control treated group (1×400 magnification). Note: Normal cytoarchitecture
(c) Photomicrographs of sperms of albino rats from KSHRM, TED treated group (1×400 magnification). Note: Normal cytoarchitecture
(d) Photomicrographs of sperms of albino rats from KSHRM, TED x 5 treated group (1×400 magnification). Note: Normal cytoarchitecture
(e) Photomicrographs of sperms of albino rats from CSHRM, TED treated group (1× 400 magnification). Note: Normal cytoarchitecture
(f) Photomicrographs of sperms of albino rats from CSHRM, TED x 5 treated group (1×400 magnification). Note: Normal cytoarchitecture.

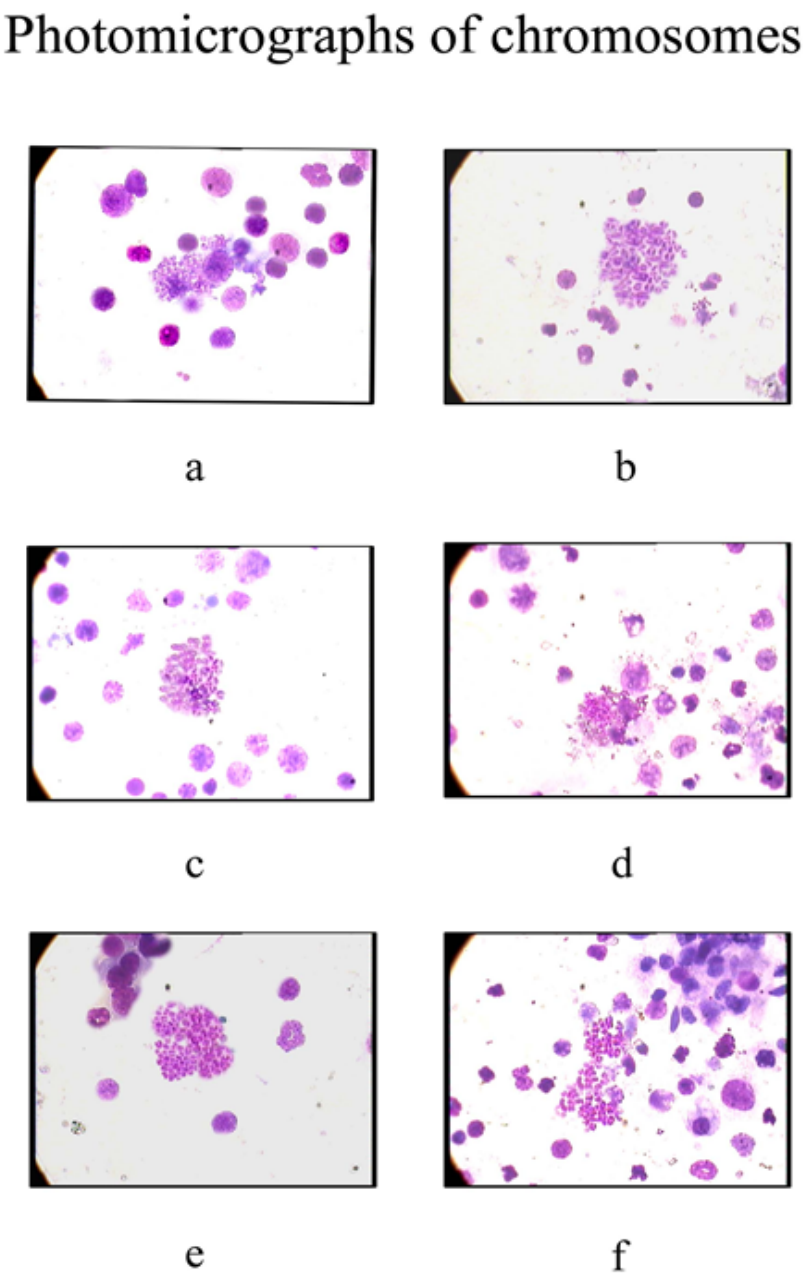


Figure 3. **(a)** Photomicrographs of chromosomes obtained from femur bone of albino rats from NC (1x1000 magnification). **(b)** Photomicrographs of chromosomes obtained from femur bone of albino rats from CP (1x1000 magnification). **(c)** Photomicrographs of chromosomes obtained from femur bone of albino rats from KSHRM, TED treated group (1x1000 magnification). **(d)** Photomicrographs of chromosomes obtained from femur bone of albino rats from KSHRM, TED x 5 treated group (1x1000 magnification). **(e)** Photomicrographs of chromosomes obtained from femur bone of albino rats from CSHRM, TED treated group (1x1000 magnification). **(f).** Photomicrographs of chromosomes obtained from femur bone of albino rats from CSHRM, TED x 5 treated group (1x1000 magnification).

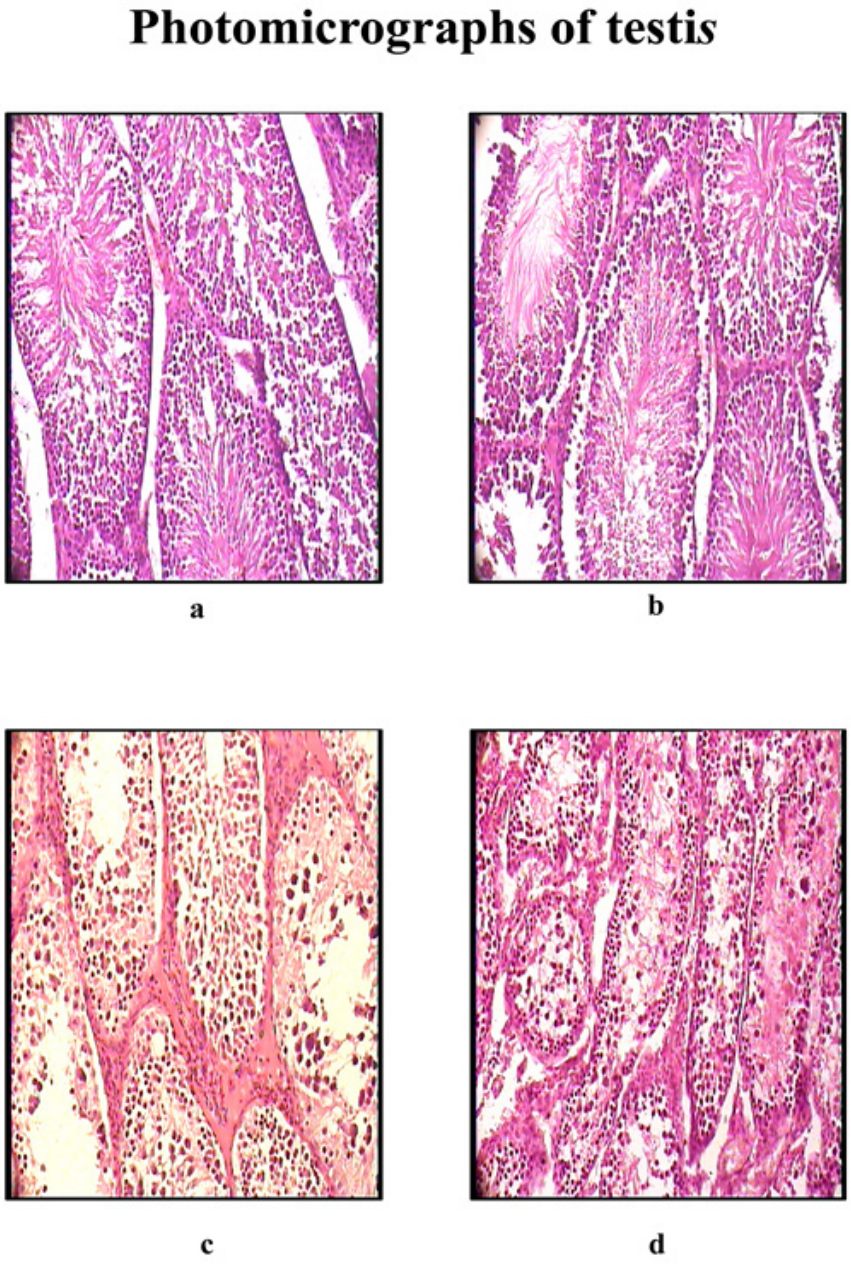


Figure 4. **(a)** Photomicrographs of representative sections of Testis of albino rats from Normal Control group (1×200 magnification) Note: Normal cytoarchitecture
(b) Photomicrographs of representative sections of Testis of albino rats from Normal Control group (1×200 magnification). Note: Normal cytoarchitecture
(c) Photomicrographs of representative sections of Testis of albino rats from Cyclophosphamide treated group (1×200 magnification). Note: Severe decrease in spermatogenesis and loss of cytoarchitecture
(d) Photomicrographs of representative sections of Testis of albino rats from Cyclophosphamide treated group (1×200 magnification). Note: Severe decrease in spermatogenesis and loss of cytoarchitecture.

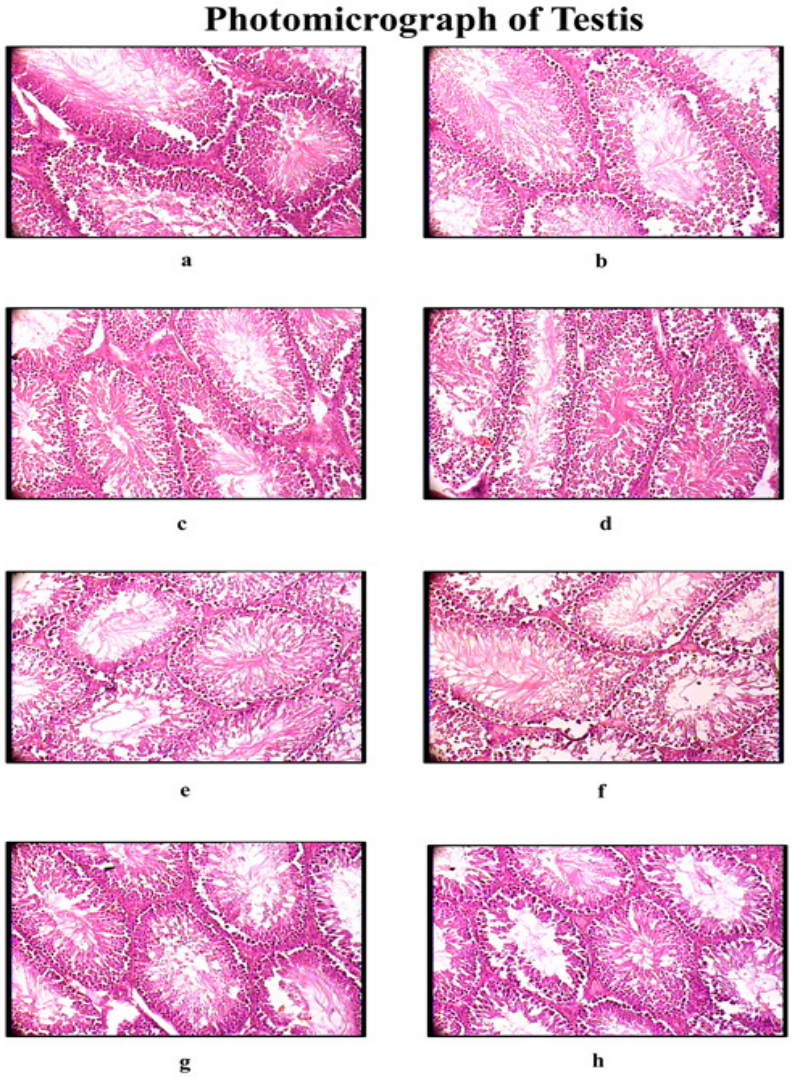


Figure 5. **(a)** Photomicrographs of representative sections of Testis of albino rats from KSHRM, TED treated group (1×200 magnification). Note: Normal cytoarchitecture
(b) Photomicrographs of representative sections of Testis of albino rats from KSHRM, TED treated group (1×200 magnification). Note: Normal cytoarchitecture
(c) Photomicrographs of representative sections of Testis of albino rats from KSHRM TEDx5 treated group (1×200 magnification). Note: Normal cytoarchitecture
(d) Photomicrographs of representative sections of Testis of albino rats from KSHRM TEDx5 treated group (1×200 magnification). Note: Normal cytoarchitecture
(e) Photomicrographs of representative sections of Testis of albino rats from CSHRM, TED treated group (1×200 magnification). Note: Normal cytoarchitecture
(f) Photomicrographs of representative sections of Testis of albino rats from CSHRM TED treated group (1×200 magnification). Note: Normal cytoarchitecture
(g) Photomicrographs of representative sections of Testis of albino rats from CSHRM TEDx5 treated group (1×200 magnification). Note: Normal cytoarchitecture
(h) Photomicrographs of representative sections of Testis of albino rats from CSHRM TEDx5 treated group (1×200 magnification). Note: Normal cytoarchitecture.

study in comparison to the control group. No mortality was observed in both the samples of *Rasamanikya* along with GG at TED and TED×5 dose levels during the experimental period. Weight gain was observed in all control and treated rats during genotoxicity study when compared with an initial body weight of rats (Table 1). But, the percent change in body weight pattern in treated groups did not differ significantly from the changes observed in the normal control group (Table 1). The effect of KSHRM and CSHRM on the shape of chromosomal aberration are shown in Tables 2 and 3. The effect of KSHRM and CSHRM on cytoarchitecture of head and tail of sperm are shown in Tables 4 and 5 respectively.

Seers of Ayurveda were well aware about the toxicity of metallic and mineral formulations when not followed pharmaceutical and clinical guidelines strictly as told by the classics. Hence, they documented possible untoward toxic effects and their treatment that can occur with the improper usage of metals or minerals[17]. They have documented specific procedures, therapeutic dosage, adjuvant and concurrent diet advice to avoid such ill effects. Being used for over a long period, these medicines are acknowledged as safe, which is the ultimate proof for their nontoxic beneficial effects. However, the use of metallic and mineral formulations has raised concerns and debate in the scientific community especially those preparations containing heavy minerals as an ingredient like arsenicals[18]. *Rasamanikya*, one of such mineral preparations is proven to be safe through acute and sub-chronic toxicity studies[19]. As a part of drug discovery, in vivo methods are second-line assays performed to support the safety of compounds. In vivo, mutagenesis detection is key for testing the genotoxic potential of anthropogenic chemicals produced for industrial or medical applications as well as of products present in our environment[20]. Guidelines are available for genotoxicity study of Ayurvedic medicines, i.e., OECD guidelines[21], CCRAS guidelines[22] and Schedule Y (D & C act)[23]. CP is well established drug to induce dominant lethal mutation, mononuclei, DNA damage and generation of free radicals or Reactive Oxygen Species (ROS) in vivo as well[24]. It is a potent genotoxicin the bone marrow micronucleus and sister-chromatid exchange assays but weakly genotoxic in the sperm morphology assay[25]. So, it is used as positive control drug in the present study. Colchicine is a microtubule disrupting agent which triggers apoptosis in rat Cerebellar Granule Cells (CGC)[26]. *Haratala* is an arsenical compound mentioned under Schedule E1 of Drugs and Cosmetics Rules 1945 and arsenic is generally considered as a poison having acute and chronic toxicity effects on the body[27]. Since, there is lack of safety reports available for such Ayurvedic formulations like *Rasamanikya* at the genetic level. The present study was undertaken to address reports of genotoxic effect due to administration of *Rasamanikya* prepared with two different media *Shodhita Haratala* for 60 days in rats. In one clinical trial, *Rasamanikya* was administered for 2 months with *Guduchi Ghana*, honey and *Ghrita* as an adjuvant. The same protocol for duration and adjuvant was followed in the current study[28]. A progressive increase in body weight of rats in all control and treated group rats suggested the anabolic effects of test drugs and devoid of any serious toxicity in rats even after administration at a higher dose level (TED×5) for 60 days indicative absence of any toxic degenerative potential.

Animals were injected with colchicine *ip* at the dose of 4 mg/kg body weight, on the 61st day to arrest dividing cells in metaphase. It is effective in causing metaphase stasis in matrix, thus used to arrest metaphase, when chromosome structure can be seen noticeably[29]. It interferes with microtubule polymerization and, therefore, affects mitosis so known as mitotic poison or spindle poison. It inhibits microtubule polymerization by binding to tubulin into microtubules leading to the inhibition of leukocyte migration and phagocytosis[30]. In vivo, CA assay in metaphase cells is one among the simplest and most sensitive test to assess the genotoxic profile of drugs in which direct visualization of the deleterious effects at the genetic level can be studied by microscopic observations. It is based on the structural unit involved that is whole chromosome or the single chromatid and the type of morphological alterations like breaking or rearrangements, etc. It is also suggested that CAs are early predictors of cancer risk. CP has produced chromosome aberrations in the given dose with relatively high frequencies of chromosome breaks, centric fusion compared with other types of chromosome abnormalities. Increased frequency of CAs like gap, ring formation and stickiness were also found in CP treated group. This may be due to the fact that almost all animals chromosomes are acrocentric[31]. These types of chromosomes have the exceptional facility to merge with each other. Only

structural aberrations were enumerated in CP treated group, with special emphasis on chromosome and chromatid gap, breaks and centric diffusions. Both the test drugs along with adjuvant did not produce any chromosomal aberration in bone marrow of albino rats at TED and TED×5 dose levels. Evaluation of bone marrow cellularity suggests that test drugs did not possess the mutagenic effect on rats at both the dose level.

Morphological abnormalities of sperm like head abnormalities (banana shaped, hookless, amorphous, folded head) and tail deformities (doubled tailed, coiled) were studied in current study. Wyrobek and others reported that large reductions in sperm number or mortality or large increases in sperm with abnormal shapes are associated with reduced fertility[32]. CP treated group observed maximum number of abnormalities in both head and tail in comparison with the control group. But, both the test drugs along with adjuvant showed almost same number of normal sperm as observed in the normal control group. They did not produce any sperm head and tail morphological abnormalities. These results may be due to *Rasayana* property of *Haratala*[33], *Guduchi* and *Ghrita*. Honey is also having *Yogawahi* and aphrodisiac (*Vrushya*) properties[34]. These drugs are known for having properties of *Rasayana* which act at the level of digestive fire (*Agni*), vitalizing the organic metabolism leading to an improved structural and functional pattern of the body.

Both the trial drugs along with adjuvant were studied on bone marrow cellularity in histopathology. The test drug did not affect the cell count of polychromatic normoblast, Erythrocytes and normoblast showing micronuclei at both dose levels studied, in comparison with the control group. In addition of this, both the drugs showed the almost same number of normal sperms as observed in the vehicle control group.

4. Conclusion

Results of the current study revealed that both the samples of *Rasamanikya* prepared with *Kushmanda Swarasa* and *Churnodaka Shodhita Haratala* along with *Guduchi Ghana* are safe at the genetic level. There is a lack of deformity in chromosomal aberration and sperm morphological abnormality assay in both samples. Hence, it can be concluded that therapeutic use of *Rasamanikya* along with *Guduchi Ghana*, honey and *Ghrita* as an adjuvantis safe at the genetic level when administered for sixty days at the therapeutic dose level.

5. Conflict of Interest

None.

6. Financial Support

ITRA, Jamnagar.

7. Acknowledgement

The authors acknowledge scholars of the department of rasa Shastra & Bhaisajya Kalpana and the department of pharmacology lab to provide all facilities. ITRA, Jamnagar.

8. References

1. Sharma SN. Rasa Tarangini, 11/88-89, Shastri K N, Hindi commentary. Varanasi: Motilal Banarasi Das Publication; 2009. p. 257.
2. Mehta M, Hundal SS. Assessment of genotoxic potential of arsenic in female albino rats at permissible dose levels. Toxicol Int. 2014; 21(1):24–28. https://doi.org/10.4103/0971-6580.128787. PMid:24748731. PMCid:PMC3989911
3. Jagtap CY, Ashok BK, Patgiri BJ, Prajapati PK, Ravishankar B. Acute and subchronic toxicity study of tamra bhasma (Incinerated copper) prepared from Ashodhita (Impurified) and Shodhita (Purified) tamra in rats. Indian J Pharm Sci. 2013; 75:346–352. https://doi.org/10.4103/0250-474X.117433. PMid:24082351. PMCid:PMC3783753
4. Sud S, Reddy S, Sujatha K, Honwad S. A comparative toxicological study of Rasamanikya prepared with three different methods. AYU. 2013; 34(3):309–315. https://doi.org/10.4103/0974-8520.123134. PMid:24501530. PMCid:PMC3902601
5. Chaudhari SY, Biradar S, Nariya M, Galib R, Prajapati PK. Acute and chronic toxicity of rasamanikya, an ayurvedic arsenical formulation in rats. Indian J Pharm Sci. 2018; 80(2):325333. https://doi.org/10.4172/pharmaceutical-sciences.1000361

6. Chaudhari SY, Nariya M, Galib R, Prajapati PK. Acute and sub-chronic toxicity of Tamra Bhasma (Incinerated copper) prepared with and without Amritikarana. J Ayurveda Integr Med. 2016; 7:23–29. https://doi.org/10.1016/j.jaim.2015.11.001. PMid:27297506. PMCid:PMC4910297
7. Gokarn RA, Nariya M, Patgiri BJ, Prajapati PK. Toxicological studies of Rasasindura, an ayuvedic formulation. Indian J Pharm Sci. 2017; 79(4):633–640. https://doi.org/10.4172/pharmaceutical-sciences.1000272
8. Deshpnde SW, Gandhi N. Drug and cosmetic Act 1940 and rules 1945. The medical devices rules 2017. Drug and magic remedies (objectionable advertisement) Act, 1954 Drugs (prices control) order 2013. Mumbai:Susmit publishers; 2018.
9. Anonymous. The Gazette of India: Extraordinary, Part II-Section 3-Sub-section (i). Ministry of health and family welfare. New Delhi; 2008. p.11.
10. Chaudhari SY, Jagtap C, Thakkar JH, Galib R, Prajapati PK. Assessment of genotoxic potential of Tamra Bhasma (incinerated copper). Int J Green Pharm. 2015; 9:175–179. https://doi.org/10.4103/0973-8258.161235
11. Jagtap CY, Chaudhari SY, Thakkar JH, Galib R, Prajapati PK. Assessment of genotoxic potential of hridayarnava rasa (A herbo-mineralo-metallic ayurvedic formulation) using chromosomal aberration and sperm abnormality assays. Toxicol Int. 2014; 21:242–247. https://doi.org/10.4103/0971-6580.155331. PMid:25948961. PMCid:PMC4413405
12. Savalgi PB, Patgiri BJ, Thakkar JH, Ravishankar B, Gupta VB. Evaluation of subchronic genotoxic potential of Swarna Makshika Bhasma. AYU. 2012; 33:418–422. https://doi.org/10.4103/0974-8520.108858. PMid:23723652. PMCid:PMC3665102
13. Paget, GE. Barnes JM. Toxicity tests. In: Laurence DR, Bacharach AL, editors. Evaluation of drug activities pharmacometries. Vol.1. New York: Academic Press, London; 1964. p. 50. https://doi.org/10.1016/B978-1-4832-2845-7.50012-8
14. OECD. Guidelines for the Testing of Chemical (483) — Mammalian spermatogonial Chromosomal Aberration Test, Published on 29 july 2016. Retrieved from: https://www.oecd.org/env/test-no-483-mammalian-spermatogonial-chromosomal-aberration-test-9789264264847-en.htm
15. Tijio JH, Whang J. Direct chromosomal preparation of bone marrow cells. Stain Technique. 1962; 37:17–20. https://doi.org/10.3109/10520296209114563. PMid:13921436
16. Wyrobek AJ, Bruce WR. Chemical induction of sperm abnormalities in mice. Proc. Nat. Acad. Sci. USA. Nov 1975; 22(11):4425–4429. https://doi.org/10.1073/pnas.72.11.4425 PMid:1060122. PMCid:PMC388734
17. Pandey S, Sharma V, Chudhari AK. Chelation therapy and chelating agent of Ayurveda. Int. J. Green Pharm. 2016; 10(3):143–150.
18. Kaushik S, Singh R, Yadav P, Galib R, Prajapti PK. Safety concerns on Ayurvedic herbomineral formulations-Myth or reality. J. Drug Res. Ayurvedic Sci. 2018; 3(4):234–241.
19. Chaudhari SY, Biradar S, Nariya M, Galib R, Prajapati PK. Acute and chronic toxicity of rasamanikya, an ayurvedic arsenical formulation in rats. Indian J Pharm Sci. 2018; 80(2):325–333. https://doi.org/10.4172/pharmaceutical-sciences.1000361
20. Guimarães AP, Guimarães AC, Alcântara DA, Cunha LR, Lima PL, Vasconcellos MC, et al. Chromosomal aberration test utilities in vitro and in vivo. Genotoxicity and DNA Repair. 2014:115–139. https://doi.org/10.1007/978-1-4939-1068-7_7. PMid:25488217
21. OECD. Guideline for testing of chemicals 483-Mammalian spermatogonial chromosomal aberration test. 2016. Retrieved from: https://www.oecd.org/env/test-no-483-mammalian-spermatogonial-chromosomal-aberration-test-9789264264847-en.htm
22. CCRAS. General guideline for safety/toxicity evaluation of ayurvedic formulations. New Delhi: Ministry of AYUSH, Govt. of India; 2018. Retrieved from: https://www.ayush.gov.in/docs/guideline_safety_toxicity.pdf
23. Deshpnde SW, Gandhi N. Drug and cosmetic AC,1940 and rules 1945. The medical devices rules 2017. Drug and magic remedies (objectionable advertisement) Act, 1954 Drugs (prices control) order 2013. Mumbai:Susmit publishers; 2018.
24. Abdella EM. Short term comparative study of cyclophosphamide genotoxicity administered free and liposome-encapsulated in mice. Iran J Cancer Prev. 2012; 5(2):51–60.
25. Simula AP, Priestly BG. Species differences in the genotoxicity of cyclophosphamide and styrene in three in vivo assays. Mutation Research/ Environmental Mutagenesis and related Subjects 1992; 271(1):49–58. https://doi.org/10.1016/0165-1161(92)90031-G

26. Bonfoco E, Ceccatelli S, Manzo L, Nicotera P. Colchicine induces apoptosis in cerebellar granule cells. Experimental Cell Research. 1995; 218(1):189–200. https://doi.org/10.1006/excr.1995.1147. PMid:7537689
27. Ballav S, Maity G, Mondal N. Toxicological and human risk assessment of Haratala (orpiment/AS2S3). Int J Res Ayurveda Pharm. 2019; 10(1):55–58. https://doi.org/10.7897/2277-4343.100113
28. Parekh D, Patgiri BJ, Bederakar P, Nariya M. Pharmaceutical standardization of Rasamanikya prepared by two different media shodhita Haratala and its clinical efficacy in Ekakustha (psoriasis). MD dissertation 2019. IPGT & RA, Gujarat Ayurved University, Jamnagar.
29. Fraser IEB. The use of colchicine and colcemid for metaphase stasis in the matrix cells of wool follicles. Aust J Biol Sci. 1963; 16(1):211–217. https://doi.org/10.1071/BI9630211
30. Mueller GA, Mary EG, Wanzer D. The effect of varying concentrations of colchicine on the progression of grasshopper neuroblasts into metaphase. Int J Cell Biol.1971; 48:259–265. https://doi.org/10.1083/jcb.48.2.253. PMid:5551659. PMCid:PMC2108180
31. Palestis BG, Brut A, Jones RN, Trivers R. B chromosomes are more frequent in mammals with acrocentric karyotypes: Support for the theory of centromeric drive. Proc Biol Sci. 2004; 271(Suppl 3):S22–S24. https://doi.org/10.1098/rsbl.2003.0084. PMid:15101408. PMCid:PMC1810000
32. Sharpe RM. Sperm counts and fertility in men: a rocky road ahead. EMBO Rep. 2012; 13(5):398–403. https://doi.org/10.1038/embor.2012.50. PMid:22491033. PMCid:PMC3343360
33. Sharma SN. Rasa Tarangini, 11/7, Shastri K N, Hindi commentary. Varanasi: Motilal Banarasi Das Publication; 2009. p. 245.
34. Bhavprakasha. Bhav Praksah Nighantu, Madhu Verga 22/3-4, Chunekar K. Varanasi: Chaukhamba Bharti Academy; 2013. p.772.