ORIGINAL ARTICLE

Genoprotective effects of *Acorus calamus* rhizome against DNA damage in peripheral blood lymphocytes

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Abstract

Background: *Acorus calamus* is a medicinal plant from India, which is used in the popular medicine for the treatment of malaria, arthritis, bronchial asthma, flatulent colic, and epilepsy. In this study, the genoprotective effect of *A. calamus* was evaluated in peripheral blood lymphocytes using cytokinesis block micronucleus assay (CBMN) and chromosome sensitivity analysis. **Materials and Methods:** Preliminary phyto chemical analysis of the extract was performed to determine its major phyto chemical constituents such as alkaloids, sterols, flavonoids, terpenoids, phenolic compounds, saponins, carbohydrates, glycosides, and tannins. Gas chromatography–mass spectrometry analysis of extract showed the presence of major bioactive compounds. Peripheral blood lymphocytes were treated with bleomycin (BLM) in the presence of methanol extract of *A. calamus* (MEAC) to observe its antigenotoxic potential. **Results:** In CBMN assay, treatment with MEAC at different concentrations in culture medium showed a statistically significant decrease in the frequency of micronucleus. A significant reduction in the chromosomal aberration frequency, especially chromosome gaps, breaks, acentric chromosomes, and dicentrics were also observed. **Conclusion:** The results clearly indicate that *A. calamus* can protect against DNA damage induced by BLM in cultured human peripheral blood lymphocytes and has proved to be a significant potential to protect cellular system from DNA damages.

Key words: *Acorus calamus*, chromatid breaks, DNA damage, lymphocytes, micronuclei

INTRODUCTION

Acorus calamus. L is a versatile medicinal plant with diverse biological activities. It is an indigenous plant to India but now found across Europe, Southern Russia, Northern Asia Minor, China, Japan, Burma, Sri Lanka, and Northern United States. The rhizomes are considered to possess anti-spasmodic, carminative, anthelmintic, sedative, and stimulant properties. It is used by almost all local people for its therapeutical efficacy against common cold, cough, fever, headache, and also used for the treatment of epilepsy, mental ailments, chronic diarrhea, dysentery, glandular, and abdominal tumors.[1]

Among the numerous biomarkers to monitor the genoprotective potential, chromosomal damage detection is suggested to be very predictive. Lymphocytes in culture constitute an ideal test system to evaluate the chromosomal damage. A large body of evidence exists regarding the role of a chromosomal break in peripheral blood lymphocytes. It was assumed that the extent of chromosomal damage evaluated in lymphocytes is likely to reflect the level of damage in cancer-prone tissues and in turn cancer risks.[2]

Mutagen sensitivity plays an important role in carcinogenesis. Tests of mutagen sensitivity have been devised as an indirect indicator of DNA repair competence and cancer predisposition.[3] A person with a slight defect in one step of DNA repair will probably accumulate more mutations and chromosomal aberrations than another individual with a completely normal repair system. The higher the number of

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Received: 25-08-2016
Revised: 24-10-2016
Accepted: 04-02-2017

mutagen induced chromosomal breaks, the poorer the DNA repair mechanisms.[4]

In the present study, with regard to mutagen sensitivity assay, bleomycin (BLM) was used as a standard mutagen. *In vitro* treatment with BLM has been proposed as an assay that allows manifestation of a higher individual sensitivity in subjects probably at a higher risk to develop cancer.[5] Here, we report the BLM-induced MN frequency and chromatid breaks, as an expression of mutagen sensitivity, was viewed as a possible means to predict the genoprotective ability of methanol extract of *A. calamus* (MEAC) against the DNA damage in light of the link between DNA repair capacity and genesis of diseases.

MATERIALS AND METHODS

Collection and Authentication of the Plant

The fresh plant of *A. calamus* was collected from Alappuzha district, Kerala, India. Identification of the plant was performed in the Department of Botany, Sanadhana Dharma College, Alappuzha. A voucher specimen (10001) is preserved as herbarium and submitted to the Department of Zoology, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore.

Preparation of Methanol Extract

Fresh rhizomes used for extraction were shade dried and powdered using a mechanical grinder. About 30 g of fine powder was subjected to sequential soxhlet extraction using methanol (300 ml) as the solvent. After extraction, the solvents were evaporated using rotary evaporator and were stored at −20°C until use.

Chemical Analysis of the Extract

Preliminary phyto chemical analysis of the extract was carried out to determine its major phyto chemical constituents. Qualitative chemical analysis was performed as per the standard procedure of Kokate *et al*.[6] Gas chromatography–mass spectrometry (GC-MS) analysis of extract was carried out on a Shimadzu QP-2010 PLUS gas chromatograph coupled to a mass spectrometer instrument (Shimadzu Corporation, Japan). The individual components were identified by computerized matching of mass spectra of peaks with those gathered in the NIST 08 and WILEY 8-Mass Spectral library of the GC-MS data software system.

Cell Viability Assay

The cell viability of MEAC on human lymphocytes was assayed using the trypan blue dye exclusion method after incubation of cells with samples of *A. calamus* at the concentrations of 10, 20, 40, 60, 80, and 100 µg/ml for 24 h. Viable cells were determined based on the ability of cells to exclude the dye.

Cytokinesis Block Micronucleus Assay (CBMN)

For CBMN analysis, the peripheral lymphocytes were incubated at 37°C for 72 h and treated with MEAC at different concentrations. Cytochalasin B was added to the cultures 44 h after PHA stimulation. At the end of incubation cells were harvested by centrifugation and slides were prepared. 1000 binucleated cells were scored and the distribution of MN among binucleated cells was recorded.

Chromosome Sensitivity Analysis

BLM treatment (0.03 u/ml) was given to peripheral blood lymphocytes, 6 h before harvesting to induce chromosomal breakage. The slides were stained with Giemsa and chromosomal lesions such as breaks, gaps, acentric fragments, double minutes, ring chromosomes, and dicentric chromosome were scored. The frequency of breaks was expressed as breaks/cell (b/c) for comparison. Any individual expression <0.8 b/c was considered hyposensitive, 0.8-1.0 b/c was considered sensitive and those >1.0 b/c was considered hypersensitive. A minimum of 100 metaphases per culture was scored.

Statistical Analysis

The data were statistically analyzed using ANOVA. The database was created in MS Word 2007.

RESULTS

Qualitative Phytochemical Analysis

Qualitative phyto chemical analysis of the plant extract provided the information regarding various types of phytoconstituents such as alkaloids, terpenoids, sterols, carbohydrates, saponins, and flavonoids.

GC-MS Analysis

The GC-MS analysis detected the presence of fourteen significant compounds in the sample. The most important compounds identified were azulene, methyl eugenol, alpha-asarone, cedranone, tetradecanoic acid, isocalamendiol, and linoleic acid.

Cell Viability Assay

Human peripheral blood lymphocytes incubated with MEAC (10-100 µg/ml) showed no important decrease in cell

viability up to a concentration of 60 µg/ml [Figure 1]. Hence, 10-40 µg/ml were selected as non-toxic concentrations in the subsequent experiments.

CBMN Assay

When MN formation was analyzed after treatment with different concentrations of MEAC, significant changes in the frequency of MN were detected for 20 µg/ml and 40 µg/ml ($P < 0.01$). The lowest extract concentration (10 µg/ml) did not induce any change in MN frequencies when compared to control [Table 1].

Chromosome Sensitivity Analysis

In normal control b/c value was found to be hyposensitive (0.6973). After the addition of mutagen (BLM) b/c value increased as sensitive. Among the treatment groups, all the treatments were found to be hyposensitive when compared to that of BLM control [Table 2]. Also, a dose-dependent decrease in b/c value was observed in MEAC treated groups.

DISCUSSION

Various repair mechanisms exist in living organisms to repair DNA damage. If not repaired, this damage may lead to mutagenesis and genetic instability, which is a hallmark of cancer.[7] Experimental and epidemiological evidence strongly suggests that oxidatively induced DNA damage may significantly contribute to human cancers. Therefore, understanding of this type of DNA damage, its repair mechanisms and biological effects is of utmost importance.

Reactive oxygen species possess high reactivity and could affect cellular functions and integrity of higher animals. Oxidative stress induced by reactive oxygen species can cause injuries in DNA such as DSBs, changes in major and minor grooves, changes in a double helix, and could destroy proteins and lipids.[8] In this situation, the biological activities of the medicinal plant such as *A. calamus* that may protect cells against DNA damage are being investigated. Here, the reduction of chromosomal damage by the addition of MEAC to peripheral blood lymphocyte culture is taken as a biomarker tool for DNA repair capacity of *A. calamus*.

The family of BLM, a widely used cancer chemotherapy antibiotics, are known to bind DNA and producing predominantly DNA single and double strand breaks as well as base loss.[9] The ability of BLM to induce specific DSB was utilized as a tool for studies on DNA repair in specific genomic locations.[10] BLM has been reported to induce DNA damage, point mutations, recombination, chromosome aberrations and micronuclei in diverse organisms, including bacteria, bacteriophage, fungi, drosophila, and mammals.[11]

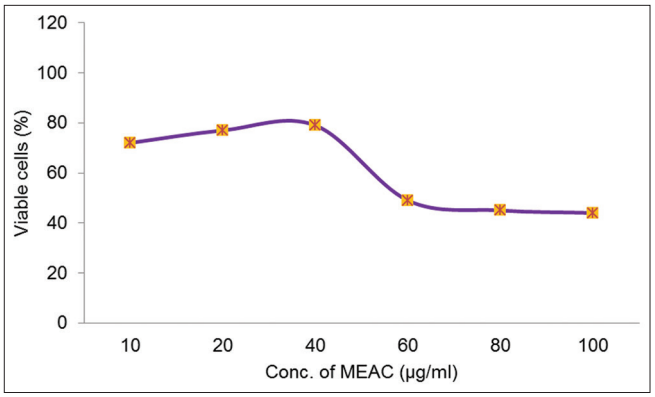

Figure 1: Cell viability of methanol extract of *Acorus calamus* in peripheral blood lymphocytes

Table 1: Micronucleus frequency in lymphocyte cultures treated with MEAC

MEAC (µg/ml)	Frequency of MN (mean±SD)
Control	12.8±0.37
10	11.4±0.6
20	9.6±0.24*
40	9.4±0.50*

ANOVA: *Significantly different from control (P<0.01), SD: Standard deviation, MEAC: Methanol extract of *Acorus calamus*

Table 2: Bleomycin induced chromatid breaks in treatment groups

Doses (µg/ml)	Mean b/c value
Control	0.6973±0.01
BLM	0.8311±0.07
10	0.7415±0.01
20	0.7275±003
40	0.7105±0.05

BLM: Bleomycin, values are mean±SD of triplicates, SD: Standard deviation

Recently, investigations on MN frequencies support the widely accepted assumption that MN is a product of early events in human carcinogenic processes.[12] Our results showed that all tested concentrations of *A. calamus* lowered the mutagenic effects of BLM by decreasing MN frequencies with the strongest activity obtained for highest concentration (40 µg/ml).

These data are in accordance with the findings of Sandeep and Nair,[13] who reported that the radiation-induced cellular damage by micronuclei formation and chromosomal aberrations were significantly reduced by alpha-asarone from *A. calamus*. This study highlights the role of alpha-asarone as a good natural radioprotective agent. Similar studies were reported by Pandey and Agarwal[14] in which the methanolic

extracts of stem bark of Bauhinia variegate significantly prevented micronucleus formation.

Considering the data from the mutagen sensitivity assay, lymphocyte cultures of control and treatment groups showed significant variation within the treatment groups. The lowest mean break per cell value was observed in the dose of 40 µg/ml (0.7105), which was found to be hyposensitive. Also, among the treatment groups, a dose-dependent decrease in mean break per cell value was observed. The treatment groups showed hyposensitivity to BLM can be considered as an efficient DNA repair systems, as they decreased the rate of chromosomal breaks.

It is believed that the main mechanisms involved in the antigenotoxic activity are related to free radical scavenging properties, iron chelation, inhibition of lipid peroxidation or stimulation of endogenous antioxidant response. Here, the protective role is mainly attributed to the free radical scavenging properties and antioxidant defense systems. This is feasible because many naturally occurring compounds are known to exhibit discrete mechanisms of protection.[15,16]

The secondary active compounds from *A. calamus,* especially, alpha-asarone was thought to play an important role in the protective activity. Anti-carcinogenic activation of α-asarone has been reported earlier on human carcinoma cells.[17] It was also reported that asarone have properties of inhibiting adipogenesis and stimulating lipolysis in 3T3-Li adipocyte.[18]

CONCLUSION

The outcome of this study highlights the genoprotective effect of MEAC against the DNA damage induced by BLM. The presence of biologically active phytocompounds such as alpha-asarone might be responsible for the protective effects rendered by the rhizome extract.

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Source of Support: Nil. **Conflict of Interest:** None declared.