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An Evaluation of Anti-Clastogenic Activities for Garlic (*Allium Sativum*), Turmeric (*Curcuma Longa*) and Tulsi (*Ocimum Tenuiflorum*) with Clastogen (Mitomycin) in Human Lymphocytes – A Case Study

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Abstract

Chromosomal aberrations based human syndrome are very critical and sometimes leads to lethality. Such syndrome or disorders are often irreversible. In this present study, we evaluated the preventing effect of herbal extracts from Ocimum (tulsi), Curcuma (turmeric) and Allium (garlic) in developing chromosomal aberrations in whole blood human lymphocytes. The results showed that the clastogenic effect was minimised by the combination of all three herbal extracts compared to the individual extract effects. The spontaneous chromosomal aberrations caused in lymphoma cells were also minimized by the herbal extracts.

Keywords: *Herbs*, *Clastogenicity*, *Chromosome*, *Turmeric*, *Garlic and Tulsi*

Introduction

Clastogenicity is an induction of changes in structure of chromosomes, which is dangerous to any organisms. In concern to mutation, the clastogenicity, a structural chromosome aberrations produce through some clastogens, which cause breaks in chromosomes and resulted in either loss or re-shifts of chromosomal segments [1]. In treating mutagenicity, compared to modern medicine, the traditional medicine has some ability to cure without worsening the condition. Globally, many herbs involved in control of mutagenic activity but selecting them for specific purpose is quite difficult and before selecting, the herbs should be validated.

Daily humans consuming normally many medicinal related products as a food but none of them were not considering about the potent properties. The foods such as turmeric, ginger, and tulsi were rich in medicinal properties such as anti-viral, anti-fungal, anticancer, anti-inflammatory, and anti-helminthic to control or to inhibit various diseases [2]. In olden days, peoples fixed on the term "Food is Medicine" and used medicinal herbs and foods products to treat disease as "Granny Cure" later it's faded due to emergence of modern medicine. Recently medicinal herbs remerged and boom in the healthcare society to treat diseases. So here, we compare the efficacy of three combinations products (turmeric, garlic, and tulsi) for anti-clastogenic activities. This case report will helps the medicinal practitioners and public for use of medicinal foods as a home remedy to control diseases.

The different types of chromosomal aberrations, which includes such as deletions, minutes, acentric rings, centric rings, inversions, reciprocal translocations and dicentric or polycentric aberrations. A chromatid or chromosome gap characterized by achromatic lesion appearing as a non-staining and constricted region in the chromatid arm. The apparently broken arm and the broken segment are in alignment. Chromatid breaks are regions where the broken arm and the broken fragment are not in the alignment. Exchanges are characterized by reciprocal translocation of broken fragments of two chromosomes, which are evidenced by the difference in size of the fragment in conjunction with the broken arm. Acentric rings are paried segments without centromere, which are joined to produce a ring shape (Figure 1). Centric rings resemble acentric rings with the presence of centromere in the ring [3].

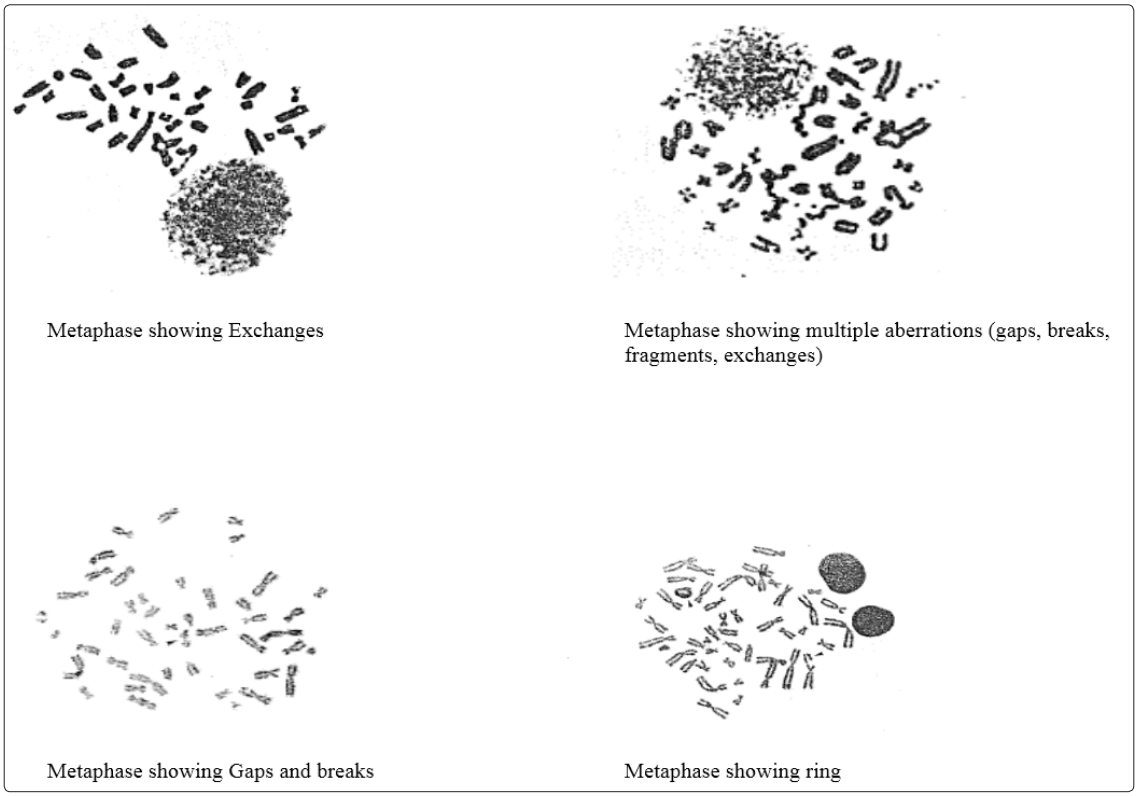


Figure 1: Chromosomal spreads whosing various abnormalities

Chromosomal aberrations based human diseases are well established. Mongolism, Klinefilter's syndrome and Turner's syndrome was due to non-dysjunction of chromosomes during meiosis process [4]. Chromosomal aberrations may alter or destroy the function of one or more genes by changing the regulation of gene expression, ie., disrupting exons or by creating gene fusion [5]. The chromosomal breaks near the site of SOX9 gene may lead to campomelic dysplasia, ie., defects in the development of skeleton bones, which may be lethal to new borns [6]. Chromosomal deletion leads to expression of pathogenic alleles in a gene causing DiGeorge/VCF syndrome leading to congenital heart problem, delayed development, frequent infections and cleft palate [7]. CEDNIK syndrome, another good example of unmasking of gene due to chromosomal deletion, is characterised by severe developmental failure of nervous system and epidermis [8].

Owing to deleterious effects of chromosomal aberrations in human health, it becomes inevitable to prevent such chromosomal aberration based syndrome and lethal conditions. Though Ayurveda texts and reviews say that these kind of genetic disorders are incurable, continuous treatment to minimize or neutralize the effects [9]. Predominantly rich medicinal value foods such as turmeric, garlic, and tulsi has therapeutic values of anti-viral, anti-fungal, anticancer, anti-inflammatory, anti-helminthic to control or to inhibit various diseases [2]. In this study, we identified the preventive effect of chromosomal aberrrations using herbal extracts of *Curcuma* (Turmeric), *Ocimum* (Tulsi) and *Allium* (Garlic) through *in vitro* culture human lymphoma cells.

Methodology

The herbal extracts were prepared and adopted the methodology of Moshi Raju et al., [10]. Fresh herbs were obtained from the local market and dried. The dried part was made in to fine powder with motor and pestle. The powder of 250g was soaked in 500 ml of ethanol for 24 hrs and the solvent was separated and concentrated through Soxhlet apparatus. The obtained extract was run through rotary evaporator for further concentration. The final extract was lyophilized to powder and stored at 4ºC for further use [11].

The human lymphoma cells were collected aseptically, cultured *in vitro* condition using DMEM medium with 10% FBS. The cells were divided in to 10 treatment groups. The first two groups as negative control and positive control (treated with mitomycin at a concentration of 0.2 mg/ml for 4h). The next four treatment groups were positive controls treated along with turmeric, garlic, tulsi extract and mixture of turmeric, garlic and tulsi extracts at a concentration of 100µl/ml. The last four treatment groups were positive controls treated along with turmeric, garlic, tulsi extract and mixture of turmeric, garlic and tulsi extracts at a concentration of 100µl/ml. After treatment, the chromosomal spreads were prepared according to Marilyn Registre and Ray Proudlock [12]. The cells were washed and grown in culture medium for 24 hours for one cell cycle. After 24 hours, colchicine was added at a concentration of 0.4 µg/ml to the culture and the cells were further harvested after 2 hours. The cells were harvested, treated with 75 mM KCl hypotonic solution, fixed using methanol acetic acid (3:1) solution and slides prepared using hanging drop method [13].

Results

The chromatid and chromosome gaps in the negative control was less (4.33 + 1.53 and 4.66 + 1.53) compared to all other treatment groups. Similarly, the chromatid and chromosome breaks were also comparatively less in negative control (6.00 + 1.00 and 4.00 +

1.73). The fragments appeared in the control (1.67 + 0.58) and the exchanges were completely absent. The total aberrations were 20.66 + 1.53 (with gaps) and 11.67 + 2.88 (without gaps). The overall percentage of aberrations in negative control with gaps of 6.89 + 0.51 and without gaps of 3.89 + 0.96 (Table 1).

Table 1: Anti-clastogenic activities of garlic, turmeric and tulsi in whole blood human lymphocytes

S. No	Concentration µl/culture	Type of aberrations									
		Chromatid		Chromosome		Fragment (ring)	exchanges	Polyploidy	Total aberration		Percentage of aberration
		gap	Break	gap	Break				With gaps	Without gaps	With gaps	Without gaps
1	0 (Negative Control)	4.33 + 1.53	6.00 + 1.00	4.66 + 1.53	4.00 + 1.73	1.67 + 0.58	0	0	20.66 + 1.53	11.67 + 2.88	6.89 + 0.51	3.89 + 0.96
2	100 (mitomycin) (0.2mg/ml) Positive control	11.67 + 1.52	22.67 + 2.51	4.33 + 1.52	12.33 + 1.52	14.33 + 2.08	8.67 + 1.52	0	74.00 + 1.73	58.00 + 1.73	24.67 + 0.58	19.33 + 0.58
3	100 (positive control) + 100 (turmeric powder extract)	8.67 + 1.15	8.67 + 3.06	4.67 + 1.53	7.67 + 1.53	4.67 + 1.15	3.33 + 1.15	0	37.67 + 1.52	24.33 + 2.08	12.56 + 0.51	8.11 + 0.69
4	100 (positive control) + 100 (Garlic extract)	8.00 + 2.64	10.67 + 1.52	4.33 + 1.52	7.67 + 1.15	7.67 + 1.53	4.00 + 1.73	0	40.33 +3.78	28.00 + 1.00	13.44 + 1.26	9.33 + 0.33
5	100 (positive control) + 100 (tulsi leaf extract)	9.67 + 1.52	11.33 + 1.54	4.33 + 1.53	4.67 + 1.15	8.66 + 1.53	2.33 + 0.57	0	42.67 +5.13	27.67 + 1.52	14.22 + 1.71	9.22 + 0.51
6	100 (positive control) + 100 (mixture of turmeric + garlic + tulsi leaf extract)	8.33 + 2.08	5.67 + 1.52	3.33 + 2.08	4.67 + 1.52	8.33 + 1.52	2.67 + 1.52	0	33.00 +4.36	21.33 + 0.57	11.00 + 1.45	7.11 + 0.19
7	Negative Control + 100 (Turmeric powder extract)	4.00 + 1.00	1.67 + 1.52	2.67 + 0.57	2.00 + 1.73	1.67 + 1.52	0	0	12.00 +6.08	5.33 + 4.62	4.00 + 2.03	1.78 + 1.54
8	Negative Control + 100 (Garlic extract)	3.33 + 0.58	4.67 + 1.15	3.67 + 0.57	1.33 + 0.57	0.67 + 0.57	0	0	13.67 + 1.15	6.67 + 2.08	4.56 + 0.38	2.22 + 0.69
9	Negative Control + (tulsi leaf extract)	2.33 + 0.58	3.00 + 1.00	3.67 + 1.15	1.67 + 0.58	1.67 + 1.15	0	0	12.33 + 2.31	6.33 + 1.53	4.11 + 0.77	2.11 + 0.51
10	Negative Control + 100 (mixture of turmeric + Garlic extract + tulsi leaf extract)	1.67 + 0.58	1.33 + 1.15	1.33 + 0.57	1.67 + 1.15	0.67 + 0.58	0	0	6.67 + 1.52	3.67 + 1.52	2.22 + 0.51	1.22 + 0.51

The positive control showed multifold increase in chromatid gaps (11.67 + 1.52), chromatid break (22.67 + 2.51), chromosome gaps (4.33 + 1.52), chromosome break (12.33 + 1.52), fragments (14.33 + 2.08) and exchanges were present (8.67 + 1.52). The total aberrations were 74.00 + 1.73 (with gaps) and 58.00 + 1.73 (without gaps). The overall percentage aberrations with gaps of 24.67 + 0.58 and without gaps 19.33 + 0.58 (Table 1).

The lymphoma cells treated with positive control along with herbal extract of *Curcuma* (turmeric) showed 8.67 + 1.15 (chromatid gaps); 8.67 + 3.06 (chromatid break); 4.67 + 1.53 (chromosome gap); 7.67 + 1.53 (chromosome break); 4.67 + 1.15 (fragments); 3.33 + 1.15 (exchanges); 37.67 + 1.52 (total aberrations with gaps); 24.33 + 2.08 (total aberrations without gap); 12.56 + 0.51 (percentage of aberration with gaps) and 8.11 + 0.69 (percentage of aberrations without gap).

The herbal extract *Allium* (garlic) showed 8.00 + 2.64 (chromatid gap); 10.67 + 1.52 (chromatid break); 4.33 + 1.52 (chromosome gap); 7.67 + 1.15 (chromosome break); 7.67 + 1.53 (fragments); 4.00 + 1.73 (exchanges); 40.33 + 3.78 (total aberrations with gap); 28.00 + 1.00 (total aberrations without gap); 13.44 + 1.26 (percentage of aberrations with gaps) and 9.33 + 0.33 (percentage of aberrations without gaps).

The herbal extract *Ocimum* (tulsi) showed 9.67 + 1.52 (chromatid gap); 11.33 + 1.54 (chromatid break); 4.33 + 1.53 (chromosome gap); 4.67 + 1.15 (chromosome break); 8.66 + 1.53 (fragments); 2.33 + 0.57 (exchanges); 42.67 + 5.13 (total aberrations with gap); 27.67 + 1.52 (total aberrations without gap); 14.22 + 1.71 (percentage of aberrations with gaps) and 9.22 + 0.51 (percentage of aberrations without gaps).

The mixture of three (*Ocimum, Allium* and *Curcuma*) showed less chromatid gaps (8.33 + 2.08), breaks (5.67 + 1.52); chromosome gaps (3.33 + 2.08) and chromosome breaks (4.67 + 1.52). The fragments and exchanges were also comparatively less when compared to the

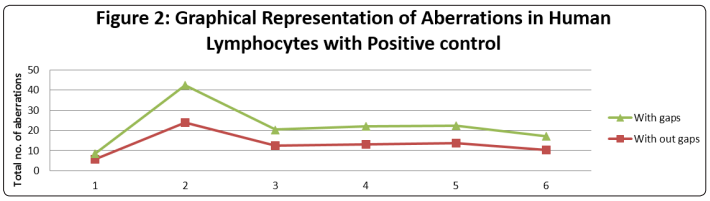
positive control. Thus the total aberrations (with gaps and without gaps) 33.00 + 4.36, 21.33+ 0.57, percentage aberration (with gap and without gap) were 11.00 + 1.45, 7.11 + 0.19; (Table 1).

The negative control cells, when treated with *Curcuma* (turmeric) showed 4.00 + 1.00 (chromatid gap); 1.67 + 1.52 (chromatid break); 2.67 + 0.57 (chromosome gap); 2.00 + 1.73 (chromosome break); 1.67 + 1.52 (fragments); 12.00 + 6.08 (total aberrations with gap); 5.33 + 4.62 (total aberrations without gap); 4.00 + 2.03 (percentage of aberrations with gaps) and 1.78 + 1.54 (percentage of aberrations without gaps).

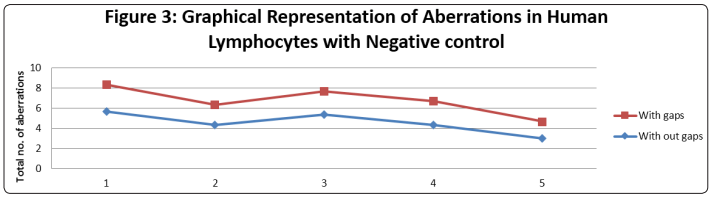
The *Allium* (garlic) extracts showed 3.33 + 0.58 (chromatid gap); 4.67 + 1.15 (chromatid break); 3.67 + 0.57 (chromosome gap); 1.33 + 0.57 (chromosome break); 0.67 + 0.57 (fragments); 13.67 + 1.15 (total aberrations with gap); 6.67 + 2.08 (total aberrations without gap); 4.56 + 0.38 (percentage of aberrations with gaps) and 2.22 + 0.69 (percentage of aberrations without gaps).

The *Ocimum* (tulsi) extract showed 2.33 + 0.58 (chromatid gap); 3.00 + 1.00 (chromatid break); 3.67 + 1.15 (chromosome gap); 1.67 + 0.58 (chromosome break); 1.67 + 1.15 (fragments); 12.33 + 2.31 (total aberrations with gap); 6.33 + 1.53 (total aberrations without gap); 4.11 + 0.77 (percentage of aberrations with gaps) and 2.11 + 0.51 (percentage of aberrations without gaps).

The mixture of all the three (*Ocimum*, *Allium* and *Curcuma*) showed 1.67 + 0.58 (chromatid gap); 1.33 + 1.15 (chromatid break); 1.33 + 0.57 (chromosome gap); 1.67 + 1.15 (chromosome break); 0.67 + 0.58 (fragments); 6.67 + 1.52 (total aberrations with gap); 3.67 + 1.52 (total aberrations without gap); 2.22 + 0.51 (percentage of aberrations with gaps) and 1.22 + 0.51 (percentage of aberrations without gaps) (Table 1). In both positive control and negative control, the cells treated along with the mixture of all the three herbal extracts showed comparatively less percentage of aberrations (Figure 2 and 3).



1 – Negative control: 2 - Mitomycin Positive control; 3 - Positive Control + Turmeric extract; 4 - Positive Control + Garlic extract; 5 - Positive Control+ Tulsi leaf c extract; 6 - Positive Control + mixture of three extracts.



1 – Negative control; 2 - Control + Turmeric extract; 3 - Control + Garlic extract; 4 - Control+ Tulsi leaf extract; 5 - Control + mixture of 3 extracts.

Discussion

In many instances, herbal extracts have proven to be anti-clastogenic, thereby proving therapeutic property for human diseases or disorders. In the present study, the herbal extracts showed less number of chromosomal breaks and gaps. The fragments and exchanges were reduced drastically in the positive controls treated with the herbal extracts (Table 1). Garlic extract showed less number of breaks and fragments compared to the positive and negative controls. Our results were coinciding with previous reports that garlic has anti-genotoxic and anti-mutagenic effects for various drugs and chemicals [14-17]. The anti-carcinogenic activity of garlic for several carcinogens were found to be effective by direct inhibition of tumour cell metabolism, inhibition of initiation and promotion phases of carcinogenesis and modulating the post immune response. Apart from these good effect, garlic acts as a scavenger of free radicals, the origin of ageing process [18]. Our results coincides with the previous reports of scavenging process.

Tulsi extract showed less number of chromatid gaps, breaks and chromosome breaks when compared to the corresponding solvent and positive controls. Many studies showed that tulsi contained many phenolic coumpounds such as cirsilineol, circimaritin, isothymusin, apigenin and rosmeric acid and eugenol. These phenolic compounds have found to be pharmacological effects [19, 20]. Khanna et al., observed the protective effect of Ocimum sanctum against chlorpyrifos induced genotoxicity under *in vitro* conditions.

In this present study, Turmeric extract showed drastic reduction of chromatid breaks, chromosome gaps, breaks, fragments and exchanges when compared with the corresponding positive and negative controls. Curcumin showed anti-mutagenic, anti-genotoxic and protective effects on various test systems [21-27]. Curcumin, as a free radical scavenger has anti-clastogenic property [23]. It protects the genome from radiation-induced damages in DNA [24]. It also protects from genotoxic effect of hydrocortisone and nicotine, which was similar to this study results observed in turmeric extract [26].

The anticlastogenic activity of herbal extracts was well observed in this study. The spontaneous chromosomal aberration was also minimized or reduced due to the effect of all the three herbal extracts (Table 1). Similar results were observed by Moshi Raju et al., showing the prevention of genotoxic damage in bone marrow cells of mice treated with garlic extract [10, 28]. Neeraj Kumar et al., also observed prevention of DNA damage in human peripheral blood lymphocytes treated with tulsi extract [29]. Palani Kumar and Paneerselvam also witnessed similar results showing the anti-mutagenic property of turmeric in onion root tip stem cells. Certain fractions of these herbal origins are chemically reactive species that are formed during the processing and ingestion of the extract, which could be acting as non-specific redox agents and scanvengers of free radicals [30]. Moreover, the present study observed in the mixture of herbal extract were similar to the previously published reports.

Conclusion

From the results, it is concluded that our present study showing anti-clastogenic activity of the herbal (*Allium*, *Curcuma* and *Ocimum*) extracts in human lymphocytes is proven. Purification and separation of various molecules of these herbal (*Allium*, *Curcuma* and *Ocimum*) extracts and evaluating the anti-clastogenic property will help the researchers to synthesize or purify such compounds and develop new drug based on those molecule for anti-clastogenic therapy.

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