IN VITRO ANTIOXIDANT AND ANTIMUTAGENIC ACTIVITY OF CURCUMIN AGAINST POTENT CHEMOTHERAPEUTIC AGENT DOXORUBICIN

DE, P.[1*] – MUKHOPADHYAY, M. J.[2]

[1] *Department of Genetics, Institute of Genetic Engineering, West Bengal, India.*

[2] *Department of Biotechnology, Institute of Genetic Engineering, West Bengal, India.*

**Corresponding author*
e-mail: puspal.dey[at]gmail.com

(Received 17th February 2023; accepted 25th April 2023)

Abstract. The chemopreventive activity of several plants derived materials have been well studied in the recent years. Among them, Curcumin, the mostly investigated dietary phyto constituents have been shown the protective effect in cardiac, renal and hepatic toxicity induced by doxorubicin in human. The protective activity of any phytochemicals mainly depends on their antioxidant property. Thus, the present study was conducted to examine the antioxidant property of laboratory extracted and pure commercial curcumin and antimutagenic action of extracted and pure curcumin against the doxorubicin induced mutagenesis in human leukocyte. To analyze the mutagenicity, parameter like micronucleus assay was conducted in peripheral human leukocytes and to evaluate the antioxidant property, parameter like DPPH free radical scavenging activity was performed. The study results demonstrated that, both the extracted and pure form of curcumin (20µg/ml) induces micronucleus in human leukocytes in an insignificant manner when compared with negative control. Doxorubicin induces significant amount of micronucleus at two variable concentrations (0.50µg/ml and 0.25µg/ml). The micronucleus induction was reduced in human leukocytes pretreated by curcumin for 48 hours in both the concentrations of doxorubucin. Pure as well as extracted Curcumin has been shown strong free radical scavenging activity in a similar pattern as concentration dependant manner. The study reveals that curcumin has antimutagenic potential against chemotherapeutic agent like doxorubicin induced micronucleus in human leukocytes. In addition, it showed strong free radical scavenging activity by DPPH method by both extracted and pure form.
Keywords: *curcumin, micronucleus assay, doxorubicin, antioxidant property, DPPH method, peripheral leukocyte*

Introduction

Doxorubicin sold with trade name Adriamycin, is an antineoplastic drug in the anthracycline class and most of this compound has been isolated from natural sources like cultures of Streptomyces peucetius. Interaction with DNA in a various ways including intercalation, DNA strand breakage and inhibition with the enzyme topoisomerase II are the general properties of drugs belonging in this class (Thorn et al., 2011; Arcamone et al., 2000). The toxicity of this particular drug has been produced by the lacking of specific antibiotics against particular microorganism. Among the available antitumor drugs in anthracycline groups, doxorubicin, one of the important members of this group, is widely used throughout the world for treating solid tumors though the drug administration shows severe side effects (Mordente et al., 2009). To overcome this problem, researcher sometimes prefer to use some alternative therapy or follow some dietary ingredients as there is no such recommended drugs for the reduction of the side effect of this particular lifesaving drug. The chemopreventive and protective activity of several plants derived materials have been well studied in the

recent years. Among them, the mostly investigated dietary phyto constituent is Curcumin, the major component of Turmeric. Curcuma longa commonly known as turmeric belongs to the Zingiberaceae family is a rhizomatous herbaceous perennial plant native to tropical South Asia. Since thousands of years Eastern medicine has used curcumin, the major component of turmeric, for a wide range of health benefits, but only in recent times has its biological action been understood (Gupta et al., 2013; Goel et al., 2008).

A wealth of scientific data shows curcumin has powerful anti-inflammatory, anti-tumor, antioxidant (Duke 2007; Kotwal and Lahiri, 2005) and antiangiogenic (Kunnumakkara et al., 2008) properties. The molecular analysis revealed the modulatory effects of curcumin in various cell cycle proteins, signal transducing kinase and transcription factors like NF-κB, AP-1 and TNF-α. It also enhances the expression of tumor suppressor proteins like p53, cell cycle inhibitors like p21 and p27 and induces apoptosis of cancer cells (Zoubková et al., 2015; Aggarwal and Shishodia, 2006; Singh and Agarwal, 2006; Singh and Khar, 2006; Šmerák et al., 2006). Curcumin exerts protective effect of doxorubicin induced myocardial toxicity (Swamy et al., 2012) and chemotherapy induced hepatotoxicity, neurotoxicity, cardiotoxicity, nephrotoxicity and genotoxicity (Liu et al., 2018). The protective effect of curcumin against radio iodine (Shafaghati et al., 2014) and anticlastogenic effect of curcumin against chemotherapeutic agent like mitomycin C (De and Mukhopadhyay, 2019) in human leukocytes was also observed previously. The clinical significance of curcumin has also been reported in many times where it played an important role in prevention and management of disease like Alzheimer's (Chen et al., 2018), diabetes, hypertension, malaria, arthritis and respiratory disorder (Rahmani et al., 2018). In Addition, different toxicity studies revealed that it is quite safe even when administered in high doses (Hewlings and Kalman, 2017; Lao et al., 2006) and as per EFSA (European Food Safety Authority) and JECFA (The Joint United Nations and World Health Organization Expert Committee on Food Additives), the Allowable Daily Intake (ADI) value of curcumin is 0-3 mg/kg body weight in human (Kocaadam and Şanlier, 2017).

In this present scenario, the study of mutagenic effect of doxorubicin in human leukocytes and comparative antimutagenic action of laboratory extracted and pure commercial curcumin against doxorubicin is the purpose of our present work as there was no such reported work where curcumin has been used to ameliorate the toxicity induced by doxorubicin, selecting micronucleus in human leukocyte as a parameter of mutagenicity. As the protective effects of any phytochemical mainly depend on its antioxidant properties, several study reports have been shown the antioxidant and free radical scavenging properties of curcumin by using different in vitro and ex vivo models and the researchers indicated the use of curcumin in the pharmacological field along with the food industry with great prospects (Bora et al., 2013; Ak and Gülçin, 2008). Thus the comparative antioxidant property of laboratory extracted and pure commercial curcumin was also evaluated in our present work.

Materials and Methods

Collection of samples

All the rhizomse of turmeric (Curcuma longa) were collected from local markets of Kolkata, India, during the month of February to March and all the plant parts were properly identified by the macro morphological characters and qualitative microscopic

method by Professor and Head, Department of Biotechnology, Institute of Genetic Engineering, Kolkata 128, West Bengal, India. After the collection and identification of plant species, the rhizomes were air dried, labeled and kept for the further use.

Chemicals

Pure curcumin was purchased from Himedia (RM1449-5G, CAS No- 458-37-7), Adriamycin, the anti-cancer drug with Doxorubicin HCL in 2mg/ml concentration was collected from local pharmacy (NDC 67457-394-00, 10ml vial, Mylan). All other chemicals and reagents used were of analytical grade supplied by Sarada Glass and Chemical, Dixon lane, Entally, Kolkata-700014, the authorized dealer of Sigma- Alrich, Merk, Loba chemicals. Central Drug House (P) Ltd. (CDH), Himedia, Sisco Research Laboratories (SRL) Pvt Ltd chemicals.

Extraction of circimin

All the dried samples of turmeric rhizome were washed twice through running tap water and distilled water respectively, cut into small pieces and finally air dried. After thorough drying, the samples were blended to fine powder, poured into dry sterile container, labeled properly and stored in room temperature for further use. 2 g (gram) dried powder were added to 60 ml of hexane (Product code-31668, SRL, Chemicals) in each pre-labeled conical flasks and the opening of the flask was sealed properly. The mixtures were kept in the BOD (Biological Oxygen Demand, Lab-Scan Instrument, India) shaker incubator at 30oC temperature in 120 rpm (Rotation Per Minute) for six hours and were filtered through Whatman filter no. 1 (Z274852, Sigma-Aldrich). The filtrate was stored into an air tight bottle. This experiment was done thrice and the concentration of the filtrate was kept in the same bottle and the absorbance was measured using UV Vis spectrophotometer (BIO-RAD) at 425nm (Nanometre). Curcumin content g/100g was measured using the following formula Eq. (1) (Bagchi, 2012):

$$\text{g/10g Curcumin} = \frac{(0.0025\text{x Abs at 425nm x V x DF x 100})}{(0.42 \text{ x weight of sample x 1000})} \qquad \text{Eq. (1)}$$

Where; Abs is absorbance; V is volume made up; DF is dilution factor; and 1. 0.42 absorbance at 425 nm=0.0025g Curcumin.

Preparation of pure and extracted curcumin solution

All the filtrate collected by the solvent extraction method was evaporated by rotator evaporator. After complete evaporation, the end product yellow colored material was extracted curcumin. Then the extract was weighted and dissolves in ethanol to make a concentration of 1mg/ml final stock solution. Pure or commercial curcumin was dissolves in ethanol and makes a final 1mg/ml stock solution for study.

Characterization of extracted curcumin

Characterization of curcumin was done after Pawar et al. (2014) TLC (Thin Layer Chromatography) of the extracted curcumin was performed on pre-coated silica gel plates which act as Stationary Phase. Mixture of n-hexane and ethyl acetate in the ratio

of 7:3 used as solvent or Mobile Phase. Commercial Curcumin was used as standard. Detection was done by spraying the plate with vanillin-sulphuric acid mixture. The retention factor (RF) values for the separated spots were calculated and compared with RF value of pure curcumin and reported values in the literature.

Antioxidant assay by free radical scavenging activity of DPPH

The percentage of Free radical scavenging activity (%) of pure or commercial and extracted curcumin was assessed by DPPH (2,2-diphenyl-1-picrylhydrazyl, Cas No-1898-66-4, Himedia) assay using ascorbic acid (CAS: 50-81-7, CDH) as reference. The measurement of the DPPH radical scavenging activity was performed according to methodology described by Brand-Williams et al. (1995). The samples were reacted with the stable DPPH radical in an ethanol solution. The reaction mixture consisted of adding 0.5 mL of test sample, 3 mL of absolute ethanol and 0.3 mL of DPPH radical solution 0.5 mM in ethanol. The mixture of ethanol (3.3 mL) and sample (0.5 mL) served as blank. The control solution was prepared by mixing ethanol (3.5 mL) and DPPH radical solution (0.3 mL). The scavenging activity percentage (%) was determined according to Mensor et al. (2001).

Collection of blood sample for lymphocyte culture

Whole blood samples were collected by venepuncture in heparinized vials (Glass transparent BD vacutainer Lithium heparin Tubes -4ml) for micronucleus assay. Peripheral venous blood was obtained from four healthy unrelated donors with no recent history of smoking, consumption of alcohol, drug therapy and exposure to mutagens with mean age of 25±2 years under sterile conditions. Informed consent was obtained from all donors and the study was approved by institutional ethical committee of Institute of Genetic Engineering (IGE/02/2019/0011).

Micronucleus assay

Micronucleus assay was done after modification of Fenech et al. (2003). 68 hours PHA (Phytohemaglutinin) stimulated leukocyte cultures were established from peripheral blood specimens contained RPMI-1640 media (Catelog No- R8758-100ml, Sigma), 15% fetal calf serum (Sigma), 1% PHA (M- form, Catalog No- 10576015, Gibco), 100 UI of penicillin and 100 µg/ml streptomycin (Sigma) with extracted and pure curcumin 100 µl (20µg/ml) from both the extracted and commercial stock solution. The concentration of curcumin was chosen based on result obtained in pilot experiment (Data not shown). Twenty-four hours prior to harvest, doxorubicin was added in final concentrations of 0.5 µg/ml and 0.25 µg/ml culture medium in replicate cultures. Only vehicle was taken as a negative control. Colchicines (Gibco, 15212-012) was added prior 2 hours of harvesting and cells were collected and treated with a mild hypotonic treatment and fixed with a mixture of methanol/glacial acetic acid (SRL)(Fenechet al., 2003; Minissi et al., 1999). The slides were prepared; air dried and then stained using conventional Giemsa staining (Sigma). In accordance with standard criteria, MN analysis was performed on coded slides by scoring 1000 lymphocytes for each subject under microscope (Olympus). The scoring has been done under 40X and the photograph has been taken in 100X magnification.

Statistical analysis

The results were expressed as Mean±SE (Standard Error) and analysis was carried out by One-way ANOVA (Analysis of Variance). P (Level of Significance)<0.05 was considered significant. To confirm the differences occurred between groups, Tukey's HSD (Honest Significant Differences) test was performed.

Results and Discussion

Extraction of curcumin

In the present study the curcumin was extracted by solvent extraction method and the concentration of curcumin was determined by spectro-photometric method which has been shown in *Table 1*. The average concentration of extracted curcumin was found 0.1663g/100g of turmeric rhizome.

Table 1. *Concentration of curcumin (in g/100g of turmeric).*

Experiment	Curcumin content (g/100g)
1	0.168
2	0.166
3	0.165
Average	0.1663

Characterization of extracted curcumin by TLC method

The extracted and commercial curcumin gives similar types of picks in the TLC plates. The Rf (Retention Factor) value obtained for the extracted Curcumin was similar with standard commercial curcumin which confirmed the identity of Extracted curcumin.

Free radical scavenging activity by DPPH assay

In the present study, three different concentrations of extracted and pure curcumin were taken from the stock solution for DPPH scavenging assay. The three different experimental concentrations of both the extracted and pure commercial curcumin have been shown good amount of free radical scavenging activity where pure curcumin has been shown slightly greater free radical scavenging activity in all the three experimental concentrations. The detailed results were depicted in *Table 2*. The graphical representation of DPPH scavenging property of Extracted and Pure Curcumin was represented in *Figure 1*.

Table 2. *Free radical scavenging activity of extracted and pure curcumin by DPPH assay method.*

Sample	Concentration (in µl)	Free radical scavenging activity (in %)
Extracted curcumin stock solution	50	48.14±3.03
	100	53.26±1.04
	200	62.31±3.09
Pure curcumin stock solution	50	49.24±2.07
	100	60.31±2.03
	200	72.58±3.02

Notes: ± means standard error (N=3)

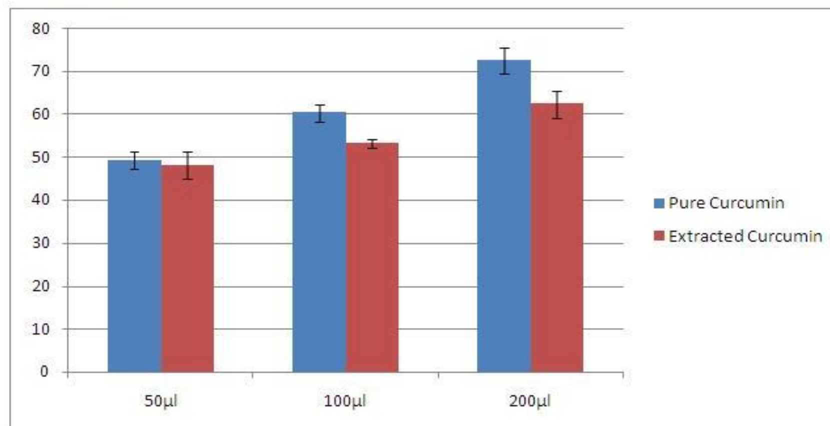

Figure 1.*Free radical scavenging activity of extracted and pure curcumin by DPPH assay method.*

Micronucleus assay

The Micronucleus Assay indicated that both extracted and pure curcumin were capable of reducing the micronucleus induction induced by Doxorubicin. The extracted curcumin showed the ameliorative property of micronucleus induction almost similar to pure curcumin. When extracted and pure curcumin were tested separately, a non-significant increase in the frequency of micronucleus induction was observed in human peripheral blood culture. Doxorubicin showed high number of micronucleus induction in human peripheral leukocytes whereas (*Figure 2*), extracted and pure curcumin both prevented micronucleus induction to some extent as revealed from decreased frequency in micronucleus induction. The result of micronucleus assay was summarized in *Table 3*. The graphical representation shows the effect of pure and extracted curcumin on micronucleus induction induced by doxorubicin in *Figure 3*.

Table 3. *Effect of pure and extracted curcumin on micronucleus induce by doxorubicin.*

Group no.	Experimental group (in µg/ml)	Induction of micronucleus
1	Doxorubicin (0.50)	0.42±0.03[a]
2	Doxirubicin (0.23)	0.40±0.02[b]
3	Doxorubicin (0.50) + PCS (20)	0.21±0.04[abc]
4	Doxorubicin (0.25) + PCS (20)	0.22±0.06[abd]
5	Doxorubicin (0.50) + ECS (20)	0.26±0.06[abe]
6	Doxorubicin (0.25) + ECS (20)	0.24±0.03[abf]
7	PCS (20)	0.07±0.02[cdef]
8	ECS (20)	0.09±0.02[cdef]
9	(-)ve control	0.08±0.01[cdef]

Notes: PCS=pure curcumin solution; ECS=extracted curcumin solution; ± mean standard error where N=3; a p<0.05=group 1 significantly different from the group 3,4,5,6 after Turkey's HSD Post Hoc Test; b p<0.05=group 2 significantly different from the group 3,4,5,6 after Turkey's HSD Post Hoc Test; c p<0.05=group 3 significantly different from the group 7,8,9 after Turkey's HSD Post Hoc Test; d p<0.05=group 4 significantly different from the group 7,8,9 after Turkey's HSD Post Hoc Test; e p<0.05=group 5 significantly different from the group 7,8,9 after Turkey's HSD Post Hoc Test; f p<0.05=group 6 significantly different from the group 7,8,9 after Turkey's HSD Post Hoc Test; p=0.05.

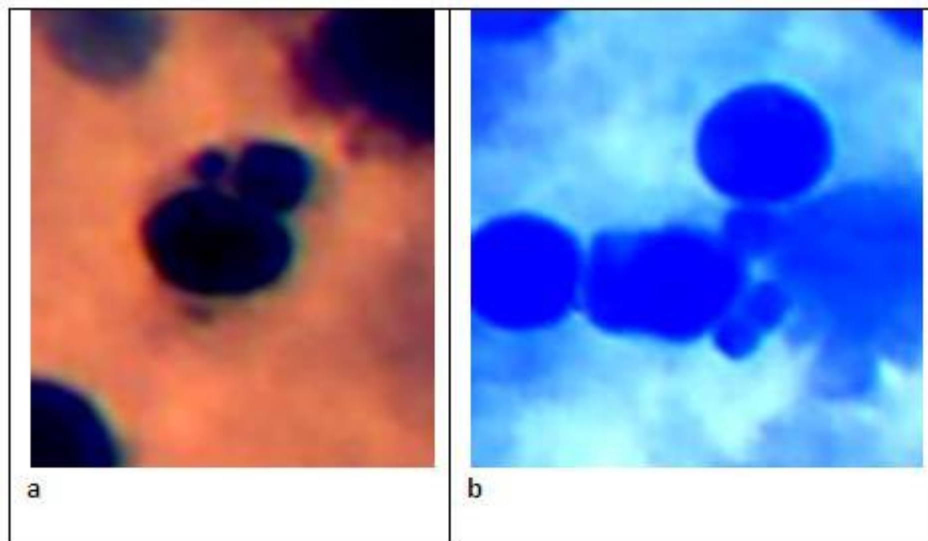

Figure 2. *Cell showing micronucleus induction by doxorubicin (a-b).*

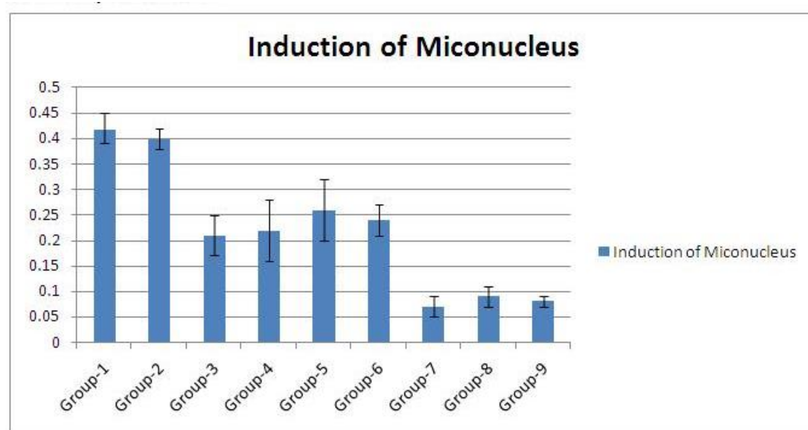

Figure 3. *The effect of pure and extracted curcumin on micronucleus induction by doxorubicin.*

Result of statistical analysis

The statistical analysis result indicated significant effect of micronucleus induction in case of doxorubicin treatment against control one where as both pure and extracted curcumin individually showed almost similar frequency of micronucleus induction against negative control and between themselves. The result of Tukey's HSD post hoc test revealed that, there was no significance difference between any two groups when Pure Curcumin Solution (PCS) and Extracted Curcumin Solution (ECS) was compared with negative control (p=0.05). Doxorubicin shows significant difference in micronucleus induction when treated with both pure and extracted curcumin. There was also significant difference in micronucleus induction in doxorubicin-curcumin combined groups with pure and extracted curcumin treated groups as well as negative control.

In the present study, the ameliorative properties of curcumin from the induction of micronucleus both in pure or commercial form and extracted form were evaluated

against a known anti neoplastic and chemotherapeutic agent doxorubicin. The incidence of micronucleus formation in the current study was not significant when pure or commercial curcumin and extracted curcumin was used alone. The induction rate of micronucleus of pure or commercial curcumin was 0.07% and extracted curcumin was 0.09% which showed almost similar frequency of micronucleus induction against negative control (0.08%). The result proved that they did not induce significant amount micro nucleated cells in comparison to negative control. This experiment indicated their non-mutagenic nature which had been proved by different researcher previously by using different parameters and different model system (Ragunathan and Panneerselvam, 2007; Nagabhushan and Bhide, 1986). Doxorubicin has the mutagenic potential as it induced micronucleus in leukocytes in both the concentrations used in a significant manner. In the experiment 0.50µg/ml doxorubicin induces micronucleus in human leukocytes as 0.42% where 0.25µg/ml doxorubicin induced 0.40%. The pure or commercial and extracted curcumin were kept constant concentration (20µg/ml) against variable doxorubicin concentration, where curcumin shows good antagonistic property against doxorubicin. The pure or commercial curcumin reduced the micronucleus induction 50% and 45% against two variable concentrations of doxorubicin (0.50µg/ml, 0.25µg/ml) respectively and the extracted curcumin reduced the same as 38% and 40% folds and this result shows strong resemblance with several reported studies. The literature review revealed that curcumin reduced the induction of micronucleus upto 52% in human culture leukocyte cells against radioiodine (Shafaghati et al., 2014) and it also restore the doxorubicin mediated impairment of different organs like heart, kidney, reproductive organs, liver, brain in a similar fashion (Liu et al., 2018). As doxorubicin was known as aneugenic and clastogenic agent, (Dhawan et al., 2003) use of positive control was omitted whereas the negative control used was the vehicle for all the compounds used. The MN or micronucleus assay is taken as a sensitive indicator or cytogenetic biomarker for monitoring mutagenicity of different chemicals or drugs as the test procedure recommended in the Committee on Mutagenicity Guidelines of the Department of Health, UK (Committee on Mutagenicity of Chemicals in Food, Consumer Products and the Environment, 2000).

Literature review revealed that, Doxorubicin can act within the neoplastic cells in two different pathways. In the cytosol, Doxorubicin converted to a very unstable metabolite semiquinone after oxidation in their metabolic pathway. The unstable semiquinone again converted back to the doxorubicin. At this process huge amount of reactive oxygen species are released which can trigger apoptotic pathway of cellular death by combinatorial effects of several processes like lipid peroxidation, DNA damage, membrane damage and oxidative stress (Doroshow et al., 1986). Genes capable for oxidation reactions such as NADH dehydrogenases, nitric oxide synthases, xanthine oxidase and genes capable for deactivating the free radicals such as glutathione peroxidase, catalase, and superoxide dismutase are the main modulatory candidate genes of this pathway (Fogli et al., 2004; Pawłowska et al., 2003). Secondly, after entering the nucleus, Doxorubicin damage DNA and can cause cellular death by poisoning Topoisomerase II. Genes capable for DNA repair and capable to control cell cycle act as candidate genes in this pathway (Oakman et al., 2009; Gewirtz, 1999; Tewey et al., 1984). According to Denard et al. (2011), Doxorubicin interrupts the cellular replication by cleaving a transcription factor named CREB3L1 by stimulating the production of ceramide molecule. CREB3L1 is a transmembrane protein residing in the endoplasmic reticulum. After the cleavage of this protein, the cytosolic NH2-

terminal domain travels to the nucleus and drives the transcription genes that suppress the cell cycle (Denard et al., 2012; 2011).

Chemotherapy most effectively kills continuous proliferating cell. Unfortunately, a chemotherapeutic agent does not know the difference between cancer cells and the normal cells. During the renewal process, the normal cells will grow back and be healthy but, in the meantime, side effects occur. The commonly affected non proliferating cells by chemotherapy are the blood cells, the cells in the mouth, stomach and bowel, and the hair follicles; resulting in low blood counts, mouth sores, nausea, diarrhea, and/or hair loss (Tobis and Hochhauser, 2014; Skeel and Khleif, 2011). To overcome these side effects, nutritionist or medical practitioners sometimes prefer to prescribe some dietary supplements for better results. The relationship between a mutagen or clastogen and diet led us believe that most lifestyle diseases like cancer and many more are preventable. A considerable emphasis also has been laid down on the use of herbal constituents to prevent the toxicity of mutagen mainly due to their antioxidant properties (Saleem and Al-Attar, 2013). The antioxidant molecules help to counter act the different endogenously produced free radicals and reactive oxygen species lead to minimize the harmful effect of causative or free radical producing agent (Birben et al., 2012). Free radical scavenging activity by DPPH Assay method showed that both pure and extracted curcumin has significant Free radical scavenging activity in a concentration dependant manner. The highest concentration (200 µl) of pure curcumin has been shown 72.58±3.02% of free radical scavenging activity while the same concentration (200 µl) of extracted curcumin has been shown 62.31±3.09%. In comparison to pure or commercial curcumin with extracted curcumin, pure form has comparatively greater Free radical scavenging activity. So, scavenging these free radicals may also help to increase the anti-mutagenic effect of this phyto-constituent. This study was carried out to check the anti-mutagenic profile of both pure and extracted curcumin whether the said herbal compound has a positive response in preventing micronucleus formation by anti-neoplastic drug like Doxorubicin. The underlying mechanism requires detail attention as it showed promising inhibitory action against a world-wide pollutant and carcinogen.

Conclusion

In conclusion the anti-mutagenic potential of pure or commercial curcumin and extracted curcumin from dried turmeric rhizome, both of the materials were evaluated against a known anti-neoplastic drug doxorubicin. The study reveals that both the pure and extracted curcumin has antimutagenic potential against doxorubicin induced micronucleus in human leukocytes. In addition, pure curcumin showed more than 70% while extracted curcumin showed more than 60% free radical scavenging activity by DPPH method in their highest experimental concentration. The antimutagenic potential of 20µg/ml curcumin is effective in both 0.50µg/ml and 0.25µg/ml concentrations of doxorubicin in human leukocytes and curcumin ameliorate the toxic effect of doxorubicin up to 50%. The study indicated that curcumin the key constituent of turmeric, widely taken as an accessory food material or colouring agent, has the potentiality to protect cellular damage in vitro. Curcumin, a major component of turmeric does not show any mutagenic activity itself, though it possesses a good antioxidant activity. It can be proven that; curcumin or turmeric can be used as a protective food accessory against several mutagenic agents like doxorubicin.

Acknowledgement

All authors acknowledge the Director and Vice Principal of Institute of Genetic Engineering for funding and affiliation. We are also thankful to other laboratory members and other associated person of the affiliated institute for their enthusiastic participation.

Conflict of interest

The authors declare that they have no conflict of interests.

REFERENCES

[1] Aggarwal, B.B., Shishodia, S. (2006): Molecular targets of dietary agents for prevention and therapy of cancer. – Biochemical Pharmacology 71(10): 1397-1421.
[2] Ak, T., Gülçin, I. (2008): Antioxidant and radical scavenging properties of curcumin. – Chemico-Biological Interactions 174(1): 27-37.
[3] Arcamone, F., Cassinelli, G., Fantini, G., Grein, A., Orezzi, P., Pol, C., Spalla, C. (2000): Adriamycin, 14-Hydroxydaunomycin, a new antitumor antibiotic from S. peucetius var. caesius. – Biotechnology and Bioengineering 67(6): 704-713.
[4] Bagchi, A. (2012): Extraction of Curcumin. – IOSR Journal of Environmental Science, Toxicol. Food. Tech. 1(3): 1-16.
[5] Birben, E., Sahiner, U.M., Sackesen, C., Erzurum, S., Kalayci, O. (2012): Oxidative stress and antioxidant defense. – World Allergy Organization Journal 5: 9-19.
[6] Borra, S.K., Gurumurthy, P., Mahendra, J., Jayamathi, K.M., Cherian, C.N., Chand, R. (2013): Antioxidant and free radical scavenging activity of curcumin determined by using different in vitro and ex vivo models. – Journal of Medicinal Plants Research 7(36): 2680-2690.
[7] Brand-Williams, W., Cuvelier, M.E., Berset, C.L.W.T. (1995): Use of a free radical method to evaluate antioxidant activity. – LWT-Food science and Technology 28(1): 25-30.
[8] Chen, M., Du, Z.Y., Zheng, X., Li, D.L., Zhou, R.P., Zhang, K. (2018): Use of curcumin in diagnosis, prevention, and treatment of Alzheimer's disease. – Neural Regeneration Research 13(4): 742-752.
[9] De, P., Mukhopadhyay, M. (2019): In vitro anticlastogenic and antioxidant properties of extracted and pure form of curcumin against mitomycin C in human peripheral leukocytes. – Journal of Advanced Pharmacy Research 3(3): 110-116.
[10] Denard, B., Lee, C., Ye, J. (2012): Doxorubicin blocks proliferation of cancer cells through proteolytic activation of CREB3L1. – Elife 14p.
[11] Denard, B., Seemann, J., Chen, Q., Gay, A., Huang, H., Chen, Y., Ye, J. (2011): The membrane-bound transcription factor CREB3L1 is activated in response to virus infection to inhibit proliferation of virus-infected cells. – Cell Host & Microbe 10(1): 65-74.
[12] Dhawan, A., Kayani, M.A., Parry, J.M., Parry, E., Anderson, D. (2003): Aneugenic and clastogenic effects of doxorubicin in human lymphocytes. – Mutagenesis 18(6): 487-490.
[13] Doroshow, J.H. (1986): Role of hydrogen peroxide and hydroxyl radical formation in the killing of Ehrlich tumor cells by anticancer quinones. – Proceedings of the National Academy of Sciences 83(12): 4514-4518.
[14] Duke, J. (2007): Turmeric-The Genus Curcuma. Medicinal and Aromatic Plants-Industrial Profiles Volume 45. – Economic Botany 61(4): 397-398.
[15] Fenech, M., Chang, W.P., Kirsch-Volders, M., Holland, N., Bonassi, S., Zeiger, E. (2003): HUMN project: detailed description of the scoring criteria for the cytokinesis-

block micronucleus assay using isolated human lymphocyte cultures. – Mutation Research/Genetic Toxicology and Environmental Mutagenesis 534(1-2): 65-75.

[16] Fogli, S., Nieri, P., CRISTINA BRESCHI, M. (2004): The role of nitric oxide in anthracycline toxicity and prospects for pharmacologic prevention of cardiac damage. – The FASEB Journal 18(6): 664-675.

[17] Gewirtz, D. (1999): A critical evaluation of the mechanisms of action proposed for the antitumor effects of the anthracycline antibiotics adriamycin and daunorubicin. – Biochemical Pharmacology 57(7): 727-741.

[18] Goel, A., Kunnumakkara, A.B., Aggarwal, B.B. (2008): Curcumin as “Curecumin”: from kitchen to clinic. – Biochemical Pharmacology 75(4): 787-809.

[19] Gupta, S.C., Kismali, G., Aggarwal, B.B. (2013): Curcumin, a component of turmeric: from farm to pharmacy. – Biofactors 39(1): 2-13.

[20] Hewlings, S.J., Kalman, D.S. (2017): Curcumin: A review of its effects on human health. – Foods 6(10): 11p.

[21] Kocaadam, B., Şanlier, N. (2017): Curcumin, an active component of turmeric (Curcuma longa), and its effects on health. – Critical Reviews in Food Science and Nutrition 57(13): 2889-2895.

[22] Kotwal, G.J., Lahiri, D.K. (Eds.) (2005): Natural products and molecular therapy (Vol. 1056). – New York Academy of Sciences 1056: 520p.

[23] Kunnumakkara, A.B., Anand, P., Aggarwal, B.B. (2008): Curcumin inhibits proliferation, invasion, angiogenesis and metastasis of different cancers through interaction with multiple cell signaling proteins. – Cancer Letters 269(2): 199-225.

[24] Lao, C.D., Ruffin, M.T., Normolle, D., Heath, D.D., Murray, S.I., Bailey, J.M., Boggs, M.E., Crowell, J., Rock, C.L., Brenner, D.E. (2006): Dose escalation of a curcuminoid formulation. – BMC Complementary and Alternative Medicine 6(1): 1-4.

[25] Liu, Z., Huang, P., Law, S., Tian, H., Leung, W., Xu, C. (2018): Preventive effect of curcumin against chemotherapy-induced side-effects. – Frontiers in Pharmacology 9: 9p.

[26] Mensor, L.L., Menezes, F.S., Leitão, G.G., Reis, A.S., Santos, T.C.D., Coube, C.S., Leitão, S.G. (2001): Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. – Phytotherapy Research 15(2): 127-130.

[27] Minissi, S., Gustavino, B., Degrassi, F., Tanzarella, C., Rizzoni, M. (1999): Effect of cytochalasin B on the induction of chromosome missegregation by colchicine at low concentrations in human lymphocytes. – Mutagenesis 14(1): 43-49.

[28] Mordente, A., Meucci, E., Silvestrini, A., Martorana, G.E., Giardina, B. (2009): New developments in anthracycline-induced cardiotoxicity. – Current Medicinal Chemistry 16(13): 1656-1672.

[29] Nagabhushan, M., Bhide, S.V. (1986): Nonmutagenicity of curcumin and its antimutagenic action versus chili and capsaicin. – Nutrition and Cancer 8(3): 201-210.

[30] Oakman, C., Moretti, E., Galardi, F., Santarpia, L., Di Leo, A. (2009): The role of topoisomerase IIα and HER-2 in predicting sensitivity to anthracyclines in breast cancer patients. – Cancer Treatment Reviews 35(8): 662-667.

[31] Pawar, H., Karde, M., Mundle, N., Jadav, P., Mehra, K. (2014): Method to prepare pure curcumin. – United State Patent Application 10p.

[32] Pawłowska, J., Tarasiuk, J., Wolf, C.R., Paine, M.J., Borowski, E. (2003): Differential ability of cytostatics from anthraquinone group to generate free radicals in three enzymatic systems: NADH dehydrogenase, NADPH cytochrome P450 reductase, and xanthine oxidase. – Oncology Research Featuring Preclinical and Clinical Cancer Therapeutics 13(5): 245-252.

[33] Ragunathan, I., Panneerselvam, N. (2007): Antimutagenic potential of curcumin on chromosomal aberrations in Allium cepa. – Journal of Zhejiang University Science B 8(7): 470-475.

[34] Rahmani, A.H., Alsahli, M.A., Aly, S.M., Khan, M.A., Aldebasi, Y.H. (2018): Role of curcumin in disease prevention and treatment. – Advanced Biomedical Research 7(1): 9p.

[35] Saleem, M.A., Al-Attar, M.S. (2013): Protective effects of mentha spicata aqueous extract against ifosfamide induced chromosomal aberrations and sperm abnormalities in male albino mice. – ZANCO Journal of Pure and Applied Sciences 28(1): 86-93.

[36] Shafaghati, N., Hedayati, M., Hosseinimehr, S.J. (2014): Protective effects of curcumin against genotoxicity induced by 131-iodine in human cultured lymphocyte cells. – Pharmacognosy Magazine 10(38): 106-110.

[37] Skeel, R.T., Khleif, S.N. (2011): Handbool of cancer chemotheraphy. – Lippincott Williams & Wilkins 875p.

[38] Singh, R.P., Agarwal, R. (2006): Mechanisms of action of novel agents for prostate cancer chemoprevention. – Endocrine-Related Cancer 13(3): 751-778.

[39] Singh, S., Khar, A. (2006): Biological effects of curcumin and its role in cancer chemoprevention and therapy. – Anti-Cancer Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Cancer Agents) 6(3): 259-270.

[40] Šmerák, P.E.T.R., Polívková, Z., Šestáková, H., Štětina, R., Bárta, I., Langová, M., Turek, B., Bártová, J. (2006). Antimutagenic effect of curcumin and its effect on the immune response in mice. – Czech Journal of Food Science 24(2): 72-83.

[41] Swamy, A.V., Gulliaya, S., Thippeswamy, A., Koti, B.C., Manjula, D.V. (2012): Cardioprotective effect of curcumin against doxorubicin-induced myocardial toxicity in albino rats. – Indian Journal of Pharmacology 44(1): 73-77.

[42] Tewey, K.M., Rowe, T.C., Yang, L., Halligan, B.D., Liu, L.F. (1984): Adriamycin-induced DNA damage mediated by mammalian DNA topoisomerase II. – Science 226(4673): 466-468.

[43] Tobis, J.S., Hochhauser, D. (2014): Cancer and its management. – Wiley-Blackwell 624p.

[44] Thorn, C.F., Oshiro, C., Marsh, S., Hernandez-Boussard, T., McLeod, H., Klein, T.E., Altman, R.B. (2011): Doxorubicin pathways: pharmacodynamics and adverse effects. – Pharmacogenetics and Genomics 21(7): 440-446.

[45] Zoubková, H., Šmerák, P., Polívková, Z. (2015): Antimutagenic Effect of the Ellagic Acid and Curcumin Combinations. – Journal of Environmental & Analytical Toxicology 5(296): 2161-0525.