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In Vitro Anticlastogenic and Antioxidant Properties of Extracted and Pure Form of Curcumin against Mitomycin C in Human Peripheral Leukocytes

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ABSTRACT

Objectives: Turmeric is native to India and is widely used in Indian cuisine. Curcumin, the active principle of turmeric used primarily as a coloring agent in food, drug and cosmetics. The medicinal properties of curcumin are well known in ancient Indian Ayurvedic Medicine. Mitomycin C (MMC) is an antineoplastic agent used to fight a number of different cancers including cancer of the stomach, colon, rectum, pancreas, breast, lung, uterus, cervix, bladder, head, neck, eye and esophagus. It is a potent DNA cross-linker. But it has severe side effect, the prolonged use of the drug may result in permanent bone marrow damage and other various types of secondary tumors in normal cells. The present study was conducted to examine the anticlastogenic action of extracted curcumin from dry turmeric and pure curcumin against the MMC-induced chromosomal aberrations. For this purpose, in the present study, clastogenic parameter like chromosomal aberration test was conducted in peripheral human leukocytes. **Methods:** The antioxidant property of both extracted and pure curcumin was also evaluated by phosphomolybdenum method. **Results:** Our results demonstrated that, extracted as well as pure Curcumin, a strong antioxidant phyto-molecule is effective in counteracting the clastogenicity in human leukocyte in vitro. **Conclusion:** These results suggest that the use of turmeric in diet may be an effective protection against the health crisis generated by harmful agents.

Keywords: *Anticlastogenecity; Antioxidant property; Chromosomal aberration; Curcumin; Peripheral blood*

INTRODUCTION

Curcuma longa commonly known as turmeric is a rhizomatous herbaceous perennial plant of the Zingiberaceae family is native to tropical South Asia. Eastern medicine has used this plant root since ages for a wide range of health benefits. It has been highly valued by both Ayuevedic and Chinese medicine and by those who practice health Yoga, for its different beneficial effects. Throughout Asia, turmeric has been used for different stomach problems, allergies, diarrhea, heartburn, bloating, colic, flatulence liver ailments.[1,2]

No longer relegated to folklore, modern science has created a large and fast growing storehouse of scientific research about this medicinal herb. The US national Institute of Health (NIH), Library of Medicine's Pubmed MEDLINE database yields a number of scientific articles about the active ingredient of turmeric. While therapeutic properties of turmeric have been known for centuries, modern science has

identified the curcuminoids (Phenolic compounds found in turmeric) for having the principal leading role for all pharmacological activities.[3,4] Turmeric contains about 2% curcumin, the main curcuminoids by weight. A number of studies revealed, curcumin having anti-inflammatory, antitumor and antioxidant properties and only in recent years it has been also claimed to have some anti-carcinogenic, cardio protective and neuro-protective activities.

Mitomycin C (MMC) is a chemically reactive antibiotic derived from *Streptomyces caespitosus.* The drug selectively inhibits DNA synthesis [8,9] and degrades cellular DNA but does not affect the synthesis of RNA or protein.[10-12] MMC induces bacteriophage production in lysogenic bacteria [13,14], increases the rate of genetic recombination among mutant forms of E. *coil* [15-17], and possesses antitumor activity[15,16]. In tissue culture systems, MMC inhibits mitosis, reduces cell viability, and produces nuclear disorganization and giant cells.[18,10] The effects of MMC on plant chromosomes. have been previously noted as Merz[19] reported that chromatid breaks in the heterochromatic regions of *Vicia faba* root tip chromosomes, induced by MC[20].

The present study was conducted to examine the anticlastogenic action of extracted curcumin from turmeric and pure curcumin against the MMC-induced chromosomal aberrations in human peripheral blood lymphocytes and to check other biological activities of curcumin.

MATERIAL AND METHODS

Collection of Samples

Fresh rhizomes of turmeric were collected from local markets of Kolkata, India, during the month of February and were properly identified.

Chemicals

Pure curcumin was purchased from Himedia (RM1449) and Mitomycin C from Sigma-Aldrich. All other chemicals and reagents used were of analytical grade.

Extraction of Curcumin

Turmeric rhizome were washed twice through running tap water followed by distilled water, cut into small pieces and finally air dried. The fully dried samples were blended to fine powder, labeled properly and stored in room temperature for future use. 2 gram of dried powder was taken in each pre-labeled conical flasks, 60 ml of hexane was added. The properly sealed flasks were kept in the BOD shaker incubator at 30oC temperature in 120 rpm for six hours and were filtered through Whatman filter no. 1 (Sigma-Aldrich) after 6 hrs. The filtrate was stored into an air tight bottle. This experiment was repeated three times keeping concentration of the filtrate same and the absorbance was measured using spectrophotometer at 425nm. Curcumin content g/100g was measured using the following formula[21].

$$\mathbf{g/10g\ Curcumin} = \frac{0.0025\text{x Abs at 425nm } x \text{ V } x \text{ DF}}{42\ x\ weight\ of\ sample\ x\ 1000} x100$$

Where:

0.42 absorbance at 425 nm = 0.0025g Curcumin
Abs= Absorbance
V= Volume made up
DF= Dilution factor

Preparation of Extracted Curcumin Solution

Finally all the filtrate collected by the solvent extraction method was evaporated. After complete evaporation, the end product was extracted as curcumin. The curcumin was dissolved in 1ml ethanol and a final 1ml stock solution was prepared.

Preparation of Pure Curcumin Solution

Pure or commercial curcumin was dissolved in ethanol to make a concentration of 1µg/ml final stock solution.

Total Antioxidant Activity Study

The total antioxidant activity of these extract were evaluated according to Prieto *et al*.[22]. 0.3 ml of each aqueous extracted and pure curcumin (50, 100, 200 µl from stock solution) were mixed with 3 ml assay mixture which contain 4 mmol/L ammonium molybdate, 0.6 mol/L sulphuric acid and 28 mmol/L sodium phosphate. The mixture along with test samples was incubated at 95ºC for 90 min in water-bath. After cooling to 25ºC, absorbance of the final solution was measured at 695 nm wave length in spectrophotometer (Beckman). Vehicle (Distilled water) was used as blank and ascorbic acid as positive control.

Calculation and Analysis of Antioxidant Data

The percentage of antioxidant property was calculated by using the following formula:

%= [(OD sample-OD blank) / (OD ascorbic acid-OD blank)] X 100.

All the experiments were performed thrice and the percentage of total antioxidant obtained from every experiment was calculated as mean standard deviation with the help of Microsoft Excel.

Chromosomal Aberration Test

Three-day PHA stimulated leukocyte cultures were established from peripheral blood specimens obtained from apparently healthy, unrelated individuals with extracted and pure curcumin 100 µl from each the

stock solution. Twenty-four hours prior to harvest, MMC was added in final concentrations of 0.5 µg/ml and 1.0 µg/ml culture medium in replicate cultures. Only vehicle was taken as a negative control. Cytological preparations were made following a method similar to that of Moorhead *et al.*[23] Coded slides were systematically scanned under microscope. Each well spread metaphase encountered was examined under oil and analyzed for chromosome number and morphology.[24]

Statistical analysis

The results were expressed as Mean ± SE and analysis was carried out by One-way ANOVA. $P < 0.005$ was considered significant. To confirm the differences occurred between groups, Tukey's HSD or Tukey's Honest Significant Differences test was performed.

RESULT 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. **Table 1.**

Table 1. Percentage of curcumin in g/100g concentration

Experiment	Curcumin content g/100g
1	0.1692
2	0.1683
3	0.1679
Average	**0.1684**

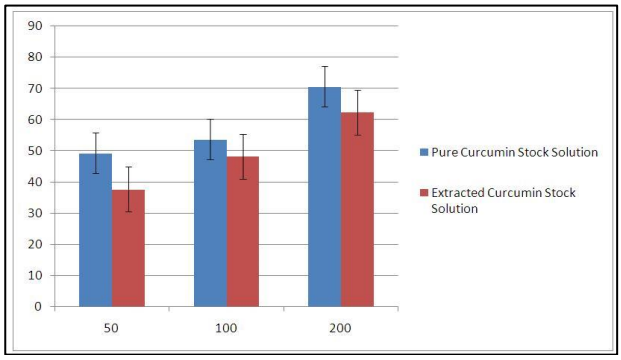

Figure 1. The graphical representation of total antioxidant property of Extracted and Pure Curcumin by Phospho-Molybdenum Method.

Result of Total antioxidant activity study

In the present study, three different concentration of each extracted and pure curcumin were taken from the stock solution for total anti-oxidant assay by phosphomolybdenum method. The detailed results were depicted in **Table 2.** The graphical representation of total antioxidant property of Extracted and Pure Curcumin by Phospho-Molybdenum Method was represented in **Figure 1.**

Result of Chromosomal Aberration Test

The chromosomal aberration test indicated that both extracted and pure curcumin were capable of reducing the chromosomal aberration induced by mitomycin C. the extracted curcumin showed the anticlastogenic properties almost similar to pure curcumin. When both tested separately, they did not show any increase in the frequency of chromosomal aberration in human peripheral blood culture. Mitomycin C showed high number of aberrant chromosomes in human peripheral leukocytes whereas (**Figure 2a; 2b**), extracted and pure curcumin both prevented chromosomal damage to some extent as revealed from decreased frequency in chromosomal aberration. The result of chromosomal aberration test was summarized in **Table 3**. The graphical representation shows the Effect of Pure and Extracted Curcumin on Chromosomal Aberration Induced by Mitomycin C in **Figure 3.**

Result of Statistical analysis

The statistical analysis result indicated a highly significant effect of aberrant chromosomes in case of MMC treatment against control one where as both pure and extracted curcumin individually showed almost similar frequency of chromosomal aberrations against negative control and between themselves. The result of Tukey's HSD post hoc test result shows no significant difference between any two groups when Pure Curcumin Solution (PCS) and Extracted Curcumin Solution (ECS) was compared with negative control ($p=0.96892>0.05$). Differenced occurred between other experimental groups were tabulated in **Table 4**.

Discussion

In the present study the anticlastogenic properties of curcumin both in pure or commercial form and extracted form were evaluated against a known clastogen or anti neoplastic agent Mitomycin C (MMC). 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 clastogens mainly due to their antioxidant properties.[25]

Table 2. Total Antioxidant activity of Extracted and Pure Curcumin by Phospho-Molybdenum Method

Sample	Concentration	Antioxidant(%)
Extracted Curcumin Stock Solution	50	37.64 ± 0.32
	100	48.23 ± 0.14
	200	62.35 ± 0.04
Pure Curcumin Stock Solution	50	49.27 ± 0.24
	100	53.62 ± 0.19
	200	70.58 ± 0.11

± = Mean SD, N=3

Table 3. Effect of Pure and Extracted Curcumin on Chromosomal Aberration Induced by Mitomycin C

Gr No	Experimental Group	Chromosomal Aberration
1	MMC (1.0 µg/ml)	0.52 ± 0.079[a]
2	MMC (0.5 µg/ml)	0.49 ± 0.081[a]
3	MMC(1.0 µg/ml) + PCS(100 µl)	0.21 ± 0.049[b]
4	MMC(0.5 µg/ml) + PCS(100 µl)	0.19 ± 0.067[b]
5	MMC(1.0 µg/ml) + ECS(100 µl)	0.23 ± 0.063[b]
6	MMC(0.5 µg/ml) + ECS(100 µl)	0.22 ± 0.033[b]
7	PCS(100 µl)	0.07 ± 0.023[b]
8	ECS(100 µl)	0.08 ± 0.029[b]
9	(-)Ve Control	0.08 ± 0.042[a]

MMC= Mitomycin C, PCS= Pure Curcumin Solution, ECS= Extracted Curcumin Solution, ± = Mean SD, N=3, [a]P < 0.005= Significantly different from the (-)Ve control, [b]P < 0.005= Significantly different from MMC.

This implies that the template competence of the DNA is destroyed. The de-polymerization of DNA and the accumulation of acid-soluble fragments implicate the DNA polymerase system as a possible target of MMC activity. The primary action of MMC is the "cross-linking" of the complementary strands of the DNA molecule and that the degradation of DNA may be of a secondary nature. [28-31]. Matsuura *et el.* [32,33] observed no breaks after MC treatment of *Trillium kamschaticum;* however, MC treatment followed by x-ray greatly increased the frequency of chromosome breaks involved in exchanges. Because MC has a profound effect on DNA synthesis and because another Streptomyces-derived antimicrobial agent (streptonigrin) caused nonrandom breakage of human chromosomes[34, 35], the present study was undertaken. The experiments to be described demonstrate that MMC preferentially produces breaks and rearrangements in the paracentric secondary constrictions of chromosomes 1, 9, and 16.

The mechanism of action of curcumin in both pure and extracted form may involve scavenging potential against free radicals. These free radicals or reactive oxygen like direct oxidizing superoxide [36] and indirectly as with hydrogen peroxide (H2O2) and hydroxyl radicals (•OH) formed from different DNA cross linking or DNA alkylating agents like MMC, which have the potentiality to break the chromosome. The antioxidant molecules help to counter act the reactive oxygen's and different endogenously produced free radicals lead to minimize the harmful effect of causative or free radical producing agent. The total anti-oxidant study with phosphomolybdenum method showed that both pure and extracted curcumin has significant anti-oxidant property. In comparison to pure or commercial curcumin with extracted curcumin, pure

Table 4. Statistical analysis (Tukey's HSD Post Hoc Test) results for the confirmation of differences between experimental groups.

Compared Groups	F value	P value	Remark
Gr-1 and Gr-4	0.33	0.0455	Significant
Gr-1 and Gr-7	0.45	0.0078	Significant
Gr-1 and Gr-8	0.44	0.0090	Significant
Gr-1 and Gr-9	0.44	0.0090	Significant
Gr-2 and Gr-7	0.42	0.0119	Significant
Gr-2 and Gr-8	0.41	0.0138	Significant
Gr-2 and Gr-9	0.41	0.0138	Significant

P=0.05, Gr-1-9 As mentioned in Table 3

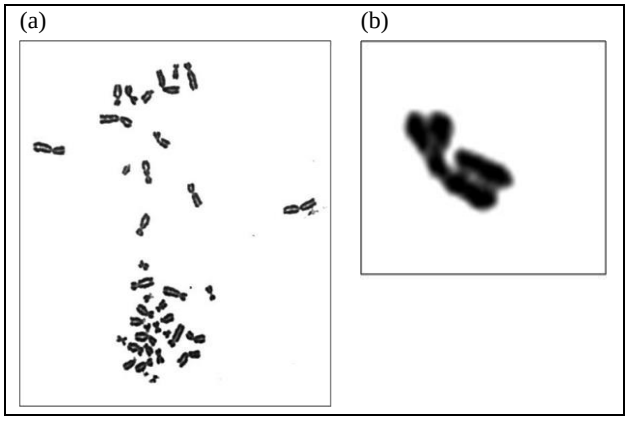

Figure 2. Metaphase showing chromosomal aberration induced by MMC (a), Enlarged version of aberrant chromosome (b)

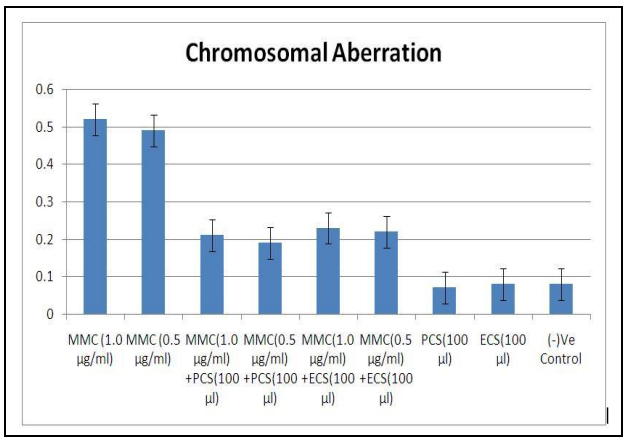

Figure 3. The graphical representation shows the effect of pure and extracted Curcumin on chromosomal aberration induced by Mitomycin C.

form has comparatively greater antioxidant percentage. So, scavenging these free radicals due to the anti-oxidation property of curcumin may also help to increase the anti-clastogenic effect of this phyto-constituent. This study was carried out to check the anti-clastogenic profile of both pure and extracted curcumin whether the said herbal compound has a positive response in preventing DNA damage induced by anti-neoplastic drug like MMC. The underlying mechanism requires detail attention as it showed promising inhibitory action against a world-wide pollutant and carcinogen.

CONCLUSION

In conclusion the anti-clastogenic 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 mitomycin C. The results showed a positive inhibitory effect of curcumin, the main constituent of turmeric in the frequency of chromosomal aberration in the human peripheral leukocytes cells. The study also revealed that, curcumin, both in pure or extracted forms, have potential antioxidant property. The study indicated that curcumin the key constituent of tumeric, widely taken as a accessory food material or colouring agent, has the potentiality to protect chromosomal damage in vitro. Curcumin, a major component of turmeric does not show any clastogenic 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 clastogenic agents. The present study revealed that pure curcumin and the curcumin extract have significant antioxidant property. Our results also demonstrated that, extracted curcumin as well as pure or commercial curcumin, are effective in counteracting the clastogenicity in human leukocyte

in vitro. So, in future it could be a possibility for a new phyto derived drug for related disease in human.

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Conflict of Interest

The authors declare that they don't have any kind of interest.

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