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Original Research Article

Evaluation of Radioprotective and Genoprotective Effect of Solaire–A Herbal Formulation

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A b s t r a c t

The present study was carried out to evaluate the radio protective and genoprotective role of Solaire—a herbal formulation on cellular changes induced by bleomycin and Ultra Violet (UV) radiation in human lymphocytes and *Saccharomyces cerevisiae* strain respectively. Chromosomal aberrations (CA) were evaluated in terms of chromosome break. Mean b/c value and CBMN frequency were also calculated. The results indicated that all Solaire supplemented lymphocytes showed a lower CAs frequency than lymphocytes treated with bleomycin only. It was observed that Solaire had a positive effect in CAs particularly at 100, 200 and 500µg concentration. Besides CBMN frequency decreased depending on applied Solaire doses. *In vitro* results showed that the Solaire supplementation decreases frequency of CAs and its protective role against cellular changes induced by bleomycin is dose-dependent. Further, Solaire treatment enhances the antioxidant status by increasing the activities of antioxidants in UV -irradiated *Saccharomyces cerevisiae* strain. The results of this study show evidence on the protective effect of Solaire against cellular and radiation damage.

K e y w o r d s

Chromosomal aberrations
Cytokinesis block micronuclei
Herbal formulation
Solaire
Ultra-violet radiation

Introduction

Radiation therapy is an essential therapeutic modality for the treatment of wide variety of tumors, but its immediate and delayed side effects on the normal tissues limit the efficacy of the therapy (Grdina et al., 2002; Malicki, 2015). Cell damage may develop, depending on dose and exposure time (Weiss et al., 1990). Prolonged human exposure to solar ultra violet (UV) radiation may result in acute and chronic health effects on the skin, eye and immune system. Sunburn is the best-known acute effect of excessive UV radiation exposure. Over the longer term, UV radiation induces degenerative

changes in cells of the skin, fibrous tissue and blood vessels leading to premature skin aging, photo-dermatoses and actinic keratoses (Zaidi et al., 2002). There are reports that both chromosome and chromatid type aberrations are observed at the first post-irradiation metaphase. In irradiated tissues where cell division is constantly taking place both chromosome and chromatid aberrations are observed (Lloyd and Dolphin, 1977; Coderre, 2007).

The development of the cytokinesis-block (CB) technique has made the human lymphocyte micronucleus assay (MN) a reliable and precise method for assessing chromosome damage. Studies have confirmed that this method is a sensitive indicator of *in vivo* radiation exposure in patients undergoing fractionated partial-body radiotherapy and rodents exposed to uniform whole-body irradiation, thus supporting the application of the cytokinesis-block micronucleus (CBMN) assay for biological dosimetry (Fenech, 1993).

Dietary antioxidants have been reported to protect cells from radiation-induced damages (Pathak and Martin, 2015; Prasad et al., 2004). Most of the plants used in ayurvedic drugs have significant antioxidant activity (Patwardhan et al., 2008; Weiss and Landauer, 2003). Many natural and synthetic chemicals have been investigated in the past for their efficacy to protect against radiation-induced damage in biological systems (Nair et al., 2001). However, the inherent toxicity of some of the synthetic agents at the effective radio protective concentration warranted further search for safer and more effective radio-protectors. In fact, no radioprotective agent is now available, either alone or in combination to meet all the requisites of an ideal radio-protector (Maisin, 1998).

The extracts of certain plants like *Ocimum sanctum*, *Panax ginseng* and *Chlorella vulgaris* have been reported to protect mice against the radiation-induced mortality (Jagetia et al., 1986; Zhang et al., 1989; Singh et al., 1995). Solaire is a herbal formulation of nine Indian medicinal plants. There are no reports regarding the radioprotective effect of Solaire. Therefore the present study was undertaken to evaluate the effect of various doses of Solaire on bleomycin induced chromosomal aberration in lymphocyte culture and UV irradiated *Saccharomyces cerevisiae* strain.

Materials and methods

Experimental design

Collection of plants: The plants were collected from different parts of Wayanad district in Kerala state. The plants were identified from Medicinal Plants Research Centre, Kottakkal, Malappuram, Kerala.

Preparation of the extracts: The extract of each plant was prepared by extracting 100 g of plant powder (powder was obtained from the dried leaves of *Tinospora cordifolia*, *Centella asiatica*, *Ocimum sanctum*, *Curcuma longa* and *Mentha piperita* and bark of *Santalum album*, *Cinnamomum zeylanicum* and *Pterocarpus santalinus* and seeds of *Vitis vinifera*) in distilled water (300ml) at 50-60°C in a Soxhlet apparatus for 72 h. The cooled liquid extract was concentrated by evaporating its liquid contents. An approximate 20% yield of the extract was obtained.

Preparation of the herbal formulation (Solaire): Solaire, the herbal formulation was prepared by mixing the extracts of the following medicinal plants in definite proportions.

Cinnamomum zeylanicum (100 mg); *Tinospora cordifolia* (200 mg); *Centella asiatica* (100mg); *Santalum album* (200mg); *Ocimum sanctum* (200mg); *Curcuma longa* (150mg); *Mentha piperita* (200mg); (200mg); *Pterocarpus santalinus* (200mg) and *Vitis vinifera* (200mg).

Subject: The study has been carried out on blood samples obtained from 10 healthy donors. The age of the donors was between 20 - 40 years.

Lymphocyte sampling and culture: For the analysis of CAs, peripheral blood samples were collected from 10 healthy donors. Ten milliliters (10 ml) of venous blood was obtained from each donor, and evacuated into heparinized tubes and transported to the laboratory on the same day. Blood culture was prepared according to Hsu et al. (1987). Briefly, 1.0 ml of whole blood was immediately added to 5 ml of RPMI 1640 medium containing 10% heat inactivated fetal calf serum, 0.3 ml phytohaemagglutinin, 300U/ml penicillin-streptomycin. Then different concentrations (100, 200 and 500 µg) of Solaire into each culture were added, and the cell culture was incubated at 37±0.5^{o}C for 72 h in a humidified atmosphere containing 5 ± 0.1%

CO_2/95% in air. At 66th hour, bleomycin (0.03 units/ml) was added to the cultures.

Experimental protocol: The six different groups of lymphocyte cultures were prepared from the blood samples obtained from healthy individual as:

Culture A: Control group did not receive mutagen (bleomycin) or Solaire.
Culture B: Lymphocytes were treated with bleomycin (0.03 units/ml).
Culture C: Lymphocytes were treated with 100 µg of Solaire and bleomycin.
Culture D: Lymphocytes were treated with 200 µg of Solaire and bleomycin.
Culture E: Lymphocytes were treated with 500 µg of Solaire and bleomycin.
Culture F: Lymphocytes were treated with 500 µg of Solaire and bleomycin at S phase
Culture G: Lymphocytes were treated only with 500 µg of Solaire alone.

Metaphase preparation: At the end of 70 hr of incubation, colchicine (0.04µg/ml) was added to block cells in metaphase during the last 2 hr of cell growth. Then standard cytogenetics procedures as hypotonic treatment, fixation (1: acetic acid/ 3: methanol), slide preparation, staining (5% Giemsa solution) and air dried preparations was performed. The metaphase preparations are made by placing 10-20 µl of cell suspension with a pipette on freezing slides.

Mitotic index: MI was determined as the percentage of dividing cells among 1000 nucleated cells in each culture. Aberrant metaphase number (AMN) was counted as the number of damaged metaphases among 100 metaphases in each culture.

Chromosomal aberration analysis: From control and positive control group 1000 metaphases, from all treatment groups 100 metaphases were counted for CAs such as break, fragment, dicentric, tricentric, acentric, ring and they were considered to be equal. CAs was scored with binocular light microscope.

Yeast strain and cell culture: The study was conducted using *Saccharomyces cerevisiae* strain. The yeast strain was obtained from local market. In order to obtain single cell suspension, cells were grown on solid yeast extract peptone dextrose (YEPD) agar plates. A single colony from plate was taken and suspended in 0.5 ml of normal saline and the cells were counted on a hemocytometer. Approximately 10^6cells were plated on to a fresh YEPD plate and incubated at 30°C for 24 h to get a lawn. Cells from the lawn were scraped and suspended in phosphate buffered saline and stored at 4°C till further use. This procedure gave single cell suspension with less than 2% budding cells and could be stored for more than 24h without any change in radiation response.

Irradiation conditions: An aliquot (1 ml) of yeast cell suspension was irradiated at ambient temperature with UV-radiation (254nm) in a UV- chamber for 30 min. After irradiation, cells were approximately diluted and plated on YEPDA plates in triplicates. Plates were incubated at 30°C for at least 3 days and the numbers of colonies were counted.

Assay of the antioxidant enzyme levels in untreated and herbal formulation treated yeast: Yeast cells (5 mg/ ml) were incubated for 12 h in phosphate buffer (pH 7.0) without or with herbal formulation under shaking conditions (120 rpm). The cells were harvested and lysed by grinding with glass beads. The extract was used for assay of different enzyme activities. The protein content of the extract was determined by Lowry's method.

Estimation of superoxide dismutase: Superoxide dismutase (SOD) was assayed with yeast culture according to the method of Misre and Fridovich (1972), based on the inhibition of the oxidation of epinephrine to adrenochrome by superoxide.

Estimation of catalase activity: The assay of catalase was carried out following the procedure of Luck (1974). Briefly, to 0.1 ml of yeast cell suspension, 1 ml of buffer and 0.4 ml of water was added. The reaction was initiated by the addition of 0.5 ml of H_2O_2 to the reaction mixture and was incubated at 37^0C for 15 min. The reaction was terminated by the addition of 2 ml of dichromate acetic acid reagent. Standard H_2O_2 solution in the range of 4-20µM was taken and treated in a boiling water bath for 15 min, cooled and OD was measured at 510 nm. Catalase activity is expressed as micromoles of H_2O_2 utilized/min/ml.

Statistical analysis

Results were expressed as mean ± S.D from three independent experiments. Differences in measured variables between experimental and control group were assessed by using the Student's t test.

Statistically significant difference was accepted at $p<0.05$.

Results

Effect of Solaire on chromosome aberration induced by bleomycin in human lymphocyte culture

The data obtained from experimental studies showed that the frequency of CAs induced by bleomycin in human lymphocytes is significantly affected by the presence or absence of herbal formulation. This phenomenon is supported by Table 1 which depicts the level of CAs in all investigated group. Culture group treated with bleomycin showed a higher frequency of total CAs when compared with the control and treated groups (as shown in Fig. 1). The level of CAs in control group was not significantly different from herbal formulation group. However, in bleomycin induced group it was found a high frequency of CAs. The number of CAs and aberrant metaphases in the group exposed to only bleomycin were found to be higher than in the treatment groups supplemented with Solaire. In Solaire-supplemented group, the maximum effect of supplementation was seen at 500 µg Solaire dose. Fig. 2 represents the distribution of mean breaks/cell (b/c) value according to different concentrations of Solaire.

Fig. 1: (A) Mutagen induced chromatid breaks; (B) Cytokinesis- block binucleated cells with Micronuclei

(A) **(B)**

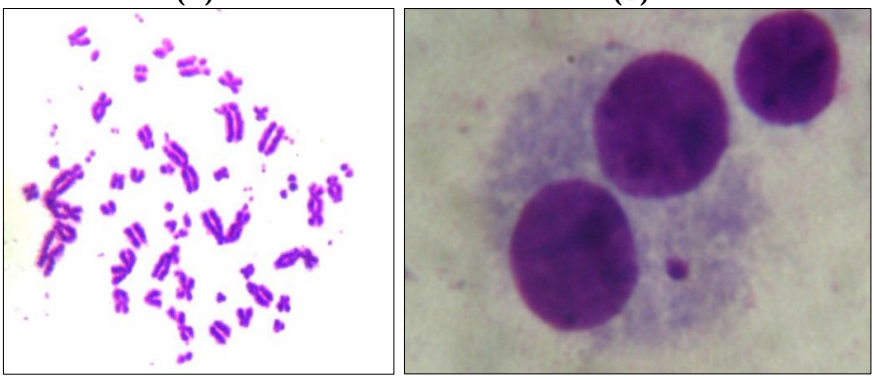


Table 1. Distribution of mean b/c value according to concentration of drug.

Culture	Substrate	Mean b/c Value
Culture A	Cells (Control)	0.5772
Culture B	Cells + Mutagen	0.8784^{a*}
Culture C	Cells + Mutagen + 100 µg Solaire	0.7621^{b}
Culture D	Cells + Mutagen + 200 µg Solaire	0.6844^{c}
Culture E	Cells + Mutagen + 500 µg Solaire	0.6011^{d*}
Culture F	Cells + 500 µg Solaire + Mutagen at S Phase	0.6385^{e}
Culture G	Cells + 500 µg Solaire only	0.5812^{NS}

Hypothesis testing method included student's t test. Values are given statistically significant at $p<0.05$; [a]BLM vs control; [b]BLM + Solaire (100 µg) vs BLM; [c]BLM + Solaire (200 µg) vs BLM; [d]BLM + Solaire (500 µg) vs BLM; [e]BLM (S phase) + Solaire (500 µg) vs BLM; [NS] Non significant.

Effect of Solaire on micronuclei yield induced by bleomycin in human lymphocyte culture

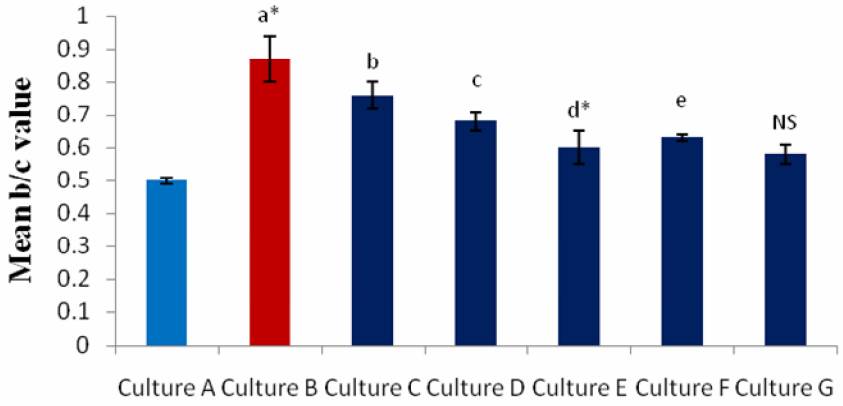
The pretreatment of blood lymphocytes with different doses of Solaire resulted in the reduction of incidence of mutagen-induced micronuclei (MN). Maximal reduction of MN was observed after treating bleomycin treated human lymphocytes with Solaire at a concentration of 500 µg. The herbal formulation has very strong protective effects as evidenced by MN analysis (Table 2). In assessing the effect of Solaire in modifying the mutagen-induced MN yield in human peripheral blood lymphocytes with cytokinesis - blocked (CB) assay, it was found that without the presence of Solaire, MN yield was 18.6 per 1000 binucleated (BN) cells (Fig. 3) in bleomycin treated lymphocytes. Different concentrations of herbal formulation treatment affect the MN yields. Treatment with Solaire, resulted in reduction in MN yields for all Solaire concentrations tested.

Table 2. Distribution of mean CBMN frequency according to concentration of drug.

Culture	Substrate	Mean CBMN Frequency
Culture A	Cells (Control)	10.2
Culture B	Cells + Mutagen	18.6[a*]
Culture C	Cells + Mutagen + 100 µg Solaire	16.0[b]
Culture D	Cells + Mutagen + 200 µg Solaire	14.4[c]
Culture E	Cells + Mutagen + 500 µg Solaire	10.8[d*]
Culture F	Cells + 500 µg Solaire + Mutagen at S Phase	12.0[e*]
Culture G	Cells + 500 µg Solaire only	10.4[NS]

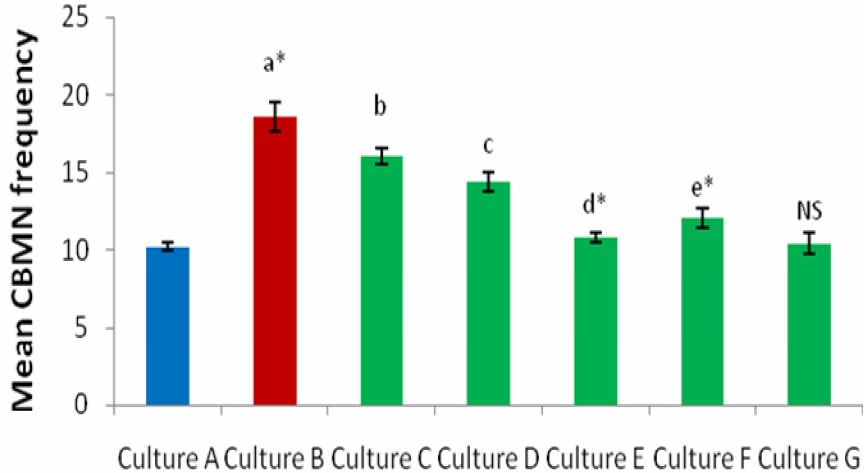
Hypothesis testing method included student's t test. Values are given statistically significant at $p<0.05$; [a]BLM vs control; [b]BLM + Solaire (100 µg) vs BLM; [c]BLM + Solaire (200 µg) vs BLM; [d]BLM + Solaire (500 µg) vs BLM; [e]BLM (S phase) + Solaire (500 µg) vs BLM; [NS] Non significant.

Fig. 2: Effect of Solaire on chromosome aberration induced by bleomycin in human lymphocyte culture.



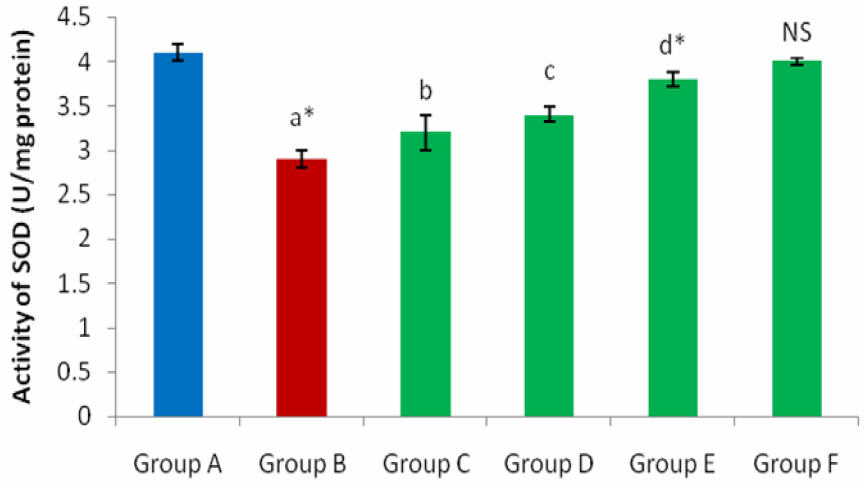
The mean b/c value of study subjects, without herbal formulation was 0.8784 whereas in control it was 0.5772. When the study subjects were supplemented with herbal formulation at conc. of 100 µg, 200µg and 500µg, the mean b/c values were reduced. Hypothesis testing method included student's t test. Values are given statistically significant at $p<0.05$; [a]BLM vs control; [b]BLM + Solaire (100 µg) vs BLM; [c]BLM + Solaire (200 µg) vs BLM; [d]BLM + Solaire (500 µg) vs BLM; [e]BLM (S phase) + Solaire (500 µg) vs BLM; [NS] Non significant.

Fig. 3: Effect of Solaire on micronuclei yield induced by bleomycin in human lymphocyte culture.



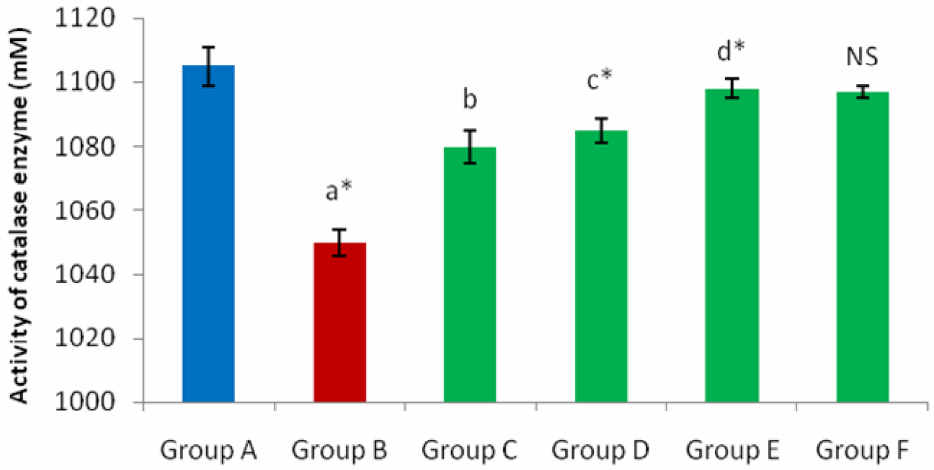
The mean CBMN frequencies of study subjects, without herbal formulation were 18.6 whereas in control it was 10.2. When the study subjects were supplemented with herbal formulation at conc. of 100 µg, 200µg and 500µg, the mean CBMN values were reduced. Hypothesis testing method included student's t test. Values are given statistically significant at $p<0.05$; [a]BLM vs control; [b]BLM + Solaire (100 µg) vs BLM; [c]BLM + Solaire (200 µg) vs BLM; [d]BLM + Solaire (500 µg) vs BLM; [e]BLM (S phase) + Solaire (500 µg) vs BLM; [NS] Non significant.

Fig. 4: Effect of Solaire on the activity of superoxide dismutase (SOD) in control and experimental group.



Radiation reduces SOD enzyme level in yeast, which was 3.00 U/mg whereas in control it was 3.89 u/mg.When the yeast cells were supplemented with herbal formulation at conc. of 20mg, 50mg, and 100mg, the level of enzyme were increased. Hypothesis testing method included student's t test. Values are given statistically significant at $p<0.05$; [a]UV-irradiated vs control; [b]UV-irradiated + Solaire (20 mg) vs UV-irradiated; [c]UV-irradiated + Solaire (50 mg) vs UV-irradiated; [d]UV-irradiated + Solaire (100 mg) vs UV-irradiated; [NS] Non significant.

Fig. 5: Effect of Solaire on the activity of catalase in control and experimental group.



Radiation reduces catalase enzyme level in yeast, which was 1050 mM whereas in control it was 1105 mM.When the yeast cells were supplemented with herbal drug at conc. of 20mg, 50mg, and 100mg, the level of enzyme were increased. Hypothesis testing method included student's t test. Values are given statistically significant at $p<0.05$; [a]UV-irradiated vs control; [b]UV-irradiated + Solaire (20 mg) vs UV-irradiated; [c]UV-irradiated + Solaire (50 mg) vs UV-irradiated; [d]UV-irradiated + Solaire (100 mg) vs UV-irradiated; [NS] Non significant.

Effect of Solaire on antioxidant status in UV-irradiated *Saccharomyces cerevisiae* strain

The effects of the 12 h incubation of the yeast cells with Solaire (10 mg/ml) on the levels of SOD and catalase are shown in Fig. 4 and Fig. 5. The activities of enzymic antioxidants were reduced in UV-irradiated yeast strain as compared to the control group. Solaire treatment enhances the activities of these antioxidants as compared with irradiated group.

Discussion

The Ayurveda widely uses the plant- derived compound formulations for the treatment of various ailments after a detailed study into the type of the disease (Jagetia and Baliga, 2003). Often the drugs formulated are such that they have the desired activity with the ample potency and are deprived of side effects. As it is observed that the desired activity is rarely present in sufficient potency in a single plant

and it may also contain unwanted activities. Therefore, several plants with the common desired activities and varied undesirable activities are selected so that the final formulation will have a concentrated desired activity and the undesired activities will be absent or diluted. Further, it is also observed that in such formulation, certain other compounds resulting in an additive positive effect, which may be of vast benefit to the patient (Kulp and Kuttan, 1996).

It is a well-established fact that ionizing radiation at cellular level can induce damage in the biologically important macromolecules such as DNA, proteins, lipids and carbohydrates in the various organs. While some damage is expressed early others are expressed over a period of time depending upon the cell kinetics and the radiation tolerance of the tissues. In the present study, a herbal formulation was prepared using extracts of nine Indian medicinal plants. Blood samples were collected from healthy donors, and mutagen (bleomycin) induced chromosomal aberration studies and cytokinesis-block micronuclei assay were performed in order to evaluate the genoprotective effect of the herbal formulation. The radio protective effect of herbal formulation was evaluated using UV-irradiated *Saccharomyces cerevisiae* strain. Biochemical assay of enzyme activities of catalase and superoxide dismutase (SOD) was also performed. The protein content of the extract was determined by Lowry's method.

All the results can be explained by the antioxidant effect of Solaire which decreases the genetic damage. The maximum level of CA incidence was observed in culture B exposed to only Bleomycin and also the lowest frequencies were observed in Solaire-supplemented culture E. With raising the Solaire doses, the frequency of CAs decreased in all groups exposed to Bleomycin. In group supplemented with Solaire, the highest frequency of CAs was observed at 100µg dose of Solaire and least frequency of CAs was observed at 500µg dose of Solaire. Briefly, there was a strong dose-effect relationship between the CAs and Solaire dose. Consequently, it was shown that Solaire has a protective effect on the frequency of CAs, but this effect related with dose. The antioxidant status of Solaire should be considered as a factor providing decrease in frequency of CAs. With the antioxidant role of Solaire the influence of free radical induced by mutagen in blood samples were decreased. The most of the damaged cells showed a large number of CAs as fragments, breaks, dicentrics, tricentrics, acentrics and rings.

The researchers found that antioxidants are significant molecules that act as free radical scavengers, and they trap the free radicals and give up own electrons. Thus, antioxidants with stated functions, molecules as protein, lipid, enzyme, chromosome and DNA were protected against free radical oxidation (Feri, 1994; Halliwell et al., 1995). It has been suggested that herbal formulations like mentat, abana, chyavanaprasha, rasayana, triphala, geriforteetc have protective effect against the harmful effects of ionizing radiations (Ashmawy et al., 2006; Scolastici et al., 2007). In another study, it was found rather decreased the percentage of CAs of supplementation with antioxidants of the human diet (Dusinska et al., 2003). Comparison of these results shows that the frequency of CAs and the protective effect of Solaire observed in this study are in agreement with the data reported in literature.

These results of cytokinesis blocked micronuclei (CBMN) assay indicated that Solaire, the herbal formulation exerts apparent cytogenetic effect on human peripheral blood lymphocyes at all concentrations. Based on the restricted quantitative information in the literature, this simple research addresses the issue of the protective effects of Solaire on mammalian cells obtained from human peripheral blood lymphocytes. Results of this study clearly indicate that the herbal formulation appears to give a protection against mutagen - induced damage. However, although the exact underlying protective mechanism of Solaire is unclear, it could be through its antioxidative capability by scavenging free radicals responsible for DNA damage.

It is well known that endogenous antioxidant enzymes like SOD and catalase act as endogenous defense mechanisms against the ROS-mediated biological damages. Any radioprotectant, thus, can exert its action by ROS scavenging and inducing the generation of the above enzymes *in vivo.* Consequently, in order to probe into the mechanism of radioprotective action of Solaire, its possible role in up-regulating the levels of SOD and catalase in yeast cells was also investigated. Earlier it has been reported that radiation alone, does not trigger SOD level in yeasts, while it can inactivate both SOD and catalase (Lee and Park, 2004). Similar findings were

reported by Uma Devi et al. (2000) after ^{60}Co whole body irradiation. This could be due to the enhanced utilization of the antioxidant system in an attempt to detoxify the free radicals generated by radiation. In the intact and healthy cells, the enzymes are restored immediately after each interaction (Sulochana and Uma Devi, 1984), which is also seen in the present experiment, where yeast cells supplemented with Solaire after radiation exposure show significant increase in SOD and catalase level. However, the levels of enzymes did not reach absolute normal level. It could be due to the fact that, in the irradiated yeast cells the normal synthesis/repair may be disrupted due to damage to DNA and membranes. As a result, restoration will be delayed till the cells recover.

Conclusion

From this study it is clear that Solaire, a plant based formulation, provided protection against cellular changes induced by Bleomycin in human lymphocytes and in UV-irradiated *Saccharomyces cerevisiae* strain. The exact mechanism of action of Solaire is not known, however it may scavenge free radicals produced by radiation and thus inhibit radiation induced damage to cellular DNA. Alternatively it may increase the level of endogenous antioxidant enzymes such as SOD and catalase providing protection against radiation-induced damage. Since significant protection is obtained at a very low non- toxic dose the formulation may be used as a radioprotector.

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