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Research Article

Antigenotoxic Evaluation of *Spathodea campanulata* Flower Extract against Doxorubicin Induced Genotoxicity by Chromosomal Aberration and Micronucleus Assay in Albino Rats

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ABSTRACT

Doxorubicin (DOX) is a broad-spectrum anthracycline chemotherapeutic agent used in human cancers but exerts myelosuppression and genotoxicity as an imperative adverse effect due to increased levels of oxidative damage, inflammation and apoptosis. Doxorubicin has a clastogenic tendency. Antioxidant materials of natural origin are reported to have anticlastogenic effects. *Spathodea campanulata* is abundant in antioxidants with high levels of phenol, alkaloids, and flavonoids. Therefore, assuming these qualitative aspects for being a probably effective chemoprotectant and antigenotoxic substance, the hydroalcoholic extract of *S. campanulata* flower (SCFE) was evaluated for genotoxicity in albino rats induced by DOX using chromosomal aberration and micronucleus assay. SCFE has been found to allay chromosomal aberrations, micronuclei formation, DNA damage and apoptosis in bone marrow forte induced by DOX administration. *In-vivo* chromosomal aberration assay showed that incidents of aberration significantly reduced post-treatment. Pre-treatment with SCFE (200 and 400 mg/kg) reduced the micronucleus polychromatic erythrocytes (MNPCE) and resulted in inhibition of micronuclei formation. PCE/NCE ratio has been found to be decreased in disease control animals while improved in the SCFE treatment group animals.

SCFE had an excellent recovery effect on the genotoxicity caused by to anticancer drug doxorubicin. The study advocates that SCFE has encouraged chemoprotective efficacy against DOX-induced toxicities. Further studies including characterization of active moiety shall shape its future indication as an adjuvant in cancer chemotherapy.

Keywords: Doxorubicin, *Spathodea campanulata,* Chromosomal aberration, Micronucleus assay, Genotoxicity, Antioxidant.

INTRODUCTION

Preclinical evaluation of novel chemical entities for antigenotoxic potential is one of the most crucial areas for safe and exerting relatively lesser adverse effects than chemotherapy in cancer treatment.[1] Genotoxicity studies are used for the determination of the magnitude of genetic risk imparted to humans or animals under a specified level of exposure or administration of chemicals or environmental agents. Drug-induced genotoxicity to normal cells leading to secondary malignancies could be one of the most undesired consequences of chemotherapy to treat cancer.[2] Uncontrolled patterns of multiplication of cancerous cells are affected by different phases of the cell cycle. Many of the anticancer drugs target the DNA of the multiplying cells, resulting in damage to cancerous cell DNA as well as the DNA of normal cells. Depurination of DNA is a type of mutagenic progression that may cause mismatches during replication and may cause mutagenic toxicity. Reactive oxygen species (ROS) radicals also cause single-strand DNA breaks (SSBs) due to the breakage of the phosphodiester backbone in one of the DNA strands. Several oxidative DNA base lesions are generated by excessive production of ROS.[3] Such damage leads to a collapse of DNA or RNA polymerase complexes, as well as recombination events during replication. Consequently, if SSBs are not repaired rapidly, they may lead to chromosomal aberrations, genetic mutations, and cell death.[4]

Doxorubicin (DOX) is a widely used chemotherapeutic agent against various human malignancies, including hematological malignancies, solid tumors, soft-tissue sarcomas and breast.[5] However, doxorubicin has been found to have a potential genotoxic effect. DOX causes mutagenicity in bacteria besides mammalian cells resulting in chromosomal abnormalities. Apart from being induced due to chemicals, chromosome aberrations may also result due to exposure to ionising radiation and genotoxic chemicals. That is why chromosome aberrations may be sought as an important biomarker in cancer. Most studies support the hypothesis that doxorubicin produces unwanted oxidative stress, leading to increased ROS generation. DOX forms a stable complex with ferric iron, which reacts with oxygen, forming superoxide anions, hydrogen peroxide, and hydroxyl radicals. DOX has been acclaimed as a potent carcinogen after being responsible for causing single-strand DNA breaks, leading to chromosomal rearrangements and mutational proceedings.[6]

DOX induces the formation of micronuclei and changes in chromatid patterns, subsequently leading to single- and double-DNA strand breaks both *in-vivo* and *in-vitro*.[7]

A potential therapeutic strategy against DOX-induced toxicity could be the attenuation of inflammation-associated oxidative stress and apoptosis.[8] Endogenous antioxidant pathways certainly could be a potential accompaniment for DOX-induced toxicity. Today, it is highly restricted due to its toxicity to various organs such as the heart, liver, lung, kidney, and testis. A strategy to prevent this undesirable effect is urgently needed.

It has been proved that plants have always been a useful and promising source of anticancer compounds. Approximately 60% of currently used anticancer agents are derived from plants.[9] The National Cancer Institute (NCI), USA, has investigated approximately 35,000 different species of plant to identify as a source of potential anticancer drugs. Nearly 3000 species have been acclaimed to contain anticancer molecules.[10] Vincristine, vinblastine, etoposide, colchicinamide, Taxol, 10-hydroxycamptothecin, elliptinium, curcumol, gossypol, lycobetaine, tetrandrine, homoharringtonine, monocrotaline, and curdione are remarkable anticancer phytochemicals derived from plant species.[11]

Spathodea campanulata P. Beauv (family Bignoniaceae) is a tall, erect tree (23-82 feet) native to tropical Africa and commonly known as the African tulip tree.[12] The fruits are 5 to 10 inches long, finger-like, 16 cm long, and point upward. Each fruit contains about 500 paper-like seeds. The tree blooms usually in the spring.[13, 14] It is distributed worldwide, but most of them occur in tropical and sub-tropical countries. It is native of Africa (i.e., in Angola, Burundi, Benin, Cameroon, Equatorial Guinea, Ghana, Gabon, Guinea, Nigeria, Liberia, Sierra Leone, Rwanda, Togo, and Zambia). It was introduced in Australia, Brazil, China, Costa Rica, Cuba, Egypt, French Guiana, French Polynesia, India, Indonesia, Kenya, Jamaica, Malaysia, Madagascar, Mexico, Papua New Guinea, Peru, Puerto Rico, Singapore, Saint Lucia, Sri Lanka, Spain, Thailand, United States (Hawaiian Is., Florida), Venezuela, and the British Virgin Islands.[15]

Through a literature survey, it has been discovered that the ethnic community dwelling at the foothills and forests of Africa prescribed this plant to cure malaria [16-18], diabetes [19], dysentery, asthma, stomach ache [20], fever [21], and fungal and bacterial infections. [22] The flower is effective as an antidote against animal poison [23] and cataracts.[24] Different extracts of flowers of *S. campanulata* may have alkaloids, tannins, saponin, steroid, terpenoids, flavonoids, glycosides, and phenolics. Banerjee and DE in 2020 showed the presence of anthocyanins in the flowers of *S. campanulata*.[25] Regarding phenolic compounds, several researchers reported the presence of a range of flavonoids or anthocyanins (36-60) in the flower. Sehab *et al.*; 2014 reported the presence of β-sitosterol-3-acetate, catechin, catechin-3-O-α-rhamnopyranoside, kaempferol, 5, 6, 4'-trihydroxy flavonol-7-O-α-rhamnopyranoside, naringenin, and chrysin [26]. Santos *et al.*; 2020 reported the presence of ferulic acids, caffeic acid, p-coumaric acid, chlorgenic acid, isoorientin, vitexin, orientin, carotenoids, lutein, and cryptoxanthin.[27] Eid *et al.*; 2014 reported the presence of limonene, camphene, sequiterpenoids, longifolene, viridoflorene and many more phytoconstituents of antioxidant potential.[28] Wagh *et al.*; 2021 reported the anticancer potential of stigmasta-5, 22-dien-3-ol, Octadecenamide, and umbelliferone.[29] On the basis of the above facts and the possible potential for the flower extracts of *S. campanulata* to be a potential antigenotoxic against doxorubicin-induced alteration in the genetic composition of normal cells, it has been evaluated in the present research work. Moreover, no study on flower extracts of *S. campanulata* has been done for antigenotoxic evaluation.

MATERIAL AND METHOD

Plant Collection, Authentication and Extraction

Approximately 5 kg of fresh flowers of *S. campanulata* were collected from different nurseries in Bhopal in winter (between December and January). The plant and its flower were authenticated by Dr. Zia-Ul-Hussian, Botanist, Safia College of Science, Bhopal, Madhya Pradesh, having voucher specimen-329/Bot/Safia/15).

The flowers were shade-dried and parts of the leaves and stem were separated. The dried flowers were coarsely ground followed by maceration for 2 days using petroleum ether and for 5 days using 70% methanol. The sticky extract with dark brown color was filtered and stored. Phytochemical screening of *S. campanulata* flower was performed and shown in Table 1.

Experimental animals

The research protocol was approved by the Institutional Animal Ethics Committee (Reference. No PBRI/IAEC/PN-434) and CPCSEA (Registration No.1283/PO/c/09/CPCSEA). A total of 36 albino rats (120-180 g; either sex) were approved for the work. The research work was performed and completed at Pinnacle Biomedical Research Institute Bhopal (M.P.), India.

Acute oral toxicity

An acute oral toxicity study was performed according to OECD guideline 423. During an acute oral toxicity study, SCFE was found to be safe and nontoxic up to the dose of 2000 mg/kg [Table 2]. Finally, $1/10^{th}$ or $1/5^{th}$ of 2000 mg/kg was selected for further study.[30]

Treatment grouping for Micronucleus Assay and Chromosomal Aberration Assay

Group1: Normal Control: saline (oral) 0.5 mL for 5 days.
Group 2: Disease Control: saline (oral) 0.5 mL for 5 days + Dox (12 mg/kg) single (I.P) at 5^{th} day.
Group 3: Extract 200 mg/kg orally for 5 days + Dox (12 mg/kg) (I.P) at 5^{th} day followed by euthanasia post 24 hours.
Group 4: Extract 400 mg/kg for 5 days + Dox (12 mg/kg) (I.P) at 5^{th} day followed by euthanasia post 24 hours.

Micronucleus assay

A micronucleus (MN) test is a test used in toxicological screening for potential genotoxic compounds. Detection of breakage and loss of normal chromosome architecture due to chemical/ionizing radiations is performed by micronucleus assay. The assay was conducted using the peripheral blood erythrocytes of rats.[31] The micronucleus formed in a damaged cell following treatment with a test chemical indicates chromosome damage. During the micronucleus assay, the polychromatic erythrocytes (PCE) were stained pinkish-blue in color, though the nuclear materials were dark purple stained.

A total of 6000 erythrocyte cells were examined and scored for each experimental point to determine the % of micronucleated polychromatic erythrocytes (MNPCE) at different magnifications of 40X and 100X with the help of light microscope.[32]

Chromosomal aberration

Chromosome aberrations could be an important biomarker post-exposure to ionizing radiation and genotoxic chemicals. In the *in-vivo* chromosomal aberration study, increased evidence of aberration like fragment, break, deletion, etc., in the chromosome. Chromosomal aberration is the other method to check the genotoxicity of any compound. A total of 100 (per animal) correctly spread chromosomes of the metaphase stage were placed on the slide in every group. A total of 600 metaphase stage chromosomes in every group were analyzed, and the chromosomal aberrations of different types like ring, fragment, break, dicentric, deletion, gap, pulverization, and polyploidy aberrations were observed. A 100X Olympus light microscope was used for analysis.[33]

Histopathology

Histopathological study has been performed using bone marrow cells of one animal from each group and observed under 100X for the formation of micronucleus and chromosomal aberration.

Table: 1 Phytochemical estimation of SCFE [34]

Carbohydrate test	*Result*
Fehling's	+ve
Benedict's	+ve
Barfoed's	+ve
Protein and amino acid test	
Biuret's	-ve
Alkaloids test	
Dargendroff's	+ve
Wagner's	+ve
Glycosides test	
Legal's	+ve
Keller-Killiani	+ve
Saponins test	
Froth formation	+ve
Flavonoids test	
Lead acetate	+ve
Alkaline reagent	+ve
Triterpenoids and steroids test	
Salkowski's	+ve
Libermann-Buchard's	+ve
Tannin and phenolic test	
Dilute iodine solution	+ve
Lead acetate	+ve

From the above table +ve mark shows confirms the presence of phytochemicals and the –ve confirms the absence of phytochemicals.

Table: 2 Acute oral toxicity of 70% methanolic extract of *S. campanulata* flower (SCME) according to OECD-423 guideline

SCFE (mg/kg/b.w) (p.o)	*Observation after 24 hours*	*Mortality*
5	Normal	Non/3
50	Normal	Non/3
300	Normal	Non/3
2000	Normal	Non/3

From the acute oral toxicity study at different doses from, the 5 to 2000 mg/kg extract dosing, all animals showed zero mortality and normal behavior according to standard protocol.

Table 3: Protective effect of 70% methanolic extract of *S. campanulata* flower (SCFE) on doxorubicin prompted MNPCEs and ratio of PCE/NCE in bone marrow

Grouping	*(MNPCE/1000 PCE)*	*(PCE/NCE)*
Group 1 (Normal Control)	3.66 ± 0.516	2.68 ± 0.420
Group 2 Dox-induced disease control)	31 ± 4.195**	1.22 ± 0.481*
Group 3 (SC Extract 200 mg/kg; p.o)	22.00 ± 3.578**	2.22 ± 0.600$^{(ns)}$
Group 4 (SC Extract 400 mg/kg;p.o)	11.33 ± 2.066*	2.56 ± 0.596$^{(ns)}$

Values are conveyed as a Mean ± SD, n=6, followed by one-way ANOVA and Bonferroni test using standard software (stat 32). The lowest value was measured as significant. *p <0.05, **p <0.001 and (ns) non-significant differences between the group when compared with the control. MNPCE- Micronucleus Polychromatic Erythrocytes; PCE- Polychromatic erythrocytes; NCE- Non-chromatic erythrocytes.

The formation of MNPCE and the ratio of PCE/NCE were examined in all groups.

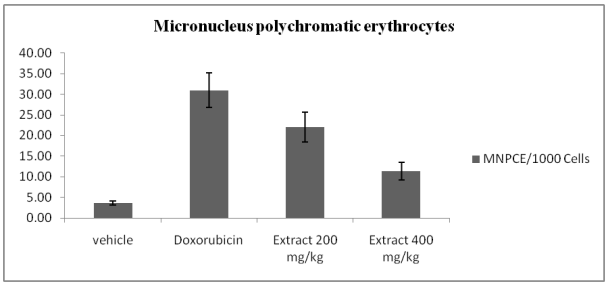

Fig. 1: All data were represented in graphic form through bar graph using simple Excel Microsoft Office 2007. Graphs represent micronucleus formation and their comparison in the groups

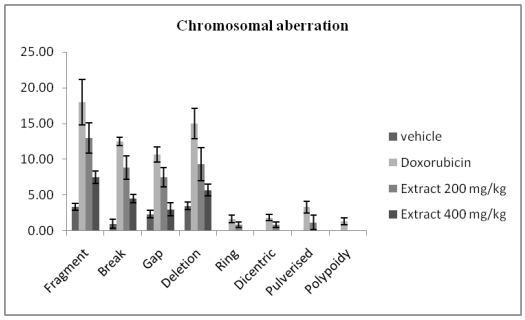

Fig. 2: All data were represented in graphic form through bar graph and the graph represents the different aberration formations in all groups and there comparison

Table 4: Protective effect of SCFE on doxorubicin generated chromosomal aberration in bone marrow

Treatment	*Chromosomal aberrations (%)*							
	Frg	*Br*	*Gap*	*Del*	*Ring*	*Dic*	*Pul*	*Poly*
Group 1 (Control)	3.33 ± 0.516**	1 ± 0.632	2.33 ± 0.516	3.50 ± 0.548	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Group 2 (Inducer)	18.00 ± 3.162**	12.50 ± 0.548**	10.67 ± 1.033**	15 ± 2.098**	1.67 ± 0.516**	1.83 ± 0.408**	3.33 ± 0.816**	1.33 ± 0.516**
Group 3 (200 mg/kg)	13 ± 2.098**	8.83 ± 1.602**	7.50 ± 1.378**	9.33 ± 2.338**	0.83 ± 0.408*	0.83 ± 0.408**	1.17 ± 0.983*	0 ± 0$^{(ns)}$
Group 4 (400 mg/kg)	7.50 ± 0.837*	4.50 ± 0.548**	3.00 ± 0.894$^{(ns)}$	5.67 ± 0.816$^{(ns)}$	0.00 ± 0$^{(ns)}$	0.00 ± 0$^{(ns)}$	0.00 ± 0$^{(ns)}$	0.00 ± 0$^{(ns)}$

Frag=fragments, Br=break, Del=deletion, Rg=ring, Dic=dicentric, Pul=pulverized, Poly=polyploidy.
All data are expressed as a Mean ± SD; one-way ANOVA is used and followed by the Bonferroni test using standard software (stat 32). The lowest value of was considered as significant. * p <0.05, **p <0.001 and (ns) non-significant difference between the group when compared with control.

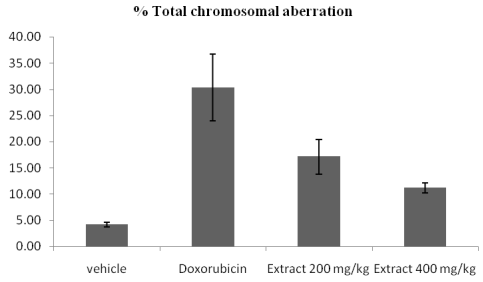

Fig. 3: All data were represented in graphic form through bar graph and the graph represents the % total aberration in all groups and their comparison

Table 5: Protective effect of SCFE on doxorubicin generated total chromosomal aberration in bone marrow

Treatment	*Total aberrations (%)*
Vehicle	4.17 ± 0.408
Inducer	30.33 ± 6.377**
Extract 200 mg/kg	17.17 ± 3.312**
Extract 400 mg/kg	11.17 ± 0.983*

All data are expressed as a Mean ± SD, one-way ANOVA is used and followed by the Bonferroni test using standard software (stat 32). The least value of was considered as significant. * p <0.05, **p <0.001 and (ns) non-significant difference between the group when compared with control.

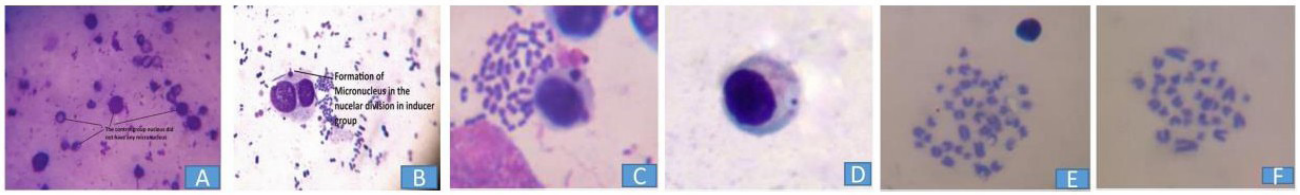

Fig 4A: Control Gp- The nucleus does not show any micronucleus formation
Fig 4B: Doxorubicin Gp- The Nuclear division shows micronucleus formation
Fig 4C (SCFE 200) & 4D (SCFE 400) : Inhibition inn the formation of micronucleus in bone marrow cells
Fig 4E (SCFE 200) & 4F(SCFE 400): Inhibition in chromosomal aberrations in bone marrow cells

RESULT

Phytochemical Analysis of Flowers of *S. campanulata*

The shade dried flowers were treated with petroleum ether and 70% methanol. A sticky dark brown mass obtained. Phytochemical screening of *S. campanulata* flower was performed and shown in Table 1. Presence of important secondary metabolites like alkaloids, glycosides, flavanoids, and phenolic compounds were found to be present in the flower extract.

Acute Oral Toxicity test

Acute oral toxicity study was performed according to OECD guideline 423 using 12 albino rats (n=3) of either sex. During acute oral toxicity study the methanolic extract of *S. campanulata* were found to be safe and nontoxic up to the NOEL dose of 2000 mg/kg [Table 2]. Finally, 1/10th or 1/5th of 2000 mg/kg was selected for experimental study.

Micronucleus Assay

The assay was conducted using the peripheral blood erythrocytes of albino rats. The micronucleus formed in a damaged cell following treatment with a test chemical was observed. The polychromatic erythrocytes (PCE) were stained pinkish-blue in color and the nuclear materials were dark purple stained. A total of 6000 erythrocyte cells were examined and scored for each experimental point and the % of micro nucleated polychromatic erythrocytes (MNPCE) at different magnifications of 40X and 100X were determined with the help of light microscope. The decrease in the formation of MNPCE in the treatment groups was clearly evident as indicated by the ratio

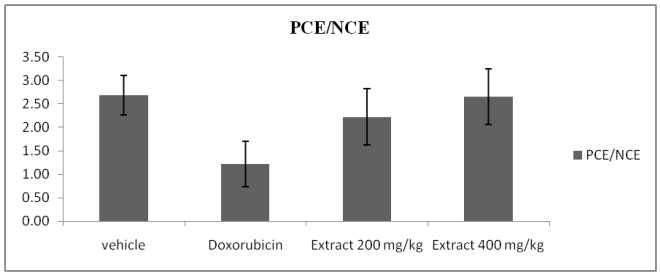

Figure 5: Ratio of polychromatic and nonchromatic micronucleus formation in all groups and their comparison. All data were represented in graphic form through bar graph.

of MNPCE and PCE (Table 3 and Fig. 1). The increased ratio of polychromatic erythrocytes (PCE) and non-chromatic erythrocytes (NCE) suggests the protective effect of SCFE on doxorubicin induced micronucleus formation in the rats (Table 3 and Fig. 1).

Chromosomal Aberration

In-vivo chromosomal aberration in the experimental animal groups has been found to be evident with incidences of fragment, break, deletion, etc. in the chromosome and confirms the genotoxicity caused by doxorubicin. Total of 600 metaphase stage chromosomes in every group (n=6; 6X100) were analysed, and the chromosomal aberrations of different types like ring, fragment, break, dicentric, deletion, gap, pulverization, and polyploidy aberrations were observed under 100X Olympus light microscope. The incidences of aberrations were very high as compared to normal control group. The treatment groups have shown protective effect against chromosomal aberration in the animals (Table 4 and Fig. 2).

The bone marrow samples were evaluated against doxorubicin induced chromosomal aberrations and depicted as total percentage of aberrations in bone marrow. The doxorubicin group was found to contain approximately 30% aberrations against 4% in normal control group. The extract treatments were found to decrease the percentage of total aberrations to 17% (SCF extract 200 mg/Kg and 11% (SCF extract 400 mg/kg), respectively (Table 5 and Fig. 3).

Histopathology

Histopathological examination showed a significant formation of micronucleus in doxorubicin group as compared to normal control group where no micronucleus formation was found (Figure 4A and 4B). The treatment groups SCFE 200 (Figure 4C) and SCFE 400 (Figure 4D) shows inhibition of micronucleus formation in bone marrow. Also the figures 4E (SCFE 200) and Figure 4D (SCFE 400) shows inhibition in chromosomal aberration in bone marrow of treated rats.

DISCUSSION

Genotoxicity determines the magnitude of genetic risk to animals or humans by environmental agents/chemicals under a specified level of exposure. Genotoxicity causes serious long-term effects on the genetic architecture of genes that affect the body in different ways. The occurrence of genotoxicity in normal cells due to therapy with anticancer drugs induces secondary malignancies as an undesired consequence of chemotherapy.[2] Doxorubicin causes malfunctions in genetic material like chromosome structure. Chromosome structure changes will cause serious disorders that can be shifted to the next cell division. Doxorubicin is a broad-spectrum anticancer drug used against various cancers, but its use is limited due to its serious and long-term side effects. Depression of bone marrow is a serious side effect that results in aberration of genetic material like DNA and chromosomes, which causes genotoxicity. A single I.P dose of doxorubicin (12 mg/kg) produced genetic material alteration of spermatic cells or bone marrow cells.[35] Different assays were designed to observe the aberration at the genetic level due to any chemical, environment, etc. Many of the well-established parameters to analyze both qualitative and quantitative deformation of genetic material, like *in-vivo* chromosomal aberration, micronucleus assay

Our plant extract from *S. campanulata* flowers contains an excellent range of antioxidants by virtue of a rich source of phytochemicals. Upon conducting a phytochemical investigation, we found that our extract contains glycosides, alkaloids, tannins, phenols, and flavonoids, as shown in Table 1. The antioxidant property of the plant has the potential to mitigate any drug toxicity side effects and aberrations in the body. Therefore, we can say that the antioxidant property is the key to unlock drug toxicity. Many herbal plants are reported to be anti-clastogenic due to their high antioxidant and excellent phytochemical range.[36,37] In acute oral toxicity, zero mortality was reported during the experiment, and at different dosages, behavior variation at specific periods was obtained and was safe up to the 2000 mg/kg dose as shown in Table 2. Hence, we put the dosage from 200 to 400 mg/kg for *in-vivo* studies.

Micronucleus assay is a known-characterized bioindicator of structural and numerical chromosomal damage, which arises from chromosomes that is not at a centre called acentric fragments or cover whole chromosome(s) that fail to combine into the daughter nuclei after nuclear division. Micronucleus (MN) test is a toxicological screening tool for impending genotoxic compounds and has been acknowledged as one of the most reliable evaluates for genotoxic carcinogens. The micronucleus (MN) test is based on the formation of a number of micronuclei in genotoxic carcinogen-treated cells.

In the inducer group number of polychromatic cells gradually declined, and increased micronucleus in polychromatic erythrocytes, which directly indicated that it causes damage to genetic material. The extract inhibited cell viability in a concentration-dependent way significantly. Various signs of apoptosis, like nuclear fragmentation, chromatin condensation, and development of apoptotic bodies, were observed in treated cells. Our data show indicated that pre-treatment of our extract of 200 and 400 mg/kg reduced the MNPCE incident and resulted in inhibition of micronuclei formation as shown in Tables 3, 4 Figs 4(a),(b). Characteristic apoptotic features such as chromatin condensation, cell shrinkage, and membrane blebbing were observed on the treated cells.

Chromosome aberrations, being a significant biomarker of chromosomal damage, may occur due to ionizing radiation and genotoxic chemicals in animals. Congenital abnormalities in newborns and neoplasia in humans could be the result of both structural and numerical aberrations. The spontaneous frequency of chromosome aberrations is roughly 0.6% of normal births. Chromosome analyses of spontaneous abortions indicate that about 50% of them are chromosomally abnormal. In the *in-vivo*

chromosomal aberration study, increased evidence of aberration like fragment, break, deletion, etc., and extract treated group of dose 200 and 400 mg/kg both were capable of reducing the aberration in the chromosome. Chromosomal aberration is the other method to check the genotoxicity of any compound. The control group animal had good chromosomal structure or number, but when we treated with the anticancer drug doxorubicin, the number of chromosomes decreased with a single exposure of doxorubicin and increased incidents of chromosomal aberration like fragment, break, gap, dicentric, deletion, etc. flower extract (200 & 400 mg/kg) pre-treatment meaningfully and dose-dependently reduced the percentage of abnormality shown in Tables 4,5 and Figs 2-5. The current study exposed that pre-treatment defends the genomic damage as evident from micronucleus assay and chromosomal aberration.

CONCLUSION

In conclusion, the present study indicates that SCFE treatment attenuates genotoxicity in the bone marrow cells caused by doxorubicin. The chemo-protective effect of possible potency might be due to the presence of several potent antioxidant phytoconstituents reported in the flower extract. Due to this fact, our extract has the potential and ability to reduce doxorubicin-induced genotoxicity and prevent associated side effects related to chemotherapeutic applications. Concluding the anticancer activity, it may be observed that the flower extract showed potent anticancer activity. In future studies the extracts could be considered for isolation of active principle and *in-vivo* investigation against a range of cell lines. The selectivity toward cancer cells could be measured during anticancer activity in future studies.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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