RESEARCH PAPER

Evaluation Of Genotoxicity Profile Of Garbhapala Rasa Prepared With Shataputa Naga Bhasma (A Lead-Based Herbo-Mineral Formulation) In Wistar Albino Rats

Bishan Kumar[1], Satish Solanki[2*], B. J. Patgiri[3], Prashant Bedarkar[4], Mukesh Nariya[5]

[1]AMO at PHC Sikri, Fridabad (Haryana)

[2]Associate Professor, Department of Rasashastra and Bhaishajya Kalpana, Jay Jalaram Ayurvedic Medical College, Godhra, Gujarat. Email: solankisatish04@gmail.com

[3]Head, Department of Rasashastra and Bhaishajya Kalpana, Institute of Teaching and Research in Ayurveda (ITRA), Jamnagar

[4]Department of Rasashastra and Bhaishajya Kalpana, ITRA, Jamnagar

[5]Head, Pharmacology Department, ITRA, Jamnagar

***Corresponding Author:** Dr. Satish Solanki, Associate Professor, Department of Rasashastra and Bhaishajya Kalpana, Jay Jalaram Ayurvedic Medical College, Godhra, Gujarat, India. Email: solankisatish04@gmail.com

ABSTRACT

The safety of herbo-mineral-metallic preparations is frequently questioned in the contemporary context of global standardization, particularly due to their heavy metal compositions. The United States (2002–2003) reported cases of adult lead poisoning linked to Ayurvedic medicines, even though these medications have been successfully utilized for therapeutic purposes without observable side effects for millennia. This study aimed to evaluate the genotoxic potential of *Garbhapala Rasa* prepared with *Shataputa Naga Bhasma* — a lead-based herbo-mineral formulation — using the in vivo micronucleus (MN) assay and chromosomal aberration assay in Wistar albino rats of both sexes. Results demonstrated no discernible increase in MN frequency or DNA damage in *Garbhapala Rasa*-treated animals compared to vehicle control groups, confirming that the formulation was not genotoxic under the experimental conditions and test system employed.

Keywords: *Garbhapala Rasa*, Genotoxicity, Herbo-mineral formulation, *Naga Bhasma*, Chromosomal aberration, Sperm abnormality

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INTRODUCTION

Garbhapala Rasa is a herbo-mineral Ayurvedic formulation first mentioned in the 17th-century text '*Rasa Chandanshu*'. It holds a reputable place in Ayurveda due to its claim to prevent abortions or miscarriages and ensure better nourishment to the fetus.[1] The formulation contains metals and minerals including '*Hingula*' (Cinnabar-HgS), '*Naga*' (Lead-Pb), '*Vanga*' (Tin-Sn) and '*Loha*' (Iron-Fe).

The herbal components of *Garbhapala Rasa* include '*Dalchini*' (*Cinnamomum cassia* L.), '*Ela*' (*Elettaria cardamomum* L.), '*Tejapatra*' (*Cinnamomum tamala* Buch-Ham), '*Shunthi*' (*Zingiber officinale* Mill.), '*Maricha*' (*Piper nigrum* L.), '*Dhanyaka*' (*Coriandrum sativum* L.), '*Chavya*' (*Piper retrofractum* Vahl.), '*Krishnajeeraka*' (*Carum bulbocastanum*), '*Draksha*' (*Vitis vinifera* L.), and '*Devdaru*' (*Cedrus deodara* Roxb.). All ingredients in equal quantity (1 *Karsh* each), except *Loha Bhasma* (1/2 *Karsha*), were triturated in the extract of *Vishnukranta* (*Clitoria ternatea* L.) for seven days. It is a *Kharaliya Rasayana* used in several complicated disorders of pregnancy, including abortions.[2]

Since antiquity, herbal remedies have played a significant role in human healthcare, and their significance has grown due to their apparently safe and efficient composition.[3] In Ayurveda, metallic herbal preparations known as '*Bhasma*' are prepared using the *Bhasmikarana* method, which is believed to transform the metal into a specially desired chemical compound, eliminating toxicity while providing requisite medicinal benefits.[4,5]

The efficacy of the *Bhasmikarana* procedure is validated using qualitative tests described in Ayurvedic texts, which guarantee that the final medication is extremely fine (tiny grains), has no metallic shine, and does not alloy with silver even at higher temperatures.[6,7,8,9]

Genotoxicity studies encompass a variety of in vitro and in vivo experiments designed to identify drugs or substances capable of damaging genetic material directly or indirectly. These assays are crucial for identifying the risk of DNA fixation and damage. Regulatory authorities worldwide require genotoxicity data as part of the safety evaluation for new drugs.[10] The same assays are used in early drug development stages to forecast potential heritable germ cell damage and carcinogenicity, given that genotoxic effects and carcinogenesis share similar mechanisms.[11]

In genetics, genotoxicity describes the property of chemical agents that damage the genetic information within a cell, causing mutations which may lead to cancer.[12] While genotoxicity is often confused with mutagenicity, all mutagens are genotoxic, whereas not all genotoxic substances are mutagenic. Chromosomal anomalies typically occur when there is an error in cell division following meiosis or mitosis.[13] They can be categorized as:

1. **Structural aberrations:** Gaps, Breaks, Dicentric chromosomes, Ring chromosomes, Deletion, Fragmentation, Stickiness/Pulverization, Exchange.
2. **Numerical aberrations:** Polyploidy (4n), Hypodiploidy (< 46 chr), Hyperdiploidy (> 46 chr).

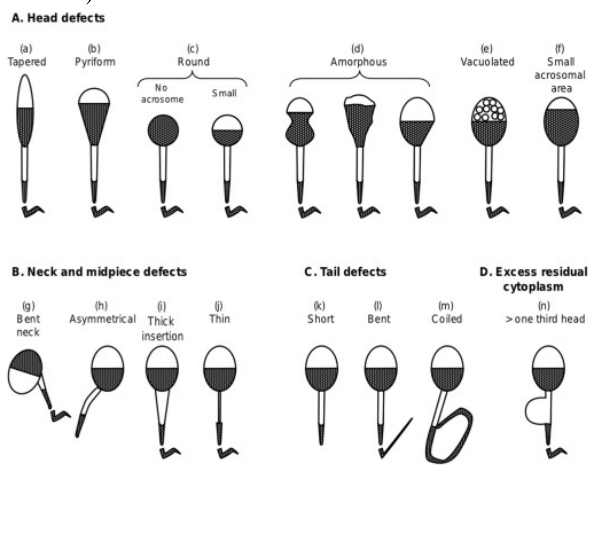

Fig. 1: Structural deformity in sperms (A. Head defects; B. Neck and midpiece defects; C. Tail defects; D. Excess residual cytoplasm)

Purpose of genotoxicity assays includes the ability to replicate tests with excellent statistical power, identify a range of genotoxic end-points, and detect a drug's potential for genotoxicity as early as the drug development stage.[14] In the current study, *Garbhapala Rasa* was evaluated for genotoxicity using sperm abnormality and chromosomal aberration experimental models to provide a sound scientific basis for its therapeutic use.

MATERIALS AND METHODS

Preparation of Garbhapala Rasa

Garbhapala Rasa was prepared according to classical guidelines in the Department of *Rasashastra* and Bhaishajya Kalpana, ITRA, Jamnagar. Raw materials — *Maricha* (*Piper nigrum* Linn.), *Tvaka* (*Cinnamomum cassia* Blume), *Tejapatra* (*Cinnamomum tamala*), *Ela* (*Elettaria cardamomum*), *Shunthi* (*Zingiber officinale*), *Pippali* (*Piper longum*), *Dhanyaka* (*Coriandrum sativum*), *Krishna Jiraka* (*Nigella sativa*), *Chavya* (*Piper chaba*), *Draksha* (*Vitis vinifera*), and *Devadaru* (*Cedrus deodara*) — were procured from the Pharmacy of IPGT & RA, Jamnagar. *Aprajita* (*Clitoria ternatea* Linn.) was collected from the Botanical Garden, GAU, Jamnagar. Mineral components — *Naga Bhasma* (100 *Puta*), *Vanga Bhasma*, and *Loha Bhasma* — were prepared in the same department following classical procedures. All herbal drugs were authenticated at the Pharmacognosy Laboratory of ITRA, Jamnagar. Powders (*Churna*) were prepared in accordance with the method described in *Sharangadhara Samhita*, Madhyama Khanda (6/1).

Animals

Wistar albino male rats (6–8 weeks old, weighing 200 ± 20 g) were procured from the animal house attached to the Pharmacology Laboratory, ITRA, Gujarat Ayurved University, Jamnagar. Animals were maintained under optimum laboratory conditions (ambient temperature: 23°C; relative humidity: 50–60%; 12-hour light/dark cycles). They were provided ad libitum access to VRK brand rat pellet feed (Keval Sales Corporation, Vadodara) and drinking water. The experiment was conducted after receiving approval from the Institutional Animal Ethics Committee (Approval No.: IAEC/23/2018/02) in accordance with CPCSEA guidelines, India.

Test Drug and Dose Fixation

The test drug was *Garbhapala Rasa*. The dose was calculated using the Paget and Barnes (1964) method based on the body surface area ratio.[15]

Clinical human dose × body surface area ratio = 1000 mg × 0.018 = 18 mg/200g = **90 mg/kg body weight**

Test drugs and vehicle were administered orally via gastric catheter fitted to a syringe nozzle, proportional to body weight.

Experimental Design

Male Wistar albino rats were randomly divided into five groups (n = 6 per group):

Group	Treatment
Group I	Vehicle Control
Group II	Cyclophosphamide (40 mg/kg, i.p.) — Positive Control
Group III	*Garbhapala Rasa* TED (33.75 mg)
Group IV	*Garbhapala Rasa* TED × 5 (168.78 mg)
Group V	*Garbhapala Rasa* TED × 10 (337.5 mg)

Vehicle and test drugs were administered for 90 consecutive days. Group II (positive control) received cyclophosphamide (40 mg/kg, i.p.) at 48 hours and 24 hours prior to termination. Body weight was recorded at weeks 2, 4, 6, 8, 10, 12, and on day 90.

Assays Performed

1. Chromosomal Aberration Assay (OECD 473 Guideline)

This assay was performed in male Wistar albino rats as per OECD 473 guideline.[16] On day 90, animals were fasted overnight. On day 91, colchicine (4 mg/kg, i.p.) was administered 1.5 hours prior to sacrifice to arrest dividing cells in metaphase.[18] Femoral bones were excised, cleaned of muscular tissue, and bone marrow was flushed into centrifuge tubes using Hank's Balanced Salt Solution (HBSS).

Hypotonic treatment was performed using 0.56% KCl for 10 minutes at room temperature, followed by fixation in cold Carnoy's fixative (methanol: glacial acetic acid, 3:1) for one hour with repeated changes. Slides were prepared by dropping cell suspension from a height of 30–40 cm onto cleaned slides, dried at 40°C, and stained with 5% Giemsa stain (pH 6.8) for 30 minutes.[20]

Slides were examined under a trinocular microscope (Carl Zeiss, Germany) using 100× oil immersion objective. A total of 1000 metaphase cells per group were analysed for chromosomal abnormalities including chromatid breaks, gaps, rings, stickiness, pulverization, centric fusion, and deletions.[21]

2. Sperm Abnormality Assay

Sperm morphological abnormalities were assessed using Wyrobek and Bruce's approach.[22] On day 91, animals were sacrificed. Both cauda epididymis were removed, placed in a watch glass containing 1 ml phosphate-buffered saline (pH 7.2), minced, and filtered through muslin fabric. A 1 ml suspension was stained with 2 drops of 10:1 Eosin Y solution for 30 minutes. Slides were prepared and stained with 5% Giemsa stain for 15 minutes. A total of 1000 sperm cells per group were analyzed for morphological abnormalities.

Statistical Analysis

Data are presented as Mean ± SEM. Differences between groups were assessed using Student's paired 't' test for paired data, and one-way ANOVA followed by Dunnett's multiple 't' test for unpaired data, using Sigma Stat software (version 3.1). A value of $P < 0.05$ was considered statistically significant.

RESULTS

Body Weight

Table 1 presents the effects of test drugs on body weight of male Wistar albino rats throughout the experimental period. A progressive and statistically significant increase in body weight was observed in all treated groups compared to their respective initial body weights.

Table 1: Effect of test drugs on body weight (g) of male Wistar albino rats during genotoxicity study

Duration	GR TED (33.75 mg)	GR TED × 5 (168.78 mg)	GR TED × 10 (337.5 mg)
Initial	204.667 ± 15.930	239.833 ± 7.530	243.833 ± 18.027
2nd Week	250.00 ± 11.240##	271.000 ± 10.405##	283.167 ± 16.290#
4th Week	250.500 ± 8.601###	276.000 ± 14.638##	243.833 ± 18.027#

6th Week	269.500 ± 9.319###	290.333 ± 12.816##	310.333 ± 11.968#
8th Week	241.500 ± 9.222#	313.833 ± 12.186##	317.000 ± 11.109#
10th Week	262.667 ± 8.523##	324.833 ± 11.640##	319.333 ± 12.060#
12th Week	295.167 ± 4.929###	333.833 ± 15.890##	326.500 ± 15.726##
% Change	44.21% ↑	39.19% ↑	33.9% ↑

Data: Mean ± SEM; #P < 0.01, ##P < 0.001 vs. initial body weight (paired 't' test). GR = *Garbhapala Rasa*; TED = Therapeutic Equivalent Dose.

Sperm Morphology

Table 2 shows the effects of test medications on the cytoarchitecture of sperm head and tail. The cyclophosphamide-treated group showed a significantly lower number of morphologically normal sperm, and a significantly higher number of hook-less, amorphous, banana-shaped, and coiled sperms compared to the control group. *Garbhapala Rasa* did not cause any significant abnormalities in sperm head or tail morphology at any dose level.

Table 2: Effects of test drugs on cytoarchitecture of sperm head and tail of Wistar albino rats

Group	Normal Shape	Amorphous Shape	Coiled Tail
Control	496.833 ± 0.792	0.00 ± 0.00	0.667 ± 0.333
CP*	398.500 ± 7.424	17.50 ± 3.074	17.833 ± 2.301
TED	492.500 ± 1.432	0.00 ± 0.00	1.833 ± 0.792
TED × 5	491.500 ± 1.765	0.00 ± 0.00	2.00 ± 0.856
TED × 10	491.000 ± 1.366	0.00 ± 0.00	2.00 ± 0.931

*CP = Cyclophosphamide (positive control). Data: Mean ± SEM; @ P < 0.01 vs. control (one-way ANOVA, Dunnett's post hoc test).

Micronuclei Assay

Table 3 presents results of the micronuclei assay in rat bone marrow. The cyclophosphamide-treated group displayed the greatest number of cells with micronuclei compared to the control group. *Garbhapala Rasa* did not significantly alter the number of cells with micronuclei at any dose level.

Table 3: Effects of test drugs on micronuclei in bone marrow of Wistar albino rats

Group	Normal Shape	Micronuclei
Control	499.500 ± 0.342	0.500 ± 0.342
CP*	490.667 ± 1.563	9.333 ± 1.563
TED	499.333 ± 0.333	0.667 ± 0.333
TED × 5	499.000 ± 0.365	1.000 ± 0.365
TED × 10	499.500 ± 0.342	0.500 ± 0.342

*CP = Cyclophosphamide (positive control). Data: Mean ± SEM; @ P < 0.01 vs. control (one-way ANOVA, Dunnett's post hoc test).

Chromosomal Aberration

Tables 4 and 5 show the effects of test drugs on chromosomal aberration in rat bone marrow. The cyclophosphamide-treated group showed the most chromosomal aberrations compared to the control group. *Garbhapala Rasa* did not cause any chromosomal abnormalities at any dose level.

Table 4: Effects of test drugs on structural chromosomal aberrations (Type I) in bone marrow of Wistar albino rats

Group	Chromatid Gap	Chromatid Break	Chromosomal Gap	Chromosomal Break	Exchange	Pulverization/ Stickiness
Control	–	–	–	–	–	–
CP*	++	++	++	++	++	++
TED	–	–	–	–	–	–
TED × 5	–	–	–	–	–	–

TED × 10	–	–	–	–	–	–

*CP = Cyclophosphamide; ++ = present; – = absent

Table 5: Effects of test drugs on structural chromosomal aberrations (Type II) in bone marrow of Wistar albino rats

Group	Deletion	Fragmentation	Ring	Centric Attenuation	Centric Fusion	Ring (secondary)
Control	–	–	–	–	–	–
CP*	++	++	++	++	++	++
TED	–	–	–	–	–	–
TED × 5	–	–	–	–	–	–
TED × 10	–	–	–	–	–	–

*CP = Cyclophosphamide; ++ = present; – = absent

Testicular Cytoarchitecture

Microscopic examination of transverse sections of testes from the normal control group revealed normal cytoarchitecture. The cyclophosphamide-treated group exhibited degeneration and partial necrosis of the tubular components (spermatocytes, round spermatids, elongated spermatids, and Sertoli cells), with tubules devoid of germ cells, and seminiferous tubules displaying multinucleated large spermatids. In comparison, *Garbhapala Rasa* treatment at all dose levels did not produce any negative effects on testicular cytoarchitecture.

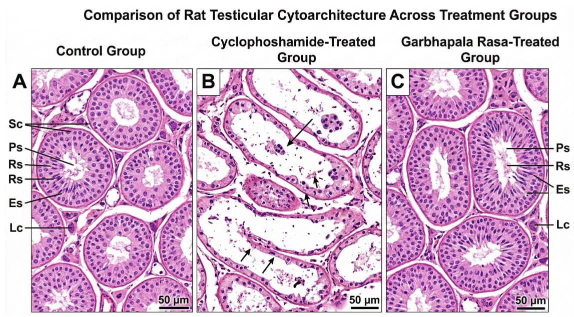

Fig. 2: Comparison of Rat Testicular Cytoarchitecture across treatment groups. (A) Control group showing normal seminiferous tubules; (B) Cyclophosphamide-treated group showing degeneration and partial necrosis; (C) *Garbhapala Rasa*-treated group showing normal cytoarchitecture comparable to control. Sc = Sertoli cells; Ps = Primary spermatocytes; Rs = Round spermatids; Es = Elongated spermatids; Lc = Leydig cells. Scale bar = 50 µm.

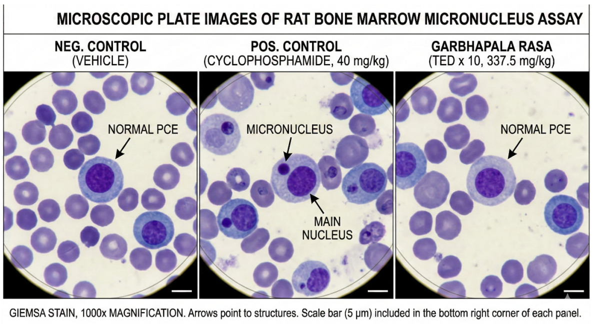

Fig. 3: Microscopic plate images of rat bone marrow Micronucleus (MN) Assay. Neg. Control (Vehicle) showing Normal PCE; Pos. Control (Cyclophosphamide, 40 mg/kg) showing Micronucleus and Main Nucleus; *Garbhapala Rasa* (TED × 10, 337.5 mg/kg) showing Normal PCE comparable to vehicle control. Giemsa stain, 1000× magnification. Scale bar = 5 µm.

DISCUSSION

The present study evaluated the genotoxic potential of *Garbhapala Rasa* a classical Ayurvedic herbo-mineral formulation containing lead-based *Naga Bhasma* — through in vivo chromosomal aberration and sperm abnormality assays. The safety of metallic preparations in Ayurveda has been a subject of global concern, particularly due to the presence of heavy metals. However, the Ayurvedic process of *Bhasmikarana* is believed to detoxify these metals through repeated incineration, converting them into biocompatible forms.

Body weight results demonstrated a progressive significant increase in all treatment groups compared to initial body weight ($P < 0.01$ to $P < 0.001$), which is consistent with healthy physiological development. No adverse effects on body weight were observed, indicating the absence of systemic toxicity at the tested doses.

Sperm morphology analysis revealed that *Garbhapala Rasa* at TED, TED × 5, and TED × 10 did not cause any significant reduction in normal sperm count, nor did it increase the number of morphologically abnormal sperms. These findings stand in clear contrast to the cyclophosphamide-treated positive control group, which showed significantly increased amorphous heads and coiled tails, confirming the sensitivity and validity of the test system.

The micronuclei assay results further support the genotoxic safety of *Garbhapala Rasa*. No significant increase in micronuclei frequency was observed in any of the treatment groups compared to the vehicle control, while cyclophosphamide produced the expected positive response with significantly elevated micronuclei counts. Similarly, chromosomal aberration assays (Tables 4 & 5) confirmed the absence of both structural and numerical chromosomal anomalies in *Garbhapala Rasa*-treated groups.(fig.1)

These results collectively indicate that the *Bhasmikarana* process effectively mitigates the genotoxic potential of lead in *Naga Bhasma*, transforming it into a therapeutically effective and non-genotoxic preparation. The findings are consistent with similar studies on other *Bhasma* preparations and support the traditional pharmacological safety claims of *Garbhapala Rasa*.

CONCLUSION

Garbhapala Rasa prepared with *Shataputa Naga Bhasma* was found to be non-genotoxic under the experimental conditions employed. Treatment at doses of TED, TED × 5, and TED × 10 for 90 consecutive days did not produce any significant chromosomal aberrations, micronuclei formation, or sperm morphological abnormalities in Wistar albino rats, as compared to the vehicle control group. These findings provide scientific validation for the traditional safety claims of this Ayurvedic herbo-mineral formulation and support its continued use as a therapeutic agent in Ayurvedic practice. Further long-term toxicological and clinical studies are recommended to fully characterize the safety profile of *Garbhapala Rasa*.

REFERENCES

1. Sharma H. *Rasa Yoga Sagara*, Vol. I. Varanasi: Krishna Academy. p. 374.
2. Anonymous. *Rastantra Rasavara Evum Siddhaprayogasangraha*, Vol. I, 17th ed. Ajmer: Krishna Gopal Granthamala; 2012. p. 278.
3. Singh SK, Jha SK, Chaudhary A, Yadava RD, Rai SB. Quality control of herbal medicines by using spectroscopic techniques and multivariate statistical analysis. *Pharm Biol*. DOI: 10.1080/13880200903059388.
4. Kumar A, Nair AG, Reddy AV, Garg AN. Availability of essential elements in *bhasma*: Analysis of Ayurvedic metallic preparations by INAA. *J Radioanal Nucl Chem*. 2006;270:173–80.
5. Wadekar MP, Rode CV, Bendale YN, Patil KR, Prabhune AA. Preparation and characterization of a copper based Indian traditional drug: *Tamra bhasma*. *J Pharm Biomed Anal*. 2005;39:951–5.
6. Kulkarni-Dudhgaonkar SB. In: *Rasaratna Samuchyaya*. Kolhapur: Shivaji University Publication; 1970. p. 158.
7. Patel NG. Ayurveda: The traditional medicine of India. In: Steiner RP, editor. *Folk Medicine: The Art and the Science*. Washington DC: American Chemical Society; 1986. p. 41.
8. Shastry KN, editor. *Rasatarangini*. Varanasi: Motilal Banarasi Das Press; 1979.
9. Svoboda RE, editor. *Prakriti: Your Ayurvedic Constitution*. Bellingham: Sadhana Publications; 1998.
10. Philomena G. Concerns regarding the safety and toxicity of medicinal plants – An overview. *J Appl Pharm Sci*. 2011;1(6):40–44.
11. Goncacak M, Mircigil C. Genotoxicity tests from biomarker studies to the regulations: National perspective. *J Pharm Sci*. 2009;34:217–32.
12. Kolle S. Genotoxicity and Carcinogenicity. BASF The Chemical Company. 2012. Available at: http://www.basf.com [Accessed 2013-03-16].
13. NHGRI. Chromosome Abnormalities. National Human Genome Research Institute; 2006. Available at: http://www.genome.gov/11508982.
14. Evans HJ. Cytological methods for detecting chemical mutagens. In: *Chemical Mutagens, Principles and Methods for their Detection*. 1976;4:1–29.
15. Paget GE, Barnes JM. Evaluation of drug activities. In: Laurence DR, Bacharach AL, editors. *Pharmacometrics*, Vol. 1. London: Academic Press; 1964. p. 50.

16. OECD Guidelines for the Testing of Chemicals, Section 4, Test No. 473: In Vitro Mammalian Chromosomal Aberration Test. Published 29 July 2016.

17. Wyrobek AJ, Bruce WR. The induction of sperm shape abnormalities in mice and humans. In: *Chemical Mutagens*. Eds. Hollander A, de Serres FJ. Vol. 5. Plenum Press; 1975. pp. 275–85.

18. Mueller GA, Mary EG, Wanzer D. The effect of varying concentrations of colchicine on the progression of grasshopper neuroblasts into metaphase. *J Cell Biol*. 1971;48:259–65.

19. Mueller GA, Mary EG, Wanzer D. The effect of varying concentrations of colchicine on the progression of grasshopper neuroblasts into metaphase. *J Cell Biol*. 1971;48:259–65.

20. Tijio JH, Whang J. Direct chromosomal preparation of bone marrow cells. *Stain Tech*. 1962;37:17–20.

21. Tijio JH, Whang J. Direct chromosomal preparation of bone marrow cells. *Stain Tech*. 1962;37:17–20.

22. Wyrobek AJ, Bruce WR. The induction of sperm shape abnormalities in mice and humans. In: *Chemical Mutagens*. Eds. Hollander A, de Serres FJ. Vol. 5. Plenum Press; 1975. pp. 275–85.