An Elsevier Indexed Journal ISSN-2230-7346

Journal of Global Trends in Pharmaceutical Sciences

IN VIVO GENOTOXIC ANALYSIS OF PURIFIED ANTHOCYANIN FROM *BEGONIA MALABARICA* AND *B. REX-CULTORUM* 'BABY RAINBOW' ON MICE BONE MARROW CELLS USING CHROMOSOMAL ABERRATION AND MICRO NUCLEUS ASSAY

Aswathy JM and Murugan K*

Plant Biochemistry and Molecular Biology Laboratory, Department of Botany, University College, Trivandrum, 695 034, Kerala, India

*Corresponding author E-mail**: harimurukan@gmail.com**

ARTICLE INFO

Key Words

Begonia malabarica, B. rex-cultorum 'Baby rainbow', anthocyanin, genotoxicity, micronucleus, mutagenic

ABSTRACT

Objective: Natural molecules are functionally safer than synthetic drugs and screening phytotherapeutic agents and phytopharmaceutical products for this puprpose have achieved exceptional growth in recent years. Scientific validations of the biological activities of phytochemicals are a pre-requisite for marketing the drug. **Methods:** Current work is targeted to analyze the *in vivo* genotoxic effects of purified anthocyanin extracted from cell suspension cultures of *Begonia malabarica* and *B. rex-cultorum* 'Baby rainbow' against mice bone marrow cells using chromosomal aberration and micro nucleus test. Two different concentrations of purified anthocyanin extracts from cell suspension cultures (200 and 400 mg kg^{-1} b.w) were tested as per OECD protocols. **Results:** The cell suspension cultures of *B. malabarica* were initiated in liquid MS medium fortified with 2, 4-D (0.1 mg L^{-1}) + BAP (0.5 mg L^{-1}) and *B. rex-cultorum* 'Baby rainbow' in BAP (1 mg L^{-1}) + 2,4-D (0.5 mg L^{-1}) . Abscisic acid (ABA) (25 mg/L) and salicylic acid (SA) (60 µM) induced significantly the anthocyanin accumulation on cell suspension cultures. Abscisic acid substantially increased anthocyanin accumulation in cell suspension (18.6g/100 mL in *B. malabarica* and 30.8 g/100 mL for *B. rex-cultorum* 'Baby rainbow'). The elicited anthocyanin was purified using amberlite column chromatography. In micronucleus test, single oral dose administration of purified anthocyanin at 200 and 400 mg kg^{-1} b.w. did not show an increase in the mean number of micronucleated polychromatic erythrocytes and decrease in PCE % in both sexes of mice indicating non clastogenicity of the molecule. Further, in chromosomal aberration analysis, a single oral administration revealed no significant variations on the mitotic indices or in the induction of numerical and structural chromosomal aberrations in the proliferative cells of the bone marrow from mice in either sex. **Conclusion:** Anthocyanin of *B. malabarica* and *B. rex-cultorum* 'Baby rainbow' showed no remarkable genotoxic impact on the chromosomes of mice of either sex. The anthocyanin extracts from *Begonia* was safe i.e., non mutagenic and also anti mutagenic and therefore may be used for further medicinal preparations.

INTRODUCTION:

Cyclophosphamide (CP) is an alkylating molecule and generally employed as antimetastatic drug in chemotherapy. Its cytotoxic impacts were the result of chemically active metabolites that alkylate DNA and protein, by producing cross-bonds. Immuno-suppression, injury to healthy cells and side effects were the draw backs of chemotherapy [1]. Oxidative stress mediated derailment of redox balance after CP exposure generates biochemical and physiological unbalance in the cell system. CP is a well-known mutagen and clastogenic agent and produces the highly reactive carbonium ion, which binds with the electron dense area of nucleic acids and proteins. CP is widely used as a genotoxic agent and results in chromosome breaks, micronucleus (Mn) formation, and cell death [2]. Many plant based phytochemicals function as antioxidants and there by counter act chemotherapic drugs as well as neutralize their adverse side effects [3]. Phytochemicals that could minimize these side effects, as well as stimulate immunity, will be of immense importance in improving cancer treatment strategies. Thus, there is an increasing demand of the search of novel herbal products that are effective against many sorts of diseases. Pratheesh kumar *et al*. [4] proved that plant based molecules function as protective agents against genotoxicity induced by CP in bone marrow cells of mice when these molecules were administrated prior to CP treatment. Hesperidin, a citrus bioflavonoid, showed antioxidative activity and also mitigated the genotoxicity induced by CP in mouse bone marrow cells by decreasing micronucleus count. Hosseinimehr *et al*.[5] reported that flavonoids and phenolic compounds had a potent chemoprotective effect against CP-induced oxidative stress and DNA damage in mice bone marrow cells. Secondary metabolites of herbals were proven as antimicrobial, insecticidal and antidiabetic but often showed toxicity [6]. Therefore, an assessment of their mutagenic and cytotoxic potential is a pre-requisite to ensure the safety to its use. Many of the drugs based on folk remedies lack ethnopharmacological validations. Various experimental models like membrane systems, plant test systems, cell culture, animal models, clinical trials are employed to study the bioactivity of these plant derived molecules. The assessment of the efficacy and safety profiles of the herbals should be based on scientific evidence-based approaches including different types of well established short-term tests when evaluating the genotoxic profile of such plants. In this scenario, the present study was undertaken to access the *in vivo* genotoxicity of purified anthocyanin from *Begonia malabarica* and *B. rex-cultorum* 'Baby rainbow' on mice bone marrow cells in terms of chromosomal aberration and micro nucleus assay.

MATERIALS AND METHODS

Plant materials

Healthy *Begonia malabarica* Lam. and *B. rex-cultorum* 'Baby rainbow' species were collected, identified by referring floras and confirmed by authenticating with the herbaria of Jawaharlal Nehru Tropical Botanical Garden and Research Institute, Palode, Kerala. The voucher plant materials were deposited at the Herbarium of Department of Botany, University College, Trivandrum (UCB 1207, UCB 1208).

Establishment and elicitation of cell suspension culture

The cell suspension was established from 1 g calli tissue derived from the leaf explants of *Begonia malabarica* and was subcultured in liquid MS culture medium fortified with 2,4-D (0.1 mg L^{-1}), NAA (0.5 mg L^{-1}) and BAP (0.5 mg L^{-1}) for

continuous and stable accumulation of biomass. Meanwhile, for *B. rex-cultorum* 'Baby rainbow' cell suspension was established in liquid MS medium (2 g calli obtained from leaf explants) supplemented with the growth regulators such as BAP (1 mg L^{-1}) + 2,4-D (0.5 mg L^{-1}), BAP (2.0 mg L^{-1}) + IAA (1.0 mg L^{-1}), KN (2 mg L^{-1}) + IAA (1 mg L^{-1}) and KN (2 mg L^{-1}) + 2,4-D (1 mg L^{-1}). Based on the results of preliminary experiments, various physico-chemical parameters at different concentrations were employed for elicitation of anthocyanin in cell suspension mainly, peptone water (0.01-0.025%), yeast extracts (0.1 – 2.5 %) phenylalanine (5-25 µM), abscisic acid (0.05-2.0 mg L^{-1}), salicylic acid (1-100 µM), zinc sulphate (50-150 µM)) and methyl jasmonate (50 µM). The suspension cultures were subcultured in the MS liquid medium at 17–20 days interval agitated on a rotary shaker (100 rpm, 25 ± 1 °C) in a 250 mL flask with 40 mL of modified liquid MS under 16 h illumination with 80 µmol m^{-2} s^{-1}. After the first cycle, the cells were harvested via a Buchner funnel, washed with distilled water to remove residual medium, and filtered again. Then the weighed fresh cells (FW) were dried at 50 °C to constant dry weight (DW). Cell growth was measured based on FW and also DW. Every 20 days, cells were subcultured to fresh media [with 5.0 g fresh weight (FW)] in 250 mL flasks.

Quantification and purification of anthocyanin

Anthocyanin content was extracted and quantified from the elicited *in vitro* cell suspension cultures and *in vivo* plants. The anthocyanin was homogenized in 3 ml methanol with 1% HCl and the extract was quantified by the standard protocol of Sutharut and Sudarat [7]. The absorbance was read at 510 and 700 nm against distilled water as blank.

The combined aqueous concentrates of anthocyanin from cell suspension culture after evaporation were purified by liquid-liquid partition against ethyl acetate followed by Amberlite XAD-7 adsorption column chromatography. Then the adsorbed anthocyanins in the column were eluted using 75% ethanol containing 7 % acetic acid (v/v) as mobile phase.

Genotoxicity analysis

Animal study

Experimental study was carried out using Swiss albino mice weighing 30-35g. The animals were procured from Biogen, Bangalore. The animals were housed in polypropylene cages. The cages were maintained clean and hygienic. Animals were acclimatized in light and temperature controlled room with a 12- 12 h dark-light cycle at temperature 25±2ºC and humidity 50±5%. The mice were fed with commercial pelleted feed and water *ad libitum*. The animal caring and handling were done according to the CPCSEA guidelines. The Institutional Animal Ethics Committee at KLE University's College of Pharmacy, Rajajinagar, II block, Bengaluru has approved the study (CPCSEA Reg.No. 202/2017).

Micronucleus test: Albino mice were divided into 11 groups consisting of 10 mice, 5 males and 5 females in each group. Group I : Vehicle control; Group II : Cyclophosphamide (CP) 50 mg kg^{-1} b.w., i.p. ; Group III : *Begonia malabarica* (BM) 200 mg kg^{-1} b.w., p.o; Group IV : (BM) 400 mg kg^{-1} b.w., p.o ; Group V : BM 200 mg kg^{-1} b.w., p.o + CP 50 mg kg^{-1} b.w., i.p. ; Group VI : BM 400 mg kg^{-1} b.w ., p.o + CP 50 mg kg^{-1} b.w., i.p.; Group VII : *B. rex-cultorum* 'Baby rainbow' (BR) 200 mg kg^{-1} b.w., p.o; Group VIII : (BR) 400 mg kg^{-1} b.w., p.o ; Group IX : BR 200 mg kg^{-1} b.w., p.o + CP 50 mg kg^{-1} b.w ,

i.p.; Group X : BR 400 mg kg^{-1} b.w., p.o + CP 50 mg kg^{-1} b.w., i.p.; Group XI: Silymarin (25 mg kg^{-1} b.w., p.o .) + CP 50 mg kg^{-1} b.w., i.p. The animals were sacrificed 24 h after the treatments by adequate dose of ketamine. Colchicine (metaphase arresting agent) (0.25 g / 50 mL DDW) was administered (i.p.) before 2 h of sacrificing the animals. Then slides were prepared essentially as per modified method of Preston. Briefly, femur bone was excised and the bone marrow was extracted in 0.56% KCl. The harvested cells were incubated at 37°C for 20 min and then centrifuged for 10 min at 1000 rpm. Cells were fixed in Cornoy's fixative (methanol: acetic acid = 3:1) and burst opened on a clean slide to release chromosomes. Smear glass slides was allowed to air dry, followed by fixation with methanol. Later stained using May- Grunwald's and Giemsa stain. Slides were dried and observed under oil immersion objective to determine the frequency of micronucleus (MN). The % of polychromatic erythrocytes (PCE) in 1000 total erythrocytes, % of micronucleus polychromatic cells in 2000 PCE (MNPCE) were evaluated. Percent of PCE = [PCE/ (PCE+NCE)] × 100, Percent of MNPCE = (MNPCE÷PCE) × 100 **Chromosomal aberration test:** Albino mice were divided into 11 groups consisting of 10 mice, 5 males and 5 females in each group.

Group I : Vehicle control; Group II : Cyclophosphamide (CP) 50 mg kg^{-1} b.w., i.p. ; Group III : *Begonia malabarica* (BM) 200 mg kg^{-1} b.w., p.o; Group IV : (BM) 400 mg kg^{-1} b.w., p.o ; Group V : BM 200 mg kg^{-1} b.w., p.o + CP 50 mg kg^{-1} b.w., i.p. ; Group VI : BM 400 mg kg^{-1} b.w ., p.o + CP 50 mg kg^{-1} b.w., i.p.; Group VII : *B. rex-cultorum* 'Baby rainbow' (BR) 200 mg kg^{-1} b.w., p.o; Group VIII : (BR) 400 mg kg^{-1} b.w., p.o ; Group IX : BR 200 mg kg^{-1} b.w., p.o + CP 50 mg kg^{-1} b.w ., i.p.; Group X : BR 400 mg kg^{-1} b.w., p.o + CP 50 mg kg^{-1} b.w., i.p.; Group XI: Silymarin (25 mg kg^{-1} b.w., p.o .) + CP 50 mg kg^{-1} b.w., i.p. After 24 h of respective single dose treatment for all the above mentioned groups, Colchicine 4 mg kg^{-1} b.w., i.p was given after respective treatments for all the animals 2 h before sacrificing. All animals were sacrificed by cervical dislocation. Femurs and tibias were separated and adhering muscle, tissue was removed. The bone marrow was extracted from both femurs using 0.56% freshly prepared KCl solution, fixed with cold Corney's fixative and flame dried. Then the slides were prepared by dropping technique and stained with 10% Giemsa solution. Slides were then air dried and observed under oil immersion objective. 100 well spread metaphase cells were screened per animal for scoring the chromosomal aberrations. MI was calculated using the formula:

$$\text{Mitotic index (I)} = \frac{P+M+A+T}{N} \times 100\%$$

P+M+A+T - the sum of all cells in phase as prophase, metaphase, anaphase and telophase, respectively; N — total number of cells.

STATISTICAL ANALYSIS

All the results were mean of 12 replications. Student's t-test and Analysis of variance (ANOVA) were employed to evaluate the significance level as 1 or 5% level.

RESULTS AND DISCUSSION

Cell suspension culture of *B. malabarica* and *B. rex-cultorum* 'Baby rainbow'

Cell suspension culture was established by culturing fresh friable calli from the leaf explants of *B. malabarica* and

B. rex-cultorum 'Baby rainbow'. Suspension was established by transferring 1 g friable callus in liquid MS media supplemented with BAP, 2, 4-D and NAA having a concentrations of 0.5, 0.1, 0.5 mg L^{-1} respectively for *B. malabarica*. BAP (1 mg L^{-1}) + 2,4-D (0.5 mg L^{-1}), BAP (2.0 mg L^{-1}) + IAA (1.0 mg L^{-1}), KN (2 mg L^{-1}) + IAA (1 mg L^{-1}) and KN (2 mg L^{-1}) + 2,4-D (1 mg L^{-1}) combinations were treated for *B. rex-cultorum* 'Baby rainbow' (from 2 g friable calli). Liquid MS medium supplemented with 2, 4-D (0.1 mg L^{-1}) and BAP (0.5 mg L^{-1}) combinations showed well established suspension cultures i.e., suspensions without any aggregation or clumps of cells for *B. malabarica* (Fig. 1a). Meanwhile maximum growth was achieved in suspension culture supplemented with BAP (1 mg L^{-1}) + 2,4-D (0.5 mg L^{-1}) for *B. rex-cultorum* 'Baby rainbow' (Fig.1 b). The *in vitro* suspension of cells revealed optimal and steady biomass accumulation on day 14 for both begonias. The same medium and growth hormone combinations were used for further analysis of growth kinetics. After 16^{th} day, cells in the suspension exhibited a marginal reduction in fresh weight as well as dry weight of cells (Table 1 and 2). The maximum fresh weight (8.0 g) and dry weight (0.85 g) was noticed at 14^{th} day of culture in liquid MS medium complimented with 2, 4-D (0.1 mg L^{-1}) + BAP (0.5 mg L^{-1}) for *B. malabarica* where as for *B. rex-cultorum* 'Baby rainbow' maximum fresh weight of 7.4 g and dry weight of 0.71 g with BAP (1 mg L^{-1}) + 2,4-D (0.5 mg L^{-1}). The time course of biomass accumulation was the typical sigmoid growth curves. MS liquid medium supplemented with other combinations of hormones NAA + BAP for *B. malabarica* and KN + IAA, BAP + IAA, KN + 2,4-D for *B. rex-cultorum* 'Baby rainbow' showed only minimum outputs with aggregated or clumped cells. Generally, the cell growth was slow during the initial 5 days of culturing. Thereafter, biomass accumulated rapidly, and reached the highest value on the 14^{th} day. Then the culture entered the stationary phase and declined marginally. Some cultures continued to grow even up to 30^{th} day.

Quantification of anthocyanin

Anthocyanin content was quantified from the *in vitro* cell suspension culture and *in vivo* plants. The *in vitro* cells showed remarkable level of anthocyanin ie., 10.4 and 20.6 g / 100 ml for *Begonia malabarica* and *B. rex-cultorum* 'Baby rainbow' respectively as compared to the *in vivo* plants (5.7 for *Begonia malabarica* and 9.8 mg g^{-1} *B. rex-cultorum* 'Baby rainbow'). From the given results it can be speculated that anthocyanin content may be effectively induced through *in vitro* culture by standardizing the culture parameters. The present results seem to be more effective and supported by other *in vitro* cultures of phytochemicals from medicinal plants.

Elicitation by chemicals on anthocyanin production

B. malabarica and *B. rex-cultorum* 'Baby rainbow' cell suspension cultures showed insignificant accumulation of anthocyanin under elicitation by peptone water and yeast treatments. Similarly, the addition of phenylalanine (10µM), the precursor of secondary metabolite synthesis enhanced the anthocyanin synthesis in the cells of *B. malabarica* and *B. rex-cultorum* 'Baby rainbow' 13.9 and 26.8 g/100 mL respectively. In fact, abscisic acid (ABA) (25 mg/L) and salicylic acid (SA) (60 µM) elicited anthocyanin synthesis remarkably i.e., 18.6 and 30.8 g/100 mL for *B. malabarica* and 19.2 and 34,4 g/100 mL for *B. rex-cultorum* 'Baby rainbow' respectively (i.e., profound effect on anthocyanin induction in cell suspension cultures). Zinc sulphate at lower dose (50µM) showed

slight rise in anthocyanin production (13.5 and 27.2 g/100 mL), but the content was lower than that of SA or ABA treatments. MeJ did not showed remarkable increase in anthocyanin production in cell cultures.

Purification of anthocyanin

25 g of fresh, homogenized cell suspension samples were extracted out from the cell suspension cultures of *Begonia malabarica* and *B. rex-cultorum* 'Baby rainbow'. The combined aqueous concentrates after evaporation were purified by partition against ethyl acetate to remove chlorophylls, stilbenoids, less polar flavonoids and other non polar compounds from the mixture. The non-aromatic compounds were removed with the use of Amberlite XAD-7 column chromatography. Amberlite XAD-7 adsorbs the aromatic compounds including anthocyanins and other flavonoids in aqueous solutions, whereas free sugars and other polar non-aromatic compounds were removed by washing with distilled water until the eluted water has a neutral pH. Subsequently, acidified ethanol with the concentration of 75% was used for the desorption of anthocyanins from the Amberlite XAD-7 HP column. Finally, the purified amberlite column eluted fraction was used for the further analysis.

Genotoxicity analysis

Micronucleus assay

Micronucleus assay in terms of percent MNPCE and percent PCE were evaluated in male and female mice and the results were shown in the table 3. Animals treated with cyclophosphamide (CP) showed a remarkable increase in the % of MNPCE and decrease of PCE when compared with the control group irrespective of sex. Mice of either sex when treated with 200 and 400 mg kg^{-1} b.w. of *B. malabarica* purified anthocyanin extract showed no significant increase in mean % of MNPCE. Meanwhile, PCE increased as compared to the CP treated group. While, 200 and 400 mg kg^{-1} b.w. of *B. rex-cultorum* 'Baby rainbow' showed a marginal increase in % of MNPCE. % of PCE showed a marginal decrease as compared to the control. Similarly, pre-treatment of purified anthocyanin from *B. malabarica* and *B. rex-cultorum* 'Baby rainbow' followed by CP mitigated the increase in MNPCE induced by CP. Similarly, % of PCE was regained significantly. The values were statistically significant with F values 1163.65**and 440.44** for *B. malabarica* and *B. rex-cultorum* 'Baby rainbow' respectively. Thus, the anthocyanin reveals its antigenotoxic potential in the animal model.

Chromosomal aberrations

In vivo chromosomal aberration assay in either sex of mice was performed at two different anthocyanin doses from *Begonia malabarica* and *B. rex-cultorum* 'Baby rainbow' and results were tabulated. Single oral dose administration of *B. malabarica* and *B. rex-cultorum* 'Baby rainbow' at 200 and 400 mg kg^{-1} b.w. after 24 h have shown no significant induction of chromosomal aberrations in the bone marrow cells of mice of both sexes when compared with the vehicle control group. Whereas, CP treated animals of both sexes showed significant levels of different types of chromosomal aberrations. Chromosome and chromatids breaks, gaps, fragments, ring formation and acentric were noticed. No remarkable aberrations were noticed with the administration of anthocyanin from *B. malabarica* and *B. rex-cultorum* 'Baby rainbow'. Single oral dosage at 200 and 400 mg kg^{-1} b.w administration of *B. malabarica* and *B. rex-cultorum* 'Baby rainbow' anthocyanin extract followed by CP (50 mg kg^{-1}) showed remarkable inhibition of

chromosomal aberration (Figure 2 and 3, Table 4). The F values of *B. malabarica* and *B. rex-cultorum* 'Baby rainbow' were 143.28** and 120.78** respectively.

Mitotic index

The mitotic index of mice bone marrow cells were used to determine the cell division rate. CP treated mice in both sexes have shown a remarkable reduction in mitotic index % when compared with vehicle control group i.e., 4.59, F = 32.3. Meanwhile, no remarkable differences in the percentages of mitotic indices were noticed in both sexes of mice with 200 and 400 mg kg^{-1} concentrations of anthocyanin from *B. malabarica* (i.e., 6.54, F = 101.2**; 6.09, F = 99.3**) and *B. rex-cultorum* 'Baby rainbow' (6.25, F = 98.52**; 5.92, F = 71.39**) as compared to control (7.14 , F = 142.6**). Mice and rats are commonly used animal models for general toxicologic, carcinogenic, pharmacokinetic and toxicokinetic evaluations. Genotoxic studies were useful to pin point the DNA damage level if any induced by the phytochemicals and its plausible clinical consequences to humans. Genotoxic agents may cause genetic damage in any cell of the organism. If the damage occurs, in somatic cells, it may lead to cancer or other degenerative disorders. Mutagenesity analysis has now been recognized as a critical event in the evaluation of the genetic toxicity of plant based drugs. The nuclear anomalies in bone marrow smear are a rapid method to validate the mutagenic potential of herbal drugs and indirectly, to others doing research in the field of mutagenesis. The mouse and rat bone marrow micronucleus test and chromosomal aberration test were commonly used to evaluate the *in vivo* genetic toxicity of test substance. In the micronucleus formation, when a bone marrow erythroblast develops into a PCE, the main nucleus is extruded; any micronucleus that has been formed may remain behind in the otherwise a nucleated cytoplasm. Visualization of micronuclei is facilitated in these cells because they lack a main nucleus. Micronuclei are cytoplasmic chromatin masses that appear as small nuclei that arise from chromosomal lagging at anaphase or from acentric chromosomal fragments. CP functions as monoabdducts and triggers the formation of bonds between DNA-DNA and DNA-proteins. They are also capable of producing ROS or free radicals. They provide a quantifiable measure of recent DNA injury that result from acentric fragments or whole chromosomes left behind the main nucleus at telophase. An increase in the frequency of micronucleated polychromatic erythrocytes (MNPCEs) in treated animals is an indication of induced chromosome damage. In chromosomal aberrations test, it is necessary to arrest the dividing cells at metaphase stage. To facilitate this, treatment with colchicine and hypotonic solution is essential. Colchicine is a mitotic arrestant and disrupts the spindle fibers thereby preventing the anaphase segregation. The action of hypotonic solution causes swelling of cells by osmosis, which allows the proper spreading of the chromosomes. Mitotic index, which represent fraction of cells in a given population that undergo mitosis at a given time, indicates the cell proliferation activity. Anthocyanins are phenolic derivatives capable of attenuating H_2O_2-triggered DNA strand breaks, protecting cell membranes against free radical-mediated oxidative burst. Most mutagenic molecules require metabolic induction through cytochromatic enzymes to express their mutagenicity. In the present study, the *B.malabarica* and *B. rex-cultorum* 'Baby rainbow' anthocyanin was evaluated for genotoxicity potential by means of *in vivo* micronucleus and chromosomal aberration test in mice bone marrow. At two different

doses of *Begonia malabarica* and *B. rex-cultorum* 'Baby rainbow'anthocyanin, there was no increase in the mean number of MNPCEs and regained the percentage of PCE in both sexes of mice indicating the non-clastogenicity of the molecule. Similarly in chromosomal aberration test at single oral administration of 200 and 400 mg kg^{-1} b.w. of purified anthocyanin from *B. malabarica* and *B. rex-cultorum* 'Baby rainbow' showed no significant changes in the mitotic indices or on the induction of chromosomal aberrations (both numerically and structurally) in proliferative cells of the bone marrow of mice in either sex. These results suggest that *B. malabarica* and *B. rex-cultorum* 'Baby rainbow' did not interact with the spindle apparatus nor with the gene function.

DISCUSSION

Costa *et al.* [8], proved non mutagenicity of *Psidium cattleyanum* extract on peripheral blood and bone marrow cells of mice. Cuzzolin *et al*. [6] evaluated the safety of phytomedicines in terms of cell DNA damage, micronucleated frequency and bone marrow toxicity. Shokrzadeh *et al*.[9] revealed that *Citrullus colocynthis* fruit extract counter act against the genotoxicity induced by cyclophosphamide in mice bone marrow cells i.e., reduced the number of MnPCEs and completely regained the normal mitotic activity. Similarly, significantly inhibited the proliferation and hypercellularity of immature emyeloid elements in mice treated with CP. Peron *et al*.[10] evaluated the cytotoxicity, mutagenicity and antimutagenicity of a natural antidepressant crude extract of *Hypericum perforatum* on vegetal and animal test systems with acute toxicity via intraperitoneal gavage and subchronic gavage. Mazzorana *et al*.[11] validated *Mikania laevigata* extract as non toxic i.e., the plant extract reduced the damage index (DI) upto 52% and 60%. Pretreatment also reduced the damage frequency. Oyeyemi *et al*.[12] analyzed the genotoxicity and antigenotoxicity study of aqueous and hydro-methanol extracts of *Spondias mombin, Nymphaea lotus* and *Luffa cylindrica* using animal bioassays. The results showed the inhibition of the extracts against somatic and germ cell mutation in mice. The extracts also ameliorated the genotoxic effect of toxic agents. Similarly, Abozed *et al*. [13] ; Idu *et al*. [14] and Mary and Begum,[15] validated the efficacy of herbal drugs as antimutagenic. Boriollo *et al*.[16] evaluated the mutagenicity and antimutagenicity of *Ziziphus joazeiro* bark via micronucleus assay. The results indicated no systemic and moderate toxicity at lower and higher doses of *Z. joazeiro*. All these data substantiates the potential of plant based compounds as nontoxic and antimutagenic.

CONCLUSION

Consumption of purified anthocyanin from *Begonia malabarica* and *B. rex-cultorum* 'Baby rainbow' was protective and reparative of DNA damage induced in mouse bone marrow cells by alkylating agents. The present results confirm the ability of the *in vivo* assay to detect genotoxicity of anthocyanin from *Begonia malabarica* and *B. rex-cultorum* 'Baby rainbow' using animal models. Further studies are warranted to analyze the molecular mechanism of anthocyanin and its mode of action to ameliorate the toxicity. Similarly, identification of genetic biomarkers would help to estimate the potential toxicity of herbals in order to revitalize medicinal plant consumption, which would be an ideal measure of public health protection.

Table 1: Fresh (FW) as well as dry weight (DW) of cells obtained in suspension culture for first 20 days of B. malabarica in liquid MS medium with 2, 4-D (0.1 mg L^{-1}) + BAP (0.5 mg L^{-1})

Days	FW -Mean (g)	DW- Mean (g)
2	0.844	0.130
4	2.268	0.366
6	3.454	0.418
8	5.388	0.504
10	6.108	0.628
12	7.106	0.738
14	8.090	0.848
16	8.082	0.836
18	7.834	0.832
20	7.800	0.820
Mean	5.697	0.612
F- FW	1735.196**	
F-DW	206.730**	
SE-FW	1.51	
SE-DW	5.57	
CD-FW	4.953	
CD-DW	1.817	

*SE-FW: Standard error between fresh weight; SE-DW: Standard error between dry weight; CD-FW: Critical difference between fresh weight; CD-DW: Critical difference between dry weight.

Table 2: Fresh (FW) as well as dry weight (DW) of cells obtained in suspension culture for first 20 days B.rex-cultorum 'Baby rainbow' in liquid MS medium BAP (1mg L^{-1}) + 2,4-D (0.5 mg L^{-1})

Days	FW -Mean (g)	DW- Mean (g)
2	0.740	0.136
4	1.814	0.366
6	2.598	0.4400
8	3.152	0.476
10	4.364	0.538
12	5.876	0.556
14	7.378	0.704
16	7.382	0.704
18	7.378	0.698
20	7.344	0.694
Mean	4.803	0.531
F-FW	1563.58**	
F-DW	196.730**	
SE-FW	6.57	
SE-DW	1.54	
CD-FW	0.185	
CD-DW	4.353	

*SE-FW: Standard error between fresh weight; SE-DW: Standard error between dry weight; CD-FW: Critical difference between fresh weight; CD-DW: Critical difference between dry weight.

Table 3: Percent of MNPCE and PCE in bone marrow cells of mice treated with purified anthocyanin of *B. malabarica* and *B. rex-cultorum* 'Baby rainbow' and / or CP

Treatment (T)	Percent micronucleus polychromatic erythrocytes (MNPCE)	% of polychromatic erythrocytes (PCE)
Control	0.57	64.76
Cyclophosphamide 50 mg kg $^{-1}$	9.18	33.48
BM 200 mg kg $^{-1}$	0.67	60.5
BM 400 mg kg $^{-1}$	0.73	58.7
BM 200 mg kg $^{-1}$+ CP 50 mg kg $^{-1}$	1.60	46.18
BM 400 mg kg $^{-1}$+ CP 50 mg kg $^{-1}$	0.91	49.60
BR 200 mg kg $^{-1}$	1.73	59.4
BR 400 mg kg $^{-1}$	2.06	54.6
BR 200 mg kg $^{-1}$+ CP 50 mg kg $^{-1}$	2.21	43.3
BR 400 mg kg $^{-1}$+ CP 50 mg kg $^{-1}$	1.42	46.14
Silymarin 25 mg kg $^{-1}$CP 50 mg kg $^{-1}$	0.53	58.5
F-BM	1163.65**	
F-BR	440.44**	
SE-T	0.19	
SE-V	9.60	
CD-T	0.547	
CD-V	0.273	

*SE-T: Standard error between treatments; SE-V: Standard error between genotypes; CD-T: Critical difference between treatments; CD-V: Critical difference between genotypes.

Fig-1 a: Cell suspension culture of *Begonia malabarica* from friable callus in MS medium fortified with 2, 4-D (0.1 mg L^{-1}**)** and BAP (0.5 mg L^{-1}) **Fig-1 b:** Cell suspension culture of *Begonia rex - cultorum* 'Baby rainbow' from friable callus in MS medium fortified with 2, 4-D (0.5 mg L^{-1}**)** and BAP (1.0 mg L^{-1})

Table 4: Effect of purified anthocyanin of *B. malabarica* (BM) and *B. rex-cultorum* 'Baby rainbow' (BR) and / or CP on the chromosomal aberrations in bone marrow cells of mice after 24 h of treatment

	Mean of total no. of aberrant metaphases /100 metaphases	Mean of Chromosomal Aberrations					Mean of total number of aberrations
		Gap	Break	Ring	Fragment	Acentric	
Control	13.6	15.6	4	1.2	1	1.8	23.6
CP 50 mg kg^{-1}	95.2	33.6	8.6	2.8	8.2	4.4	57.6
BM 200 mg kg^{-1}	14.6	11.2	2.6	2.2	4.6	3	23.6
BM 400 mg kg^{-1}	22.4	11.4	7.2	2.6	3.4	3.8	28.2
BM 200 mg kg^{-1} + CP 50 mg kg^{-1}	25.2	16.2	7.8	1.2	3.2	1.6	30
BM 400 mg kg^{-1} + CP 50 mg kg-1	29.2	14.4	4.8	2.2	1.8	1.4	24.6
BR 200 mg kg^{-1}	16.8	12.8	4.2	3.2	6	3.6	29.8
BR 400 mg kg^{-1}	21.8	13	6.2	3.4	2.6	4.8	30.2
BR 200 mg kg^{-1} + CP 50 mg kg^{-1}	26	14	5.2	1.2	2.8	3.4	26.6
BR 400 mg kg^{-1} + CP 50 mg kg^{-1}	30	15	4.2	1	2	1.8	24
Silymarin 25 mg kg^{-1} + CP 50 mg kg^{-1}	22.8	16.4	7.4	1	1.2	2.2	28.2
F-BM	143.28**						
F-BR	120.78**						
SE-T	0.51						
SE-V	0.25						
CD-T	1.468						
CD-V	0.734						

*SE-T: Standard error between treatments; SE-V: Standard error between genotypes; CD-T: Critical difference between treatments; CD-V: Critical difference between genotypes

Fig-2: Bone marrow cells treated with cyclophosphamide showing chromosomal aberrations

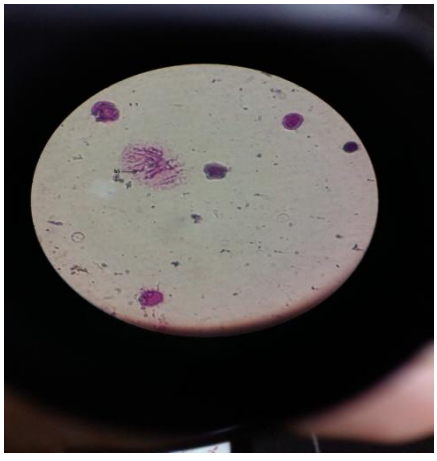
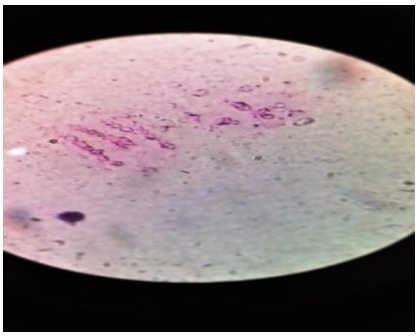
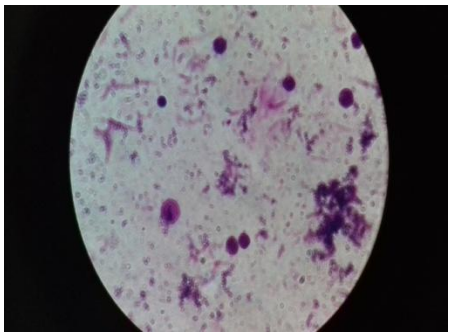


Fig-3 a & b: Bone marrow cells pre treated with *Begonia malabarica* and *B. rex-cultorum* 'Baby rainbow' followed by cyclophosphamide.





ACKNOWLEDGMENTS

The authors are indebted to Kerala State Council for Science Technology and Environment, Govt. of Kerala for funding the major project. Similarly, thanking for the animal facility support provided by KLE University's College of Pharmacy Rajajinagar, Bengaluru.

REFERENCES

1. Chakraborty P, Sk UH, Jayanta N, Das K, Pal S, Bhattacharya S. Modulation of cyclophosphamide-induced cellular toxicity by diphenylmethyl selenocyanate in vivo, an enzymatic study. Journal of Cancer Molecules. 4 (6), 2009, 183–189.
2. Ahmadi A, Hosseinimehr SJ, Naghshvar F, Hajir E, Ghahremani M. Chemoprotective effects of hesperidin against genotoxicity induced by cyclophosphamide in mice bone marrow cells. Archives of Pharmacal Research. 31 (6), 2008, 794– 797.
3. Sanadgol N, Najafi S, Ghasemi LV, Motalleb G, Afsharimoghadam A. A study of the inhibitory effects of *Citrullus colocynthis* (CCT) using hydro-alcoholic extract on the expres- sion of cytokines: TNF-α and IL-6 in high fat diet-fed mice towards a cure for diabetes mellitus. Journal of Pharmacognosy and Phytotherapy. 3 (6), 2011, 81–88.
4. Pratheesh kumar P, Kuttan G. Ameliorative action of *Vernonia cinerea* L. on cyclophosphamide-induced immune sup- pression and oxidative stress in mice. Inflammopharmacology. 2010; 18 (4): 197–207.
5. Hosseinimehr SJ, Ahmadashrafi S, Naghshvar F, Ahmadi A, Ehasnalavi S, Tanha M. Chemoprotective effects of *Zataria multiflora* against genotoxicity induced by cyclophos-phamide in mice bone marrow cells. Integrative Cancer Therapies. 9(2), 2010, 219–223.
6. Cuzzolin L, Zaffani S, Benoni G. Safety implications regarding use of phytomedicines. European Journal of Clinical Pharmacology. 62, 2006, 37-42.
7. Sutharut J, Sudarat J. Total anthocyanin content and antioxidant activity of germinated colored rice. International Food Research Journal. 19(1), 2012, 215-221.
8. Costa TDA, Vieira S, Andrade SF, Maistro EL. Absence of mutagenicity effects of *Psidium cattleyanum* Sabine (Myrtaceae) extract on peripheral blood and bone marrow cells of mice. Genetics and

Molecular Research. 7 (3), 2008, 679-686.

9. Shokrzadeh M, Chabra A, Naghshvar F, Ahmadi A. The mitigating effect of *Citrullus colocynthis* (L.) fruit extract against genotoxicity induced by cyclophosphamide in mice bone marrow cells. The Scientific World Journal. Article ID 980480, 2013, 1-8.
10. Peron AP, Mariucci RG, de Almeida IV, Düsman E, Mantovani MS, Pimenta Vicentini VE. Evaluation of the cytotoxicity, mutagenicity and antimutagenicity of a natural antidepressant, *Hypericum perforatum* L. (St. John's wort), on vegetal and animal test systems. BMC Complementary Alternative Medicine. 13, 2013, 97.
11. Mazzorana DM, Nicolau V, Moreira J, Amaral PA, Andrade VM. Influence of *Mikania laevigata* extract over the genotoxicity induced by alkylating agents. Toxicology. 2013, 2013, 1-7.
12. Oyeyemi IT, Yekeen OM, Odusina PO, Ologun TM, Ogbaide OM, Olaleye OI, Bakare AA. Genotoxicity and antigenotoxicity study of aqueous and hydro-methanol extracts of *Spondias mombin* L., *Nymphaea lotus* L. and *Luffa cylindrica* L. using animal bioassays Interdisciplinary Toxicology. 8(4), 2015, 184–192.
13. Abozed SS, El-kalyoubi M, Abdelrashid A, Salama MF. Total phenolic contents and antioxidant activities of various solvent extracts from whole wheat and bran. Annals of Agricultural Science. 59(1), 2014, 63–67.
14. Idu M, Obaruyi GO, Erhabor JO. Ethnobotanical uses of plants among the Binis in the treatment of ophthalmic and ENT (EAR, Nose and Throat) ailments. Ethnobotanical Leaflets. 13, 2008, 480–496.
15. Mary RR, Begum SMI. Antimicrobial activity of diff erent solvent ex- tracts of *Tridax procumbens* (Asteraceae) from leaf and stem against Human pathogens. International Journal of Scientific Research. 3(8), 2014, 487–489.
16. Boriollo MFG, Resende MR, da Silva TA, Publio JY, Souza LS, dos Santos Dias CT, de Mello Silva Oliveira N, Fiorini JE. Evaluation of the mutagenicity and antimutagenicity of *Ziziphus joazeiro* Mart. bark in the micronucleus assay. Genetics and Molecular Biology.37 (2), 2014, 428-438.