Research Article

Evaluation of radioprotective properties of *Curcuma longa* rhizome extract: A cytogenetic analysis in cancer

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ABSTRACT: This is aimed to examine radio protective effect of *Curcuma longa* rhizome extract (CLE) on radiation induced chromosomal aberrations in cancer treatment. The 50% hydroalcoholic extract was used to treat blood sample (taken from a healthy volunteer). In-vitro cytogenic analysis was performed by arresting metaphase using colchicine. The chromosomal aberrations were observed using light microscopye. For evaluation of the radioprotective role of CLE the abnormal metaphase aberrations were analyzed and their percentage was calculated. Pre-treatment and post treatment with *Curcuma longa* extract (CLE) at 200 mg/bodyweight mg/body weight, resulted in a significant reduction in the abnormal metaphases, as well as in the different types of aberration. It was concluded that *Curcuma longa* extract (CLE), when given prior to radiotherapy lead to a decrease in all types of chromosomal aberrations. CLE may therefore have applications to as an adjuvant to radiotherapy in cancer treatment due to its radioprotective potential.

KEY WORDS: Curcuma longa, turmeric, cancer, radioprotective effect, metaphase, cytogenic analysis.

INTRODUCTION

Curcuma longa is a medicinal plant extensively used in ayurveda, *unani* and *siddtha* medicine as a home remedy to treat various diseases.^[1] In old Hindu medicine it was extensively used for the treatment of sprain and swelling caused by injury.^[2] Turmeric is native to the monsoon forests of South East Asia. It is perennial herb, 1 M tall with underground rhizomes. The chemical composition of turmeric rhizome consists of protein (6.3%), fat (5.1%), minerals (3.5%), carbohydrate (69.4%), moisture (13.1%) and curcumin ($C_{21}H_{20}O_{6}$, 50-60%).^[3-6] Curcumin is very useful in joint related concerns, liver protection, lowering cholesterol, as a digestive support, controlling obesity, induces the flow of bile, as anti-inflammatory agent, skin complaints, circulatory disorders as well as tumors in the uterus and menstrual problems.^[7-9] In Chinese Medicine it

*Correspondence: E-mail: swapnilsharma1978@gmail.com DOI: 10.5530/pc.2012.2.7 is used for chest, stomach and periodic pain, removing stagnation, lifting both depression and mania, while relaxing the gall bladder and to treat jaundice and gall stones.^[10] It has good antioxidant and anti-cancer properties, protecting DNA from cigarette smoke hazards.^[11]

There is a review on suppression of tumor initiation, promotion and metastasis by curcumin in the prevention and therapy of cancer.^[12] Oral administration of turmeric oil in 9 healthy volunteers revealed that there was no clinical, hematological, renal or hepatic-toxicity.^[13-15] A Phase 1 clinical trial in 25 patients with a high-risk or premalignant lesions revealed that it was not toxic to humans up to 8,000 mg/day.^[16] The suppression of cyclooxygenase-2 expression by inhibiting extra cellular signal-regulated kinase activity and NF-kappa B activation represented molecular mechanisms of the reported antitumor promoting effects of CLE in mouse skin tumorigenesis.^[17] Dietary curcumin administration (2% w/v, 30 days) to male ddY mice increased the activities of glutathione peroxidase, glutathione reductase, glucose-6-phosphate dehydrogenase and catalase.^[18] Nitrosodiethylamine (NDEA) treated female Wistar rats receiving 1 or 5% turmeric before, during and

after carcinogen exposure showed a decrease in gamma glutamuyl transpeptidase positive foci measuring >500 or >1000 micron and decreases in NDEA-induced focal dysplasia and hepatocellular-carcinomas.^[19] Gavage administration of tumeric at 200 mg/kg or 600 mg/kg effectively suppressed diethylinitrosamine-induced liver inflammation hyperplasia and cellular gene products and cell-cycle related proteins rate, thus playing a potential role in prevention of hepatocellcular carcinoma.^[20] It's administration showed immunomodulatory effects in Balb/c mice which include increase in an total WBC count (from normal 10, 130 to 15, 290), circulating antibody titre against SRBC, plaque forming cells in the spleen, bone marrow cellularity and alpha-esterase positive cells.[21] Application of curcumin at concentration of 200 µM to human cancer cell cultures HeLa, K-562 and IM-9 with X-irradiation showed a radiation sensitising effect.^[22] Curcumin prevented photodynamic treatment induced JNK activation, mitochondrial release of cytochrome c, capase - 3 activation and cleavage of p21-activated kinase 2 in human epidermal carcinoma A431 cells.^[23] It was found to be a potent radiosensitizer in p53 mutant prostate cancer cell line PC-3 and it acts by overcoming the effects of radiation-induced prosurvival gene expression in prostate cancer.^[24] An herbal detoxification formula Smoke Shield which contains extracts of turmeric and extracts of green tea and other spices, inhibit mutagenic response in vitro and in vivo in smokers.^[25] Curcumin was cytotoxic in vitro, for B16-R melanoma cells resistant to doxorubicin.[26] The cellular and molecular changes induced by curcumin, lead to the growth inhibition and induction of apoptosis in human lung cancer cell lines-A549 and H1299.^[27] The potential of antisense inhibition of antiapoptotic proteins as an effective strategy to tackle chemoresistant cancers with curcumin was revealed by the ectopic expression of Bcl-XL or Ku70 which protects human colon cancer cells against curcumin-induced apoptosis.^[28] The potential use of curcumin to sensitize prostate cancer for tumor necrosis factor-related apoptosis inducing ligand mediated immunotherapy was demonstrated in androgen sensitive human prostate cancer cell line LNCaP.^[29] The inhibitory effect of curcumin on the expression of glutathione S- transferase P1-1 mRNA as well as protein synthesis was examined and this inhibition was correlated with the apoptotic effect on K562 leukemia cells.^[30] Curcumin inhibits the proliferation of bovine aortic endothelial cells induced by fetal bovine serum and tumor conditioned medium.[31] Interleukin (IL-2 and IL-6), mRNAs levels were not influenced markedly by curcumin in stimulated human peripheral blood mononuclear cells, but reduced in stimulated Jurkat T cells which confirms the anti-neoplastic potential of curcumin.^[32] The anticancer effect of zerumbone from Zingiber aromaticum was compared with that of curcumin in an in-vitro MTT tetrazolium salt assay using HT-29, CaCo-2 and MCF-7 cancer cells.[33]

MATERIALS AND METHODS

Collection, authentication of identity, and storage of plant material

Rhizomes of *C. longa* were collected from the herbal garden of Rajasthan University, Jaipur (Rajasthan, India) and botanical authentification was carried out at the Department of Botany, University of Rajasthan (voucher specimen no. RUBL20695). Rhizomes of *C. longa* were collected, dried, powdered and extracted with 50% hydro-alcohol using the soxhlet apparatus for 48 hours. The hydro alcoholic extract was filtered through Whatmann filter paper no.1. The filtrate was dried under vacuum and stored in a desiccators. The extract obtained was powdered and weighed. The yield was found to be 24% of dried mass. The dried *C. longa* rhizome extract (CLE) was than subjected to qualitative phytochemical analysis.^[44]

Cytogenic analysis: Chromosomal aberration studies in human blood sample:

5-10 ml of venous blood was drawn from a adult male (bearing normal body mass index 22.5) (JLNCRH, Bhopal, India) with a sterile disposable needle (1.10x 38mm) and syringe (10ml) aseptically in to a sterile Bisou bottle containg 30 units of heparin (1000IU/MI). The blood was allowed to settle by gravity sedimentation. Roswell Park Memorial Institute medium 1640 (RPMI, USA), (Himedia, Bombay) was prepared and sterilized and transferred in to the cultured bottle. 1 ml of phytohaemagglutin (PHA) was added with 9 ml media into the autoclaved bottles. The blood samples were divided into five groups and exposed to radiation treatment of 4 Gy for 10 mins.

Five groups were prepared for analysis which contains:

- I. Blood sample
- II. Blood sample + Rhizome extract (200 mg/kg)
- III. Blood sample + Radiation (Co-60)
- IV. Blood sample + Rhizome extract (200 mg/kg) + Radiation(Co-60) (after 1 hr)
- V. Blood sample + Radiation (Co-60) + Rhizome extract(200 mg/kg) (after 1 hr)

The sample were then kept for seventy two hr for developing of culture in carbon di-oxide $(0.5\% \text{ CO}_2)$ incubator. Following a seventy two hr incubation colchicines was added to each bottle to arrest the metaphase and again incubated for 2 to 3 hr. The blood samples were then transferred into separate test tubes and centrifuged at 500 rpm/10 min. Cells were sedimented in tubes and supernatant was discarded. Pellets were fixed in freshly prepared chilled Cornoys fixative for 30 min (methanol and acetic acid). After fixation, cells were centrifuged and the supernatant was discarded. Fresh Cornoys fixative was added and the process was repeated accordingly to debris in the cell sample so as to get clear solution. Finally the cell suspension was dropped on clean, pre chilled slides using Pasteur pipettes, from a height of 6 inches. The excess of fixatives was allowed to run off by fitting the slides which were gently heated to help in the spreading of chromosomes and dried at 40-60 °C. The slides were stained with Giemsa (0.1%)Qualigens) for 10-15 min and rinsed in tap water. The slides were dried and mounted in DPX and observed by light microscopye. Five slides from different groups were selected. The remainders were stored at -10°C. The aberrations scored were breaks, (chromatid breaks and chromosomes breaks), asymmetrical exchanges (dicentrics, rings and fragments), severely damaged cells, polyploidy and anaphase aberrations. One hundred metaphases per groups were counted and the percentage of chromosomal aberrations was calculated.

RESULTS

Phytochemical screening:

Phytochemical screening was carried out on *Curcuma longa* rhizome extract which revealed the presence of alkaloids, saponins, flavonoids, terpenes and steroids (Table 1).

Cytogenic analysis

The present research work explained the mutagenic behavior of radiation and the radio protective role of hydroalcoholic extract of *Curcuma longa* (CLE) in *in-vitro* lymphocyte cultures.

In group I, normal blood was analysed and the 100 metaphases were found to be entirely safe without any chromosomal aberration (Figure 1 and Table 2). In group II, normal blood was treated with CLE (200 mg/kg), a total

Table 1: Result of the phytochemical screening on the rhizome extracts of *Curcuma longa*

Constituents Analyzed	Remarks
Tannins	++
Flavonoids	++
Sterols	++
Terpenes	++
Saponins	++
Polyphenol	++
Alkaloids	++
Cardiac glycosides	
Gums	

++ indicates presence detected; -- indicates lack of presence detected.



Group IV: Blood sample + CLE + Radiation

Group V: Blood sample + Radiation + CLE extract

Figure 1: Effect of CLE on radiation (4 Gy for 10 min) induced chromosomal abbberation in human lymphocyte culture.

100 metaphase were counted. All metaphase were found to be normal indicating the CLE (200 mg/kg) had no deleterious effect on the observed chromosomes. In group III, (blood exposed to Co-60 at a dose of 4 Gy for 5 minutes), 30 chromosomes were normal and 70 were aberrated out of 100 in which 13 of these were dicentric, 8 were acentromeric fragments, 15 were rings, 10 showed premature centromeric division, 6 showed polyploidy, 9 showed double minutes,4 showed deletion and 5 showed acrocentric association (Figure 1 and Graph 1). It was noticed that in IV group when CLE (200 mg/kg) was given prior to radiation exposure out of 100 metaphase, 80 were normal and 20 were abnormal. Of these 4 were dicentric, 4 showed acentromeric fragments, 2 were rings, 2 showed premature centromeric division,3 showed polyploidy, 2 showed double minutes, 1 showed deletion, 2 showed acro centric association (Figure 1). Finally in group V, 60 metaphases were normal out of 100. Of those showing aberrations, 12 were dicentric, 12 showed acentromeric fragments, 10 were rings, 3 showed premature centromeric division, 3 showed polyploidy, 2 showed double minutes 2, 3 showed deletion, 3 showed acro centric association (Figure 1 and Graph 1).

DISCUSSION

It has long been known that ionizing radiation can interact directly with biological chromophores such as deoxyribonucleic acid (DNA) and in doing so, can damage those molecules. The use of ionizing radiation has become an integral part of modern medicine. It is used for diagnostic as well as therapeutic purposes. The therapeutic differentiation may be achieved with chemical radiation sensitizers or protectors. The development of radiation protectors is important not only to enhance the effectiveness of cancer treatment, but also for the study of the underlying mechanisms of radiation cytotoxicity.^[34] Ionizing radiation causes cellular damage mainly by formation of reactive oxygen species. Therapeutic exposure to 131-Iodine is known to generate oxidative stress and cause cellular damage in target organs involved in concentrating radioiodine.[35] Many Indian herbs have potential medicinal values which covers various aspects of medication. However finding a radio protective herb is rare. Turmeric consists of water soluble turmerin and lipid soluble curcumin with potent antioxidant properties.[36-37] Turmeric and its constituents have also shown cellular protection against H2O2 induced renal epithelial cell injury.[38-39]

Table 2: Effect of 50% hydroalcoholic extract of turmeric rhizome (CLE) on radiotherapy induced chromosomal abberations in human lymphocyte culture

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Groups	ТМ	NM	AM	Types of Aberrations							
				DC	AF	R	PCD	PP	DM	D	ACA
I	100	99	Nil	Nil	Nil	1	Nil	Nil	Nil	Nil	Nil
II	100	99	Nil	Nil	Nil	Nil	Nil	Nil	1	Nil	Nil
111	100	30	70	13	8	15	10	6	9	4	5
IV	100	80	20	4	4	2	2	3	2	1	2
V	100	60	40	12	4	10	3	3	2	3	3

TM = total metaphase, NM = normal metaphases, AM = abberent metaphase, DC= dicentric, AF = acentromeric fragements R = rings, PCD = premature centromeric division, PP = polyploidy, DM = double minute D = deletion, ACA = acrocentric association



Graph 1: Effect of 50% hydroalcoholic extract of turmeric rhizome on radiotherapy induced chromosomal aberrations in human lymphocyte culture.

In the present study the radioprotective role of hydroalcoholic extract of rhizomes of Curcuma longa was evaluated using a peripheral blood lymphocyte culture. It was evident that chromosomal aberrations had occured due to radiotherapy at 4 Gy for 10 mins (Group III). However in group IV, a dose of 200 mg/kg body weight Curcuma longa rhizome extract showed excellent protection via reduction of abnormal metaphase when the drug is administered one hour prior to radiotherapy. When the same extract was administered after one hour of radiation (Group V), the reduction in aberrated metaphase was observed but it was lesser to that of group IV. In both the groups it showed that the frequency of double minute and acrocentric association showed hardly any difference in comparison to group III, although a remarkable reduction in all other types of aberration were observed. This directly indicates that rhizome extract may have potent radioprotective effects. Furthermore, the greatest radioprotective effect was observed in group IV, when the CLE was administered one hour prior to radiotherapy.

When CLE was administered as an adjuvant, has shown radio protective properties in radiotherapy (Graph 1 and Table 2). The results of this study demonstrated the radio protective effects of hydroalcoholic extract of CLE against genotoxicity and toxicity induced by radiotherapy in human peripheral lymphocyte culture. Preliminary phytochemical screening indicated the presence of flavonoid, triterpenoids, alkaloids and tannins, phytosterols, in the extract. It has been previously reported that some flavonoids possess antimutagenic and antimalignant effects.[40] Moreover, flavonoids have a chemopreventive role in cancer through their effects on signal transduction in cell proliferation and angiogenesis.^[41] Phytosterols are able to be incorporated into the cell membrane, where they alter membrane fluidity and the activity of membrane-bound enzymes. They also alter signal transduction in pathways leading to tumor growth and stimulate apoptosis in tumor cell lines.^[38,39] This supports the indirect cytotoxic mechanism of the extract. The results also revealed that the extract showed significant radioprotective activity; this may be due to the presence of triterpenoids, alkaloids and tannins which might have enhanced the radioprotective property of the flavonoids in the extract. Flavonoids, alkaloids and other active compounds which are present in CLE rhizomes extract had shown excellent scavenging property to scavenge free radicals due to high reactivity of hydroxyl substituents. Hence it is possible that the extract may protect chromosomal and genomic damages with its antioxidant property. Nevertheless extract may have certain different other mechanism probably to contribute its radioprotective effects. It would be of interest to evaluate the effects of CLE on different cancer cell lines e.g. breast cancer, lung cancer. Further studies are required to evaluate its potential in chemotherapy as well. It may be important finding if this extract can protect healthy cells in cancer radiotherapy and chemotherapy, but this would be less useful if it also protects cancer cells. Therefore studies to check for a potential protective effect against cancer cells are also required.

CONCLUSION

It was concluded that hydroalcoholic extracts of CLE have been significant radioprotective properties as supported by chromosomal morphology. Further study is essential with reference to different doses to understand the better radio protective effect of CLE.

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