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Genotoxic, antigenotoxic and protective evaluation of Musalyadi churna by chromosomal aberration assay in Swiss albino mice

Research Article

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Abstract

Ayurveda is an ancient science of life that uses a wide range of formulations or herbal compounds to treat various diseases. Each drug must be scientifically, pre-clinically, and clinically evaluated in order to gain global acceptance. *Musalyadi Churna* (MSDC) is a classical Ayurvedic formulation in herbal powder dosage form consisting of eight ingredients of herbal origin and indicated in the treatment infertility. This herbal combination may be useful, however mixture of substances can be dangerous and genotoxic, causing harm to living cells' genetic material and resulting in mutations that induce serious issues for the host, such as cancer and birth defects; consequently, genotoxicity testing is essential. The study aimed to evaluate the genotoxic, antigenotoxic and protective properties of MSDC by using "Mammalian Bone Marrow Chromosome Aberration assay," Mice were administered MSDC orally for four weeks at a dose of 25 mg/25 g mouse. Cyclophosphamide was utilized as a mutagen, and a single dose of 50 mg/kg b.w. was given intraperitoneally 24 hours after the final dose of MSDC in the pre-treatment regimen and 24 hours before the first dose of MSDC in the post-treatment regimen. The study revealed that the MSDC at 1000 mg/Kg body wt. had no genotoxic activity against the bone marrow of Swiss albino mice. Using the same experiment, it demonstrated both protective and antigenotoxic properties against Cyclophosphamide- induced mutagenicity in mice. Further research should be to capitalize on *Musalyadi Churna'*s potential as an alternative source of natural compounds to treat genotoxic problems.

Key Words: Genotoxicity*, Musalyadi Churna,* Chromosomal aberration, Mice, Cyclophosphamide, Antigenotoxicity.

Introduction

Preclinical toxicity studies are used to determine the basic toxicological profile of novel chemical entities. To assess the safety and efficacy of novel chemical entities, toxicological data are employed. The evaluation of new chemical entities' genotoxicity potential is one of the most essential areas of safety pharmacology. (1) Genotoxicity research is carried out in both nonclinical and clinical settings. As part of the safety evaluation process, regulatory bodies all over the world demand data on NCE's genotoxic potential.(2) Furthermore, OECD guidelines mandate that a medicine molecule's genotoxicity be assessed in order to ensure its safety.(3) For global acceptance, each drug must be scientifically, pre-clinically, and clinically evaluated, according to modern science.(4) The primary goal of genotoxicity studies is to determine the safety and efficacy of new chemical entities prior to their commercialization, to establish a quantitative estimate of chemical agents' contribution to the prevalence of genetic illnesses, and to describe cancer risk. Ayurveda is an ancient science of life which cites many formulations or herbal compounds to treat various types of ailments. Due to the limitations of modern medicine and therapies, it is in need of scientific and research documentation on traditionally used and newly derived herbal substances, and Ayurveda medicine provides a ray of hope. *Churna* is the most commonly prescribed dosage type in Ayurvedic system of medicine.(5) *Musalyadi Churna* (MSDC) is the most widely used classical Ayurvedic formulation in herbal powder dosage form mentioned in *Bharat Bhaishajya Ratnakar* treatise and indicated in the treatment of impotency and infertility, both of which are major global concerns.(6) It is a polyherbal blend of seven different herbs, including *Shweta Musali* (*Chlorophytum borivilianum* Sant. & Fern.)*, Vidarikanda* (*Pueraria tuberosa* DC), *Guduchi* (*Tinospora cordifolia* (Willd)Miers ex Hook. f. & Thom), *Kapikacchu* (*Mucunna prurita* Hook.), *Gokshura* (*Tribulus terrestris* Linn.), *Shalmali* (*Bombax malabaricum* DC) and *Amalaki* (*Phyllanthus emblica* Linn). It was suggested that this formulation be taken orally with a vehicle such as cows' ghee or cows' milk. (7) This herbal combination could be beneficial, but chemical concoctions can be detrimental and genotoxic,

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causing damage to the genetic material of living cells and leading in mutations that create major issues for the host, such as cancer and birth defects; therefore, genotoxicity testing is essential. However, research on some of the ingredients in *Musalyadi Churna* has shown that they have antioxidant, immunomodulator, anticancer and genoprotective properties.(8)(9) and also has been characterized to contain glycosides, protein, amino acids, saponins, steroids, flavonoids alkaloids, carbohydrates, fixed oils and fats, tannins and phenols. (10) But there have been no scientific study carried out to assess the genotoxicity and genoprotective properties of *Musalyadi churna.* Hence, in the current study, *Musalyadi churna* was evaluated for its genotoxicity against bone marrow cell by using chromosomal aberration assay in Swiss albino mice. The study also uses the same assay to see if the *Musalyadi churna* has any antigenotoxic or protective properties against cyclophosphamide (CYP)-induced genotoxicity in mice and the generated evidence will be useful for future studies.

Materials and Methods

Experimental Animals

This study was conducted as per "OECD guideline for the testing of chemicals - Mammalian Bone Marrow Chromosome Aberration Test-475."(11) After taking the approval of Institutional Animal Ethical Committee (IAEC approval Research Project No. {10/1920} dated 13/07/2019), Swiss Albino Mice of both sexes weighing 25-30 g and aged 10-14 weeks were used in the study. They were supplied by Animal House of the APT research Center (registration number 40/PO/ReBiRc/S/99/CPCSEA), Pune, India where also the experimental study was conducted. Mice were acclimated for one week prior to the start of the experiment. The animals were kept in stainless steel polypropylene hanging cages with wood chip bedding in an experimental room with temperature (37.2^{o}C), humidity (50±5%), and light regulated conditions (12:12 light and dark cycle). The animals were fed a conventional solid pellet diet and were given water ad libitum. At the start of treatment and every week following that, food consumption were assessed per cage. Each cage was given specific amounts of food and the leftovers were measured the next day to determine the difference, which was then used to compute the daily food consumption

Chemicals

Cyclophosphamide [(Sigma Aldrich PVT LTD) (CAS no. 6055-19-2)] was procured and dissolved in distilled water from Sigma-Aldrich PVT LTD (Banglore, India). A single intraperitoneal dose of 50 mg CYP/Kg b.wt was administered to mice.

Preparation of test drug

Musalyadi Churna was formulated according to the *Bharat Bhaishajya Ratnakar*, a classical Ayurvedic treatise.(12) Table No. 1 lists the *Musalyadi Churna* ingredients, along with the parts employed and their proportions. To prepare *Musalyadi Churna*, all of the raw ingredients were acquired from a trusted source and powdered separately to a fine powder before being completely blended together.

Table 1: Ingredients and quantity of *Musalyadi Churna*:

Sr. No.	Ingredients	Part Used	Quantity
1	*Shweta Musali* (*Chlorophytum borivilianum* Sant. & Fern.)	Rhizome	1 Part
2	*Vidarikanda* (*Pueraria tuberosa* DC	Rhizome	1 Part
3	*Guduchi Satwa* (*Tinospora cordifolia* (Willd)Miers.)	Stem Starchy extract	1 Part
4	*Kapikacchu* (*Mucunna prurita* Hook.)	Seed	1 Part
5	*Gokshura* (*Tribulus terrestris* Linn.)	Fruit, Root	1 Part
6	*Shalmali* (*Bombax malabaricum* DC)	Root	1 Part
7	*Amalaki* (*Phyllanthus emblica* Linn.)	Fruit	1 Part
8	*Khanda Sharkara* (*Saccharum officinarum* L.)	-	1 Part

Dose selection and preparation

The prepared powder of *Musalyadi Churna* (MDC) weighed precisely in the required dose in mg/kg b.wt. It was then dissolved in 10 ml of a vehicle, such as Cows-Ghee and Cows-Milk in equal parts. The dose for this experiment was calculated using the limit test dose range of the OECD 475 guideline and converted to an animal dose using the mice conversion factor based on body surface area ratio(13) i.e 1000 mg/kg/body weight/day dose of study drug used in the study. Prior to dosing, the weight of each individual rat was recorded, and the dosage volume was adjusted accordingly.

Experimental Design

The experimental design for the evaluation of the genotoxic, antigenotoxic and protective activities of the MSDC is summarized in Table 2. Mice were randomly assigned to five main experimental groups. Negative control group animals received distilled water (T1) and Vehicle Control (T2) were treated with *Goghrita* (Cows Ghee) and *Godugdha* (Cows Milk). The mutagen Cyclophosphamide was used as a positive control (T3) at a dose of 50 mg/kg mouse. For genotoxicity testing, the mice were given a higher limit test dose of MSDC at 1000 mg/kg mouse (T4) for four consecutive weeks. Mice were given two types of treatment, pre- and post-treatment, based on the time of administration, in an antigenotoxicity study to assess MSDC's preventive and therapeutic potential. To assess the preventive potential of MSDC, mice were given MSDC for four weeks in a row, followed by CYP treatment 24 hours later. To assess MSDC's therapeutic potential, mice were given CYP first, followed by MSDC treatment (for four weeks) after 24 hours from the end of the CYP injection.

Table 2: Grouping and posology for Genotoxicity study and Anti-genotoxicity study

Treatment Groups(n=6)	Drug specifications	Dose	Study Duration	Route of Administration
T1- Negative Control	-	-	28 days	-
T2- Vehicle control	*Godugdha, Goghrita*	10 ml/Kg b. wt	28 days	Oral
T3- Positive control	CYP Single dose administration and cell harvesting after 24 hrs.	50 mg /Kg b. wt	28 days	Oral + i.p
T4-Only Test group	MSDC	1000 mg/ Kg b. wt	28 days	Oral
T5- Pre-treatment (Preventive group) (MSDC+CYP)	To evaluate preventive potential of MSDC mice were given first MSDC then CYP treatment after 24 hr from the end of treatment of MSDC	1000mg/Kg+ 50 mg/ kg b. wt	28 days	Oral + i.p
T6- Post-treatment (Therapeutic group) (CYP+MSDC)	To evaluate therapeutic potential of MSDC, mice were given first CYP, then MSDC treatment after 24hr from the end of injection with CYP	50 mg/kg+ 1000mg/Kg b. wt	28 days	i.p+ Oral

i.p- Intraperitoneally, CYP- Cyclophosphamide, b.wt- Body Weight

Chromosome aberrations (CAs) assay:

Mice were sacrificed one day the after the end of treatment. The standard technique for preparing bone marrow for metaphase cells was used.(14) In brief, mice were given an intraperitoneal injection of colchicine two hours before being killed by cervical dislocation. Bone marrow cells were extracted from both femurs after flushing with 0.56 % KCl and incubated for 20 minutes at 37°C. Fresh and chilled Carnoy's fixing was used to disperse centrifuged cells. The slides were developed by air drying the cells after placing them on clean, cold slides and stained them with 5% buffered Giemsa (pH. 6.8). A light microscope at 2500 X magnification was used to evaluate 100 well-spread metaphases per animal for chromosomal aberrations.

Statistical Analysis

Statistical methods, such as the paired t-test, were used to assess the change in body weight. For all treated groups, the results were presented as mean standard error of mean for six mice of both sexes in each group using SPSS Software (Version 27). To compare different treatment groups, the data was statistically evaluated utilizing analysis of variance (ANOVA) and the Bonferroni multi range testing. $P<0.05$ was used as the statistical significance level. The following formula was used to estimate the suppression of mutagenicity: (Percentage of aberrant cells in group A– Percentage of aberrant cells in group B)/ (percentage of aberrant cells in group A) ×100 whereas A: represents CYP treated groups only, and B: represents CYP + MSDC treated groups.(15)

Observations and Results

Table 3: Body weight of Swiss Albino Mice during Genotoxicity and Antigenotoxicity study

Groups	Male		Female		Total		Actual change in body weight (g)	Actual Change in %
	Before treatment	On 28th Day	Before Treatment	On 28th Day	Before treatment	On 28th Day		
NC (T1)	26.83±3.25	42±2.65	30±2.65	38±3.46	28.42±3.17	40±3.21	11.58±0.04	28.95%
VC (T2)	31±0.5	38±1.73	27.83±4.25	34±2	29.42±3.22	36±2.52	6.58±0.7	23.24%
PC (T3)	28.17±1.15	43.67±2.52	25.83±0.6	36±3.61	27±1.55	39.83±4.6	12.83±3.05	25.07%
Only Drug (T4)	27.67±2.02	34.33±1.15	26.83±2.75	36.67±3.06	27.25±2.21	35.5±2.22	8.25±0.01	7.31%
Pre Treatment (T5)	29.17±0.76	30.33±3.51	25.83±2.02	29±5.2	27.5±2.28	29.67±3.68	2.17±1.4	32.21%
Post Treatment (T6)	27.33±3.55	38.33±3.06	28.67±0.29	36.4±0.69	28±2.37	37.37±2.05	9.37±0.32	18.28%

NC - *Negative Control,* ***VC-****Vehicle control,* ***PC-*** *Positive control*

Figure 1: Body weight of Swiss Albino Mice during genotoxicity and antigenotoxicity study

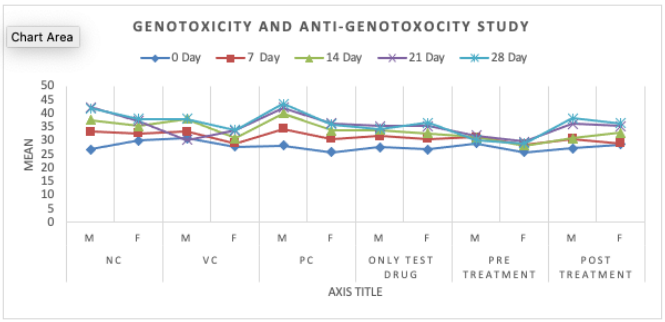


Figure 2: Data of food consumption by Swiss albino mice during Genotoxicity and Antigenotoxicity study

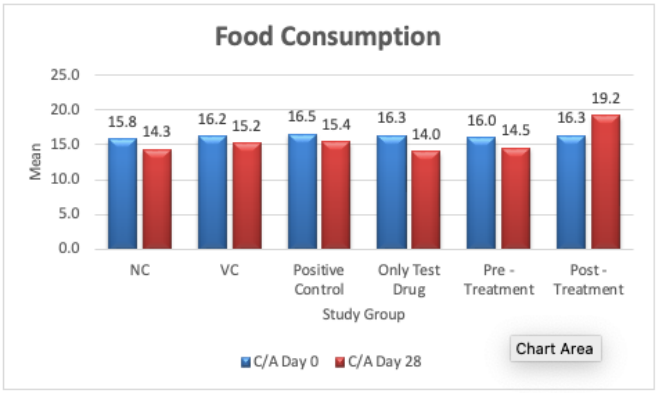


Table 4. The recorded frequency of chromosomal aberrations in somatic cells of mice treated with Cyclophosphamide and primed with MSDC:

Treatment Groups	Normal Metaphases (%)	Abnormal Metaphases (%)
T1- Negative Control	559(93.16)	41(6.83)
T2- Vehicle control	562(93.66)	38(6.33)
T3- Positive control	422(70.33)	178(29.66)
T4-Test group	561(93.5)	39(6.5)
T5 Pre-treatment	567(94.5)	33(5.5)
T6- Post-treatment	568(94.66)	32(5.33)

Table no. 5 Total no. and percentage of metaphases with different types of CAs

Treatment	Gap no. (%)	Chromatid break/fragment no.(%)	Chromosome Break/fragment no.(%)	Chromatid Exchange no.(%)	Chromosome Exchange no. (%)	Mean% ±SEM	Reduction %
NC	13(2.16)	14(2.33%)	5(0.83)	5(0.83)	4(0.66)	8.2±2.177	-
VC	12(2)	11(1.83)	6(1)	4(0.66)	5(0.83)	7.6±1.631	-
PC	58(9.66)	55(9.16)	32(5.33)	19(3.16)	14(2.33)	35.6±9.037	-
MSDC	15(2.5)	8(1.33)	11(1.83)	2(0.33)	3(0.5)	7.8±2.437	-
Pre -treatment	12(2)	7(1.16)	8(1.33)	3(0.5)	3(0.5)	6.6±1.691	81.46%
Post- treatment	10(1.66)	11(1.83)	5(0.83)	3(0.5)	4(0.66)	6.7±1.631	80.92%

Total 600 metaphase cell were examined in 6 animals in each experimental group. , PC- Positive control, VC-Vehicle control. NC - Negative Control, CAs-chromosomal aberrations

Table no 6: Percentage of Chromosomal Aberration in mice of both sexes|

Groups	Maximum % Aberration in Male	Maximum % Aberration in Female
Negative control	11%	8%
Vehicle control	8%	7%
Positive control	31%	35%
Test group (only MSDC)	8%	7%
Pre-treatment group	7%	7%
Post-treatment group	7%	7%

Figure 3: Graph showing percentage of Chromosomal aberration in male and female mice

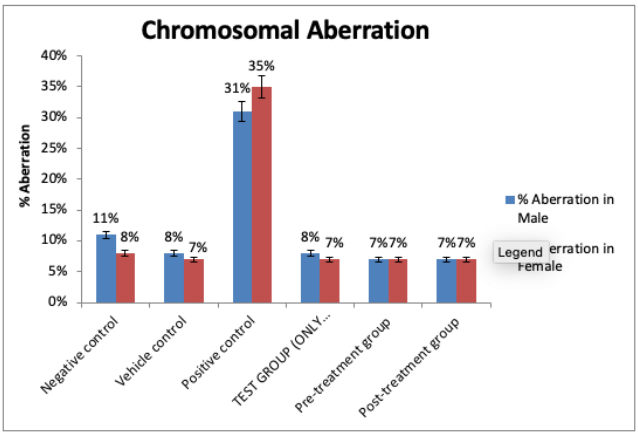
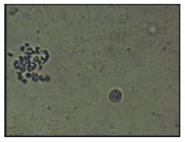
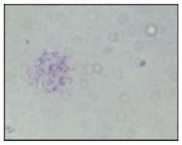
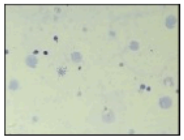
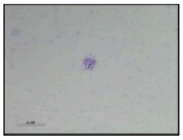
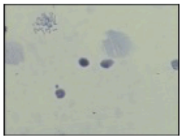
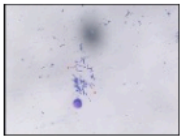
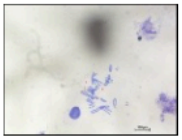


Figure 4: Images during Chromosomal abberation study in Swiss albino mice

(a)- NC chromosomes	*(b)- VC chromosomes*	*(c)- Test group chromosomes*	*(d)- Pre-treatment chromosomes*	*(e)- Post-treatment chromosomes*	*(f)- Positive control treated group*	*(g)- Positive control treated group*
						

Positive control (Cyclophophamid) treated groups showing abnormal chromosomes

Discussion

Plant-based medications have long been used in ancient and modern societies to treat various ailments. (16) Genotoxicity and mutagenicity testing is an essential part of evaluating a chemical's hazard for regulatory purposes. Different endpoints must be considered when assessing genotoxicity and/or mutagenicity: aside from inducing point mutations, a compound can also cause changes in chromosomal number or chromosomal structure (Splits, deletions, and rearrangements). Genotoxicity is a term used in genetics to describe a harmful effect on a cell's genetic material (DNA, RNA) that impair its integrity.(17) In current study genotoxicity and anti-genotoxicity activity of *Musalyadi churna* were assessed in Swiss albino mice of both sexes using the OECD-475 guideline's 'Mammalian Bone Marrow Chromosome Aberration assay.' This method is used to determine structural chromosome abnormalities in animal bone marrow cells, most commonly in rodents, caused by test drug. Bone marrow cells that are actively proliferating provide the most information on the effect of any test compound. It takes roughly seven cell division cycles to shift from proerythroblast to erythrocytes. Changes in chromosome structure caused by chromosome material breakage or exchange are referred to as chromosomal aberrations. Although the mass of chromosomal aberrations is harmful, many of their associated aberrations are viable and can have somatic or inherited genetic consequences. The presence of chromosomal aberrations in a high frequency suggests the possibility of carcinogenesis.(18) Cyclophosphamide (CYP) was utilized as a positive control in this study since it is used throughout many genotoxicity screening systems to assess drug toxicity. It is a well-known bifunctional alkylating substance that is commonly used in cancer chemotherapy, antineoplastic therapy, and the treatment of non-malignant disorders.(19) It also has a broad spectrum of cytotoxicity to normal cells in experimental animals and humans as well as possess genotoxicity when metabolically activated.(20) Body weight is an important factor in determining an animal's health. Body weight loss is frequently the first sign of an adverse effect. When a dose causes a 10% or greater decrease in body weight, it is considered toxic. In the current study, all of the mice of both sexes gained comparable amounts of weight, and no animal lost weight or became overweight, implying that *Musalyadi Churna* had no adverse effects on the mice's overall health, metabolic growth, or appetite. (Table 3, Figure 3). It was also revealed that the mice's food consumption followed a consistent and consistent pattern from the time of acclimatization to the end of the experiment. It illustrates that the study drug had no adverse effects on the mice growth and development. (Figure 2). In this analysis, various types of chromosomal abnormalities were detected. These anomalies are divided into structural, numerical, and other types. Chromosome aberrations (Gap, Chromatid break/fragment, Chromatid exchange, Chromosome exchange, etc.) evidenced in mice bone marrow cells from all groups were counted and tabulated in Table 5. Figure 3 depicts a graphical representation of the data.

The suppression of mutagenicity was calculated shown in Table 5. The percentage of CAs reached 35.6 % after treatment with CYP compared with the control group (8.2%). After treatment, this value increased by 4.34 folds when compared to the control group's value. CYP is a mutagenic agent in somatic cells, per this finding. According to the results of MSDC pre-treatment experiments, MSDC significantly reduced the incidence of CAs during treatment periods when compared to the corresponding group treated with CYP alone. After one day, the suppression rate reached 81.46 %, indicating that MSDC had a significant protective effect. The results of the MS post-treatment experiments, on the other hand, revealed that the frequency of CAs was significantly reduced after one day of treatment with MS compared to the corresponding groups treated only with CYP. During the treatment period, the reduction rate reached 80.92 %, indicating that MSDC had a potential therapeutic effect. The results revealed that the mutagen CYP as the positive control (T3) had the highest incidence of chromosomal aberrations in the experiment, whereas the genotoxic treatment test group (T4) at 1000 mg/Kg including the negative control treatments (T1) had the lowest incidence aberrations. Notably, both the protective (T5) and therapeutic (T6) treatment groups showed lower chromosomal aberration counts than the positive control group. The frequency of chromosomal aberrations in the control and treated groups was compared using Bonferroni's multiple comparisons test. As CYP was used to induce mutations, it was expected that as a mutagen, it would result in a higher number of chromosomal aberrations. The Bonferroni test

confirmed that the positive control (T3) had a significantly higher count than the other treatments, demonstrating its potent mutagenicity, which effectively generated chromosomal aberration in the mice. In previous research, cyclophosphamide at a dose of 135 mg/kg caused a significant rise in the frequency of micronuclei in male mice's polychromatic erythrocytes. (21) Furthermore, the percentage of chromosomal abnormalities was 59.33 in mice given 50 mg/kg body weight cyclophosphamide.(22) The chromosomal aberration score for the genotoxic test group (T4) was the lowest, but the count was not significantly different from the negative controls (T1). It was also lower than the positive control and the other treatments by a significant margin. This result indicated that the study drug *Musalyadi churna* had no significant genotoxic activity against the mice chromosomes, even at a greater dose of 1000 mg/kg. One of the MSDC ingredients, *Gokshura* (*Tribulus terrestris*), has been reported to have genotoxic properties.(23) However, due to the synergistic effect of all MSDC ingredients, the genotoxic effect of *Tribulus terrestris* may be reduced.

The small estimate of chromosomal aberrations spotted in the control treatments was most likely due to naturally occurring chromosomal aberrations in the cell. (24) Prior to MSDC treatment, the mice were given a CYP mutagen to test for antigenotoxicity. After MSDC administration, the frequency of chromosomal abnormalities was lowered. It indicates that the MSDC's endogenous compounds must have repaired the DNA damage caused by CYP, demonstrating antigenotoxicity. Moreover, the T6 group showed decreased chromosomal aberration frequencies which were significantly lower than the positive control group (Table 4). DNA metabolism and repair, inhibiting tumour progression, detoxifying carcinogenic chemicals, or regulating gene expression, altering replication, and inducing apoptosis are only some of the processes through which natural compounds might cause antigenotoxic effects.(25) The mice in the protective group (T5) received MSDC before being exposed to the mutagen. When mice erythrocytes are treated with CYP, the MSDC should inhibit the formation of chromosomal aberrations, indicating protective effect. The MSDC protective compounds may be intended to function by altering the mutagen CYP's activation or by limiting the actions of superactive oxygen species or detoxifying by modifying xenobiotic metabolism through absorption. The powder's active components may interact directly with CYP without altering DNA molecules. Several human genetic illnesses are caused by chromosomal damage and related events, although there is strong evidence that these lesions and events induce cancer in individuals and experimental systems by triggering mutations in oncogenes and tumour suppressor genes. *Amalaki,* (*Phyllanthus emblica*) one of the ingredients in MSDC, was reported to protect against adriamycin and chromium-induced genotoxicity in a previous study. Another study found that *Phyllanthus emblica* could be a viable solution for protecting mouse somatic cells from cyclophosphamide-induced genotoxicity.(26) It is a *Rasayana* herb that has anti-oxidant activities. Antioxidants protect the body from the harmful effects of free radicals. Emmiconin A, embliconin B, punigluconin, and peunculagin are tannoid principles that have been found to exhibit antioxidant action in vivo and in vitro.(27) Previous research has shown that other MSDC ingredients, such as *Chlorophytum borivilianum* Sant. F, *Tinospora cordifolia* (Willd)Miers, and *Bombax malabaricum* DC, have an antigenotoxic or antimutagenic effect. (28)(29)(30)

Conclusion

The chromosomal aberration assay significantly revealed that the herbal compound formulation *Musalyadi Churna* had no genotoxic activity against Swiss albino mice bone marrow. By the same assay, the formulation, on the other hand, demonstrated both protective and antigenotoxic properties against Cyclophosphamide-induced mutagenicity in mice. Based on the findings, *Musalyadi Churna* is a potential candidate as a protective agent against cyclophosphamide-induced genotoxicity in mice somatic cells. Cyclophosphamide and *Musalyadi Churna* combined treatment hold promise as a safe and effective chemotherapeutic strategy. Further research is necessary to capitalize on *Musalyadi Churna'*s potential as an alternative source of natural compounds to treat genotoxic problems.

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Acknowledgement: Nil

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