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RESEARCH ARTICLE

TOXICOLOGY

MITIGATION OF GENOTOXIC EFFECTS OF CISPLATIN THROUGH SOME DIETARY ANTIOXIDANTS IN HUMAN LYMPHOCYTE CHROMOSOMES *IN VITRO*

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ABSTRACT

Curcumin has been used in traditional Indian medicine for many centuries for its anti-inflammatory and anticarcinogenic properties and Garlic is a popular spice added to several edible preparations and is a remedy for a variety of ailments. The protective effects of Curcumin and Garlic on cisplatin induced chromosomal aberrations have been determined in the human peripheral lymphocyte chromosomal aberration test *in vitro*. The results of treatments with Curcumin and Garlic indicated that they statistically significantly decrease the number of chromosomal aberrations and number of metaphases with aberrations induced with cisplatin, but they cannot completely protect cells from damage. The test concentrations of Curcumin (50 µg/ml) and Garlic (100 µg/ml) showed limited antimutagenic effect on cisplatin (1.0 µg/ml and 2.0 µg/ml) at 0, 24 and 48 hour of duration in reverse order. Curcumin and Garlic showed the most efficient anticlastogenic effect during simultaneous treatment with cisplatin. The result further strengthened the findings about the protective and antigenotoxic properties of these two natural dietary products.

KEYWORDS

Cisplatin, Curcumin, Garlic and Chromosomal aberration, etc.

INTRODUCTION

Antineoplastic drugs (ANDs) have been in clinical use for five decades. The carcinogenic properties of many of these drugs have been studied extensively in animal models and in follow-up studies of patient populations. Comparatively less research has evaluated the possible ameliorating compounds, which can at least minimize the genotoxic effect of ANDs. Cisplatin is a chemotherapeutic drug which is used to treat cancers including: sarcoma, small cell lung cancer, germ cell tumors, lymphoma, and ovarian cancer. While it is often considered an alkylating agent, it contains no alkyls groups and does not instigate alkylating reactions, so it is properly designated as an alkylating-like drug. Cisplatin is platinum-based and was the first medicine developed in that drug class. Other drugs in this class include carboplatin, a drug with fewer and less severe side effects introduced in the 1980s, and oxaliplatin, a drug which is part of the FOLFOX (FOL– Folinic acid (leucovorin), F – Fluorouracil (5-FU), OX – Oxaliplatin) treatment for colorectal cancer. The other names for cisplatin are DDP, cisplatinum, and cis-diamminedichloridoplatinum(II) (CDDP). Cisplatin was actually first created in the mid 19th Century and is also known as Peyrone's chloride (The disoverer was Michel Peyrone). It wasn't until the 1960s that scientists started getting interested in its biological effects, and cisplatin went into clinical trials for cancer therapy in 1971. By the late 1970s it was already widely used and is still used today despite the many newer chemotherapy drugs developed over the past decades.

Alkylating agents work by three different mechanisms: 1) attachment of alkyl groups to DNA bases, resulting in the DNA being fragmented by repair enzymes in their attempts to replace the alkylated bases, preventing DNA synthesis and RNA transcription from the affected DNA, 2) DNA damage via the formation of cross-links (bonds between atoms in the DNA) which prevents DNA from being separated for synthesis or transcription, and 3) the induction of mispairing of the nucleotides leading to mutations.

Natural products have been widely used since age long in different parts of the world for medicinal purpose, but scientific study on their mode of action has only been attempted recently. Extensive research in the last few years has revealed that regular consumption of certain fruits and vegetables can reduce the risk of acquiring specific cancers. Phytochemicals derived from such fruits and vegetables, referred to as chemopreventive agents include genistein, resvertrol, diallyl sulfide, S-allyl cysteine, allicin, lycopene, capsaicin, curcumin, 6-gingerol, ellagic acid, ursolic acid, silymarin, anethol, catechins and eugenol. Because these agents have been shown to suppress cancer proliferation, inhibit growth factor signaling pathways, induce apoptosis, and inhibit NF-Kb, AP-1 and JAK-STAT activation pathways, inhibit angiogenesis, suppress the expression of anti-apoptotic proteins, inhibit cyclooxygenase-2, they may have untapped therapeutic value [1]. These chemopreventive agents have also very recently been found to show reverse effects. Thus, these chemopreventive agents have potential to be used as adjunct to current cancer therapies [2].

Several epidemiological studies have suggested an association between garlic consumption and a reduced risk of different kinds of human cancer [2]. The main constituent of garlic is allicin. It has potent antioxidant

properties, including the scavenging of oxygen radicals and lipid radicals. Many *in vitro* models have been used to test flavonoid antioxidant activity. However, the absorption and mechanism of action of these compounds in cells such as lymphocytes, which are primary targets of oxidative damage, have not been yet evaluated. Cell culture has been used mainly for studying chemopreventive action of catechins and natural products at relatively high concentrations whereas it seems that the antioxidant function occurs at lower levels [3-5].

Turmeric (*Curcuma longa)* is a medicinal plant extensively used in Ayurveda, Sidha and Unani medicine in India. It has been used in traditional medicine as household remedy for various diseases. Curcumin (diferuloyl methane) the main yellow bioactive component of turmeric has been shown to have a wide spectrum of biological actions. These include its anti-inflammatory, antioxidant, anticarcinogenic and antimutagenic activities also [6]. Curcumin has been reported to inhibit skin, forestomach, stomach and colon cancer and mammary tumerogenesis in rats [7-9]. It is anti retroviral in human epithelial cells, HIVI[10] having antiinflammatory and antioxidant activity[11]. Curcumin and isoflavonoids in combination have been reported antitumerous in estrogen receptor (ER) positive and ER negative human breast cancer cell and have protection against steroid induced breast cancer[12]. Its anticancer effect is mainly mediated through induction of apoptosis. Safety evaluation studies indicate that both turmeric and curcumin are well tolerated at a very high dose without any toxic effect.

There has been enough evidence for anticarcinogenic potential of garlic (*Allium sativum)* used in powder form [13]. *Allium sativum* consist of 6 organo sulphur compounds [14]. viz. diallyl sulfide, diallyl disulfide and diallyl trisulfide. Milner, et. al., [15] using water soluble S-allyl cysteine diallyl disulfide, noticed antigenotoxic action against N-methyl -N-nitrosourea compound [16].

It is, therefore, quite natural to evaluate the antigenotoxic potential of these natural products in human test system *in vitro.*

MATERIALS AND METHODS

Cisplatin was dissolved in dimethyl sulfoxide (DMSO 5μl/ml; E. Merck, Mumbai, India) at a final concentration of 1.0 μg/ml and 2.0 μg/ml; curcumin and garlic (Sigma) was prepared by dissolving their compounds in DMSO at the concentrations viz. 50 μg/ml and 100 μg/ml respectively.

(i) Chromosomal Aberrations Analysis

Lymphocyte culture was planted as per the method of Moorehead, et. al., [17]. Cultures were planted in previously cleaned and sterile chamber of laminar flow. About 0.4 ml of blood was transferred to a culture vial, already containing 5-7 ml of culture medium and 0.2-0.3 ml of phytohaemagglutinin (PHA), under the sterile conditions of laminar flow. Then the vials were closed tightly and were put at 37°C for 72 hour in water bath. A final concentration of 1.0 and 2.0 μg/ml of Cisplatin was added to the culture vials at 0 hour, 24 and 48 hour and Curcumin and Garlic, prepared at the concentration of 50μg/ml, 100μg/ml respectively were added at the same time to examine probable reduction of these effects. The DMSO was added to the simultaneously kept culture for 72 hour of incubation as negative control. For positive control, cyclophosphamide was added for 0, 24 and 48 hour of incubation. 0.15 ml of colchicine (2μg/ml) was added to each vial 1 hr prior to harvesting to arrest the cells at metaphase stage. After 1 hour of colchicine's action in the medium, the cells were spun down by centrifugation (10 min, 1000 rpm) and the button of cells was saved by discarding the supernatant. Hypotonic treatment (0.075 M KCl) was carried out for 10-20 minutes at 37°C and the cells recollected by centrifugation. The cell pellet was suspended in 7 ml freshly prepared chilled fixative (3: 1, methanol: acetic acid). The contents, then, were again centrifuged at 1000 rpm for 10 minutes and the same two or

three washings with fresh fixative were given before preparing the slide. Slides were prepared by flame drying technique. Giemsa stain was used for staining metaphase chromosomes.

Detectable and finely spread metaphase chromosome spots were analyzed under 100X oil immersion, position of the desired spot was marked by reading scales over the microscope. Photographs were taken with an automatic digital camera attached in a Nikon 80i microscope.

(ii) Statistical Analysis

Data are expressed as the mean ±S.E. Student's two tailed "t" test was used for calculating the statistical significance for CA with the help of SPSS 18. The level of significance was set at $P < 0.05$.

RESULTS

Table I, II and III present the data showing genotoxic effects of Cisplatin and its amelioration by curcumin and garlic extract at 24, 48 and 72 h in reverse order. The mean frequency of cells with aberrations was 0.04±0.02, 0.16±0.08, 0.08±0.04 and 0.07±0.05 for control, 1.0µg/ml of cisplatin, 1.0µg/ml of cisplatin + 50 µg/ml curcumin and 1.0 µg/ml + 100 µg/ml garlic respectively at 0 hour. The mean frequency of cells with aberrations was 0.04±0.02, 0.24±0.10, 0.15±0.07 and 0.11±0.04 for control, 2.0µg/ml of cisplatin, 2.0µg/ml of cisplatin + 50 µg/ml curcumin and 2.0 µg/ml + 100 µg/ml garlic respectively at 0 hour **(Table-1 and Figure-1)**. The mean frequency of cells with aberrations was 0.04±0.02, 0.16±0.07, 0.07±0.03 and 0.06±0.03 for control, 1.0µg/ml of cisplatin, 1.0µg/ml of cisplatin + 50 µg/ml curcumin and 1.0 µg/ml + 100 µg/ml garlic respectively at 24 hour. The mean frequency of cells with aberrations was 0.04±0.02, 0.26±0.11, 0.17±0.08 and 0.16±0.07 for control, 2.0µg/ml of cisplatin, 2.0µg/ml of cisplatin + 50 µg/ml curcumin and 2.0 µg/ml + 100 µg/ml garlic respectively at 24 hour **(Table-2 and Figure-2)**. The mean frequency of cells with aberrations was 0.04±0.02, 0.10±0.04, 0.06±0.03 and 0.05±0.02 for control, 1.0µg/ml of cisplatin, 1.0µg/ml of cisplatin + 50 µg/ml curcumin and 1.0 µg/ml + 100 µg/ml garlic respectively at 48 hour. The mean frequency of cells with aberrations was 0.04±0.02, 0.21±0.10, 0.15±0.07 and 0.14±0.07 for control, 2.0µg/ml of cisplatin, 2.0µg/ml of cisplatin + 50 µg/ml curcumin and 2.0 µg/ml + 100 µg/ml garlic respectively at 48 hour **(Table-3 and Figure-3)**. Maximum protective effect was observed in case of Garlic treated cultures. The difference between control and treated values are statistically significant showing quite significant amelioration at both the concentrations. The values mentioned above showed a significant result when compared to the control ($P<0.05$).

Table: 1

Chromosomal Aberrations in Human Lymphocytes after Cisplatin Treatment and its amelioration with curcumin (C) and garlic extract (G) for 0 hr Duration

Drug Conc.	Chromatid type Aberrations			Chromosome type Aberrations			No. of cells with Aberrations	Aberrations/ cell ± SE
	Gaps	Breaks	Exchanges	Break	Dicentrics	Rings		
Cisplatin								
1.0 µg/ml	8	1	1	1	2	3	16	0.16±0.08
1.0 µg/ml+C	4	0	1	1	2	0	8	0.08±0.04
1.0 µg/ml+G	5	0	1	0	1	0	7	0.07±0.05*
2.0 µg/ml	4	3	6	3	6	2	24	0.24±0.10
2.0 µg/ml+C	2	1	4	2	5	1	15	0.15±0.07**
2.0 µg/ml+G	1	2	3	1	3	1	11	0.11±0.04**
Controls								
Positive (CP)	10	6	4	8	7	2	37	0.37±0.16***
Negative	2	1	0	1	0	0	4	0.04±0.02

CP = Cyclophosphamide SE = Standard error
**Significant at P<0.05 **Significant at P<0.02 ***Significant at P<0.001*

Table: 2

Chromosomal Aberrations in Human Lymphocytes after Cisplatin Treatment and its amelioration with curcumin (C) and garlic extract (G) for 24 hr Duration

Drug Conc.	Chromatid type Aberrations			Chromosome type Aberrations			No. of cells with Aberrations	Aberrations/ cell ± SE
	Gaps	Breaks	Exchanges	Break	Dicentrics	Rings		
Cisplatin								
1.0 µg/ml	5	3	1	4	2	1	16	0.16±0.07
1.0 µg/ml+C	2	2	0	2	1	0	7	0.07±0.03**
1.0 µg/ml+G	3	1	1	0	1	0	6	0.06±0.03*
2.0 µg/ml	4	6	5	4	7	0	26	0.26±0.11
2.0 µg/ml+C	3	2	5	2	5	0	17	0.17±0.08*
2.0 µg/ml+G	2	3	4	2	5	0	16	0.16±0.07**
Controls								
Positive (CP)	9	6	12	5	9	6	47	0.47±0.19***
Negative	3	0	1	0	0	0	4	0.04±0.02

CP = Cyclophosphamide SE = Standard error
**Significant at P<0.05 **Significant at P<0.02 ***Significant at P<0.001*

Table: 3

Chromosomal Aberrations in Human Lymphocytes after Cisplatin Treatment and its amelioration with curcumin (C) and garlic extract (G) for 48 hr Duration

Drug Conc.	Chromatid type Aberrations			Chromosome type Aberrations			No. of cells with Aberrations	Aberrations/ cell ± SE
	Gaps	Breaks	Exchanges	Break	Dicentrics	Rings		
Cisplatin								
1.0 µg/ml	3	2	0	2	2	1	10	0.10±0.04
1.0 µg/ml+C	1	0	2	1	2	0	6	0.06±0.03
1.0 µg/ml+G	1	0	1	1	2	0	5	0.05±0.02
2.0 µg/ml	8	1	2	6	2	2	21	0.21±0.10
2.0 µg/ml+C	6	1	3	4	1	0	15	0.15±0.07
2.0 µg/ml+G	5	1	2	5	1	0	14	0.14±0.07*
Controls								
Positive (CP)	14	9	6	5	9	1	44	0.44±0.20***
Negative	2	0	1	0	0	0	4	0.04±0.24

*CP = Cyclophosphamide SE = Standard error *Significant at P<0.05 ***Significant at P<0.001*

Fig: 1

Graphical Representation of Chromosomal Aberrations in Human Lymphocytes after Cisplatin Treatment and its Amelioration with curcumin (C) and garlic extract (G) for 0 hr Duration

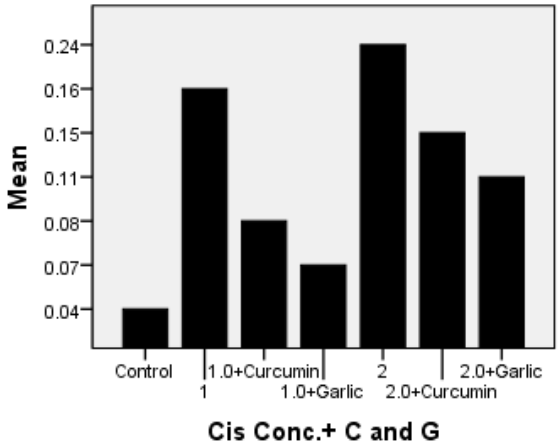


Fig: 2

Graphical Representation of Chromosomal Aberrations in Human Lymphocytes after Cisplatin Treatment and its Amelioration with curcumin (C) and garlic extract (G) for 24 hr Duration

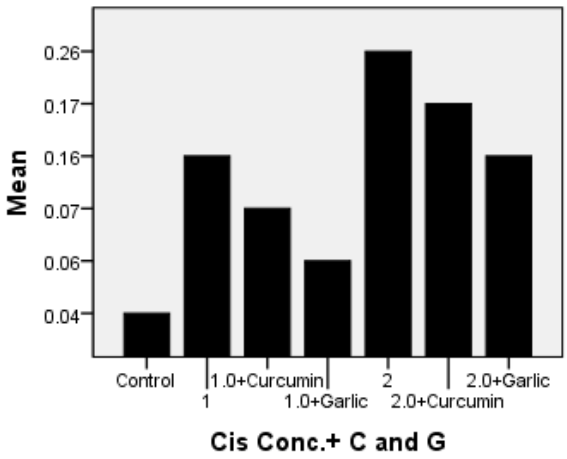
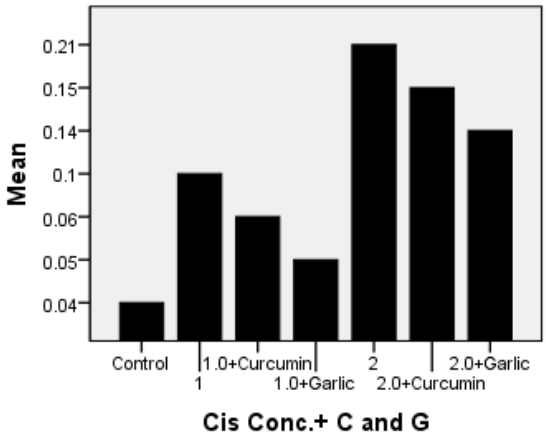


Fig: 3

Graphical Representation of Chromosomal Aberrations in Human Lymphocytes after Cisplatin Treatment and its Amelioration with curcumin (C) and garlic extract (G) for 48 hr Duration



DISCUSSION

All classes of ANDs have been shown to be mutagenic in *in vitro* assays [18]. Numerous ANDs have been found to be carcinogenic in animal models indications that AND-therapy was associated with second primary malignancies were initially reported in case reports and case series. In the mid-1970s, large-scale follow-up studies of cancer patients undergoing AND therapy were first reported in the literature. These reports strongly and consistently associated several alkylating agents with the development of leukemia and myelodysplastic syndromes [19-34]. Studies that evaluated histological subtypes found that acute non-lymphocytic leukemia predominated. Case series and longitudinal studies also specifically implicated cyclophosphamide (and chlornaphazine used in Europe) with the development of bladder cancer[35-42]. In animal models and patient populations, various ANDs have been associated with myocardial cardiac conduction defects; pulmonary fibros gastrointestinal disturbances includi stomatitis, nausea, vomiting, and diarrhe changes in liver function; neurotoxici myelosuppression; and alopecia (hair loss) [59]. The data presented demonstrate seve major points' viz. Cucumin and Garlic c ameliorate the genotoxic effect by reducing C The exact mechanism of action of Curcumin their antimutagenicity induced by progeste has not been fully explored. Many biologic and medicinal properties of curcumin are now well recognised. It is reported to possess anti-inflammatory [60-61], antitumor [62] and antioxidant properties [63-64]. Toxicologically, it is relatively inert and does not appear to be toxic to either animals [65] or humans [66], even at high doses. Curcumin inhibits the formation of potentially mutagenic benzo(a) pyrene derived DNA adducts both *in vitro* [67] and *in vivo* [68]. It is also capable of inhibiting the singlet oxygen induced damage to plasmid DNA [69]. Most of the biological effects of curcumin are considered to be due to its antioxidant and radical scavenging properties [70]. Curcumin has been shown to influence the activities of enzymes such as cyclogenase, Lipoxygenase, phospho-lipase A2 and phospholipase C-gama 1 [71]. Other feasible mechanisms of action of curcumin could involve suppression of the activities of protein kinases, (e.g. kinase C and tyrosine kinase [72]. These results support the concept that a combination of natural chemopreventive nutrients is more potent than individual compounds against ameliorating the genotoxicity induced by antineoplastic drug or other mutagen. Further, the *in vitro* data presented in this paper constitute the framework for further studies in animal models and clinical trials. Data on natural plant compounds may further be helpful in developing new therapeutic agents.

CONCLUSION

This study was aimed at finding the genotoxic effects of Cisplatin and mitigation of these effects using some natural dietary antioxidants and anticancerous principles in human cells. The study was helpful in suggesting therapeutic application of these agents in dose dependant manner. Such principles will also help toward low cost chemopreventive medication to the cancer patients specially from the third world countries undergoing chemotherapy.

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REFERENCES

1. Islam N, Haqqi TM, Jepsen KJ, Kraay M, Welter JF, Goldberg VM and Malemud CJ, Hydrostatic pressure induces apoptosis in human chondrocytes from osteoarthritic cartilage through up-regulation of Tumor Necrosis Factor-a, inducible nitric oxide synthase, p53, c-myc and bax-á and suppression of bcl-2. J. Cell Biochem, 87: 266-78 (2002).
2. Dorai T, Aggarwal BB, Role of chemopreventive agents in cancer therapy. Cancer Letters, 215: 129-140 (2004).
3. Islam S, Islam N, Kermode T, Johnstone B, Moskowitz RW, Goldberg VM, Mukhtar H, Melamud CJ, Haqqi TM, Activation of Caspase-3 in human chondrosarcoma cells by Tumor Necrosis Factor-α and epigallocatechin-3-gallate *in vitro*. Biochem Biophys Res Comm, 270(3): 793-797 (2000).
4. Islam Najmul, Kanost Andrew R, Teixeira Luciella, Johnson John, Hejal Rana, Aung H, Wilkinson RJ, Hirsch CS, Toossi Z, Role of cellular activation and tumor necrosis factor-alpha in the early expression of Mycobacterium tuberculosis 85B mRNA in human alveolar macrophages. The Journal of infectious diseases, 190(2): 341-51 (2004).
5. Wilkinson RJ, DesJardin LE, Islam N, Gibson BM, Kanost RA, Wilkinson KA, Poelman D, Eisenach KD, Toossi Z, An increase in expression of a Mycobacterium tuberculosis mycolyl transferase gene (fbpB) occurs early after infection of human monocytes. Molecular microbiology, 39(3): 813-21 (2001).
6. Chattopadhyay I, Biswas K, Bandyopadhyay U, Banerjee RK, Turmeric and curcumin: Biological actions and medicinal applications. Current Science, 87: 44-53 (2004).
7. Verma SP, et al., The inhibition of the estrogenic effects of pesticides and environmental chemicals by curcumin and isoflavonoids. Environ Health Perpect, 106: 807-12 (1998).
8. Stoner GD, Mukhtar H, Polyphenols as cancer chemo-preventive agent. J Cell Biochem Suppl, 22:169-180 (1995).
9. Rao CV, Simi B, Reddy BS, Inhibition by dietary curcumin of azoxymethane-induced ornithine decarboxylase, tyrosineproteinkinase, arachidonic acid metabolism and aberrant cryptic loci formation in the rat colon. Carcinogenesis, 14: 2219-2225 (1993).
10. Mazumder A, Wang S, Neamati N, Nicklaus M, Sunder S, Chen J, *et al*; Antiretroviral agents as inhibitors of both human immunodeficiency virus type1integrase and protease, J Med Chem, 39: 2472-81 (1996).
11. Cohli HHP, Asad S, Das SK, Angel MF, Rao M, Effect of antioxidant (turmeric, turmerin and curcumin) on HIV. Int. J. Mol. Sci., 4: 22-33 (2003).
12. Ruby AJ, Kuttan G, Anti-tumour and antioxidant activity of natural curcuminoids. Cancer Lett, 94: 79-83 (1995).
13. Liu JZ, Lin RI. Milner JA, Inhibition of 7, 12-dimethylbenz (a)-anthracene induced mammary tumors and DNA adducts by garlic powder. Carcinogenesis, 13:1847-1851 (1992).
14. Sundaram SG, Milner JA, Impact of organosulfur compounds in garlic on canine mammary tumor cells in culture. Cancer Lett, 74:85-90 (1993).
15. Milner JA, Garlic: its anticarcinogenic and antitumorigenic properties. Nutr. Rev, 54: S82-S86 (1996).
16. Schaffer EM, Milner JA, Impact of dietary fatty acids on 7, 12-dimethylbenz[a] anthracene-induced mammary DNA adducts. Cancer Lett, 106: 177-183 (1996).

17. Moorhead PS, Nowell PC, Mellman WJ, Battips DM, Hungerford DA, Chromosomes preparations of leucocytes cultured from human peripheral blood. Exp Cell Res, 20: 613-616 (1960).
18. Benedict WF, Baker MS, Haroun L, Choi E, Ames BN, Mutagenicity of cancer chemotherapeutic agents in the *Salmonella*/microsome test. Cancer Research, 37: 2209-2213 (1977).
19. Bartolucci AA, Liu C, Durant JR, Gams RA, Acute myelogenous leukemia as a second malignant neoplasm following the successful treatment of advanced Hodgkin's disease. Cancer, 52: 2209-2213 (1983).
20. Bersagel DE, Bailey AJ, Langley GR, MacDonald RN, White DF, Miller AB, The chemotherapy of plasma-cell myeloma and the incidence of acute leukemia. New England Journal of Medicine, 301: 743-748 (1979).
21. Boice JD, Jr, Greene MH, Killen JY, Jr, Ellenberg SS, Keehn RJ, McFadden E, Chen T, Fraumeni JF, Jr, Leukemia and preleukemia after adjuvant treatment of gastrointestinal cancer with semustine (methyl-CCNU). New England Journal of Medicine, 309: 1079-1084 (1983).
22. Boivin JF, Hutchison GB, Lyden M, Godbold J, Chorosh J, Schottenfeld D, Second primary cancers following treatment of Hodgkin's disease. Journal of the National Cancer Institute, 67: 751-760 (1984).
23. Chak LY, Sikic BI, Tucker MA, Horns RC, Jr., Cox RS, Increased incidence of acute nonlymphocytic leukemia following therapy in patients with small cell carcinoma of the lung. Journal of Clinical Oncology, 2: 385-390 (1984).
24. Coleman MP, Bell CMJ, Fraser P, Second primary malignancy after Hodgkin's disease, ovarian cancer and cancer of the testis: A population-based cohort study. British Journal of Cancer, 56: 349-355 (1987).
25. Curtis RE, Hankey BF, Myers MH, Young JL, Jr., Risk of leukemia associated with the first course of cancer treatment: An analysis of the Surveillance, Epidemiology, and End Results Program Experience. Journal of the National Cancer Institute, 72: 531-544 (1984).
26. Devereux S, Selassie TG, Hudson GV, Hudson BV, Linch DC, Leukemia complicating treatment for Hodgkin's disease: The experience of the British National Lymphoma Investigation, British Medical Journal, 301: 1077-1080 (1990).
27. Haas JF, Kittelmann B, Mehnert WH, Staneczek W, Mohner M, Kaldor JM, Day N, Risk of leukemia in ovarian tumour and breast cancer patients following treatment by cyclophosphamide. British Journal of Cancer, 55: 213-218 (1987).
28. Hancock SL, Hoppe RT, Horning SJ, Rosenberg SA, Intercurrent death after Hodgkin disease therapy in radiotherapy and adjuvant MOPP trials. Annals of Internal Medicine, 109: 183-189 (1988).
29. Pedersen-Bjergaard J, Ersboll J, Sorensen M, Keiding N, Larsen SO, Philip P, Larsen MS, Schultz H, Nissen NI, Risk of acute nonlymphocytic leukemia and preleukemia in patients treated with cyclophosphamide for non-Hodgkin's lymphomas. Annals of Internal Medicine, 103: 195-200 (1985a).
30. Pedersen-Bjergaard J, Osterlin K, Hansen M, Philip P, Pedersen AG, Hansen HH, Acute nonlymphocytic leukemia, preleukemia, and solid tumors following intensive chemotherapy of small cell carcinoma of the lung. Blood, p 66: 1393-1397 (1985b).
31. Pedersen-Bjergaard J, Philip P, Larsen SO, Andersson M, Daugaard G, Ersboll J, Hansen SW, Hou-Jensen K, Nielsen D, Sigsgaard TC, Specht L, Osterlind K, Therapy-related myelodysplasia and acute myeloid leukemia. Cytogentic characteristics of 115 consecutive cases and risk in seven cohorts of patients treated intensively for malignant diseases in the Copenhagen Series. Leukemia, 7: 1975-1986 (1993).

32. Pedersen-Bjergaard J, Larsen SO, Incidence of acute nonlymphocytic leukemia, preleukemia, and acute myeloproliferative syndrome up to 10 years after treatment of Hodgkin's Disease. New England Journal of Medicine, 307: 965-971 (1982).
33. Tester WJ, Kinsella TJ, Waller B, Makuch RW, Kelley P, Glatstein E, DeVita VT, Second malignant neoplasms complicating Hodgkin's disease: The National Cancer Institute experience. Journal of Clinical Oncology, 2: 762-9 (1984).
34. Tucker MA, Coleman CN, Cox RS, Varghese A, Rosenberg SA, Risk of second cancers after treatment for Hodgkin's disease. New England Journal of Medicine, 318: 76-81 (1988).
35. Baker GL, Kahl LE, Zee BC, Stolzer BL, Agarwal AK, Medsger TA, Jr., Malignancy following treatment of rheumatoid arthritis with cyclophosphamide. American Journal of Medicine, 83(1): 1-9 (1987).
36. Dale GA, Smith RB, Transitional cell carcinoma of the bladder associated with cyclophosphamide. Journal of Urology, 112: 603-604 (1974).
37. Elliott RW, Essenigh DM, Morley AR, Cyclophosphamide treatment of systemic lupus erythematosus--Risk of bladder cancer exceeds benefits. British Journal of Medicine, 284: 1160-1161 (1982).
38. Fairchild WV, Spence CR, Solomon HD, Gangai MP, The incidence of bladder cancer after cyclophosphamide therapy. Journal of Urology, 122: 163-4 (1979).
39. Kinlen LJ, Incidence of cancer in rheumatoid arthritis and other disorders after immunosuppressive treatment. American Journal of Medicine, 78(Suppl1A): 44-49 (1985).
40. Pearson RM, Soloway MS, Does cyclophosphamide induce bladder cancer? Urology, 11: 437-47 (1978).
41. Plotz PH, Klippel JH, Decker JL, Grauman D, Wolff B, Brown BC, Rutt G, Bladder complications in patients receiving cyclophosphamide for systemic lupus erythematosus or rheumatoid arthritis. Annals of Internal Medicine, 91: 221-223 (1979).
42. Wall RL, Clausen KP, Carcinoma of the urinary bladder in patients receiving cyclophosphamide. New England Journal of Medicine, 293: 271-273 (1975).
43. Buckner JC, Edmonson JH, Ingle JN, Schaid DJ, Evaluation of menogaril in patients with metastatic sarcomas and no prior chemotherapy exposure. American Journal of Clinical Oncology, 12(5): 384-386 (1989).
44. D'Alessandro N, Gebbia N, Crescimanno M, Flandina C, Leto G, Tumminello FM, Messina L, Effects of Amsacrine (*m*-AMSA), a new aminoacridine antitumor drug, on the rabbit heart. Cancer Treatment Reports, 67: 467-474 (1983).
Coleman RE, Maisey MN, Knight RK, Rubens RD, Mitoxantrone in advanced breast cancer--a phase II study with special attention to cardiotoxicity. European Journal of Cancer and Clinical Oncology, 20: 771-776 (1984).
45. Drago JR, Goldman LB, Evaluation on nonhormonal cytotoxic chemotherapy in the Nb Rat (Pr-90) prostatic carcinoma. Cancer, 45: 757-763 (1980).
46. Henderson BM, Dougherty WJ, James VC, Tilley LP, Noble JF, Safety assessment of a new anticancer compound, Mitoxantrone, in beagle dogs: Comparison with doxorubicin. I. Clinical observations. Cancer Treatment Reports, 66: 1139-1143 (1982).
47. Kimler BF, Mansfield CM, Svoboda DJ, Cox GG, Ultrastructural evidence of cardiac damage resulting from thoracic irradiation and anthracyclines in the rat; International Journal of Radiation Oncology, Biology and Physics, 10: 1465-1469 (1984).
48. Kojima S, Hzuka H, Yamaguchi H, Tanuma SI, Kochi M, Ueno Y, Antioxidative activity of benzylideneascorbate and its effect on Adriamycin-induced cardiotoxicity.

Anticancer Research, 14: 1875-1880 (1994).
49. Kris MG, Gralla RJ, Kelsen DP, Casper ES, Phase II trial of oral 4-demothoxydaunorubicin in patients with non-small cell lung cancer. American Journal of Clinical Oncology, 8: 377-379 (1985).
50. Lindpaintner K, Lindpaintner LS, Wentworth M, Burns CP, Acute myocardial necrosis during administration of amsacrine. Cancer, 57: 1284-1286 (1986).
51. Martoni A, Pacciarini MA, Pannuti F, Activity of 4-demethoxydaunorubicin by the oral route in advanced breast cancer. European Journal of Cancer and Clinical Oncology, 21: 803-806 (1985).
52. McCarthy GM, Skillings JR, Orofacial complications of chemotherapy for breast cancer. Oral Surgery Oral Medicine and Oral Pathology, 74: 172-178 (1992).
53. Shaw NJ, Tweedale PM, Eden OB, Pulmonary function in childhood leukemia survivors. Medical and Pediatric Oncology, 17:149-154 (1989).
54. Shinar E, Hasin Y, Acute electrocardiographic changes induced by amsacrine. Cancer Treatment Reports, 68: 1169-1172 (1984).
55. Tamatsu H, Nakazawa M, Imai S, Watari H, ^{31}P-topical Nuclear magnetic resonance (^{31}P-TMR) studies of cardiotoxic effects of 5-fluorouracil (5-FU) and 5'-deoxy-5-fluorouridine (5'-DFUR). Japanese Journal of Pharmacology. 34: 375-379 (1984).
56. Tan CTC, Hancock C, Steinherz P, Bacha DM, Steinherz L, Luks E, Winick N, Meyers P, Mondora A, Dantis E, Niedzwiecki D, Stevens YW, Phase I and clinical phamacological study of 4-demthoxydaunorubicin (Idarubicin) in children with advanced cancer. Cancer Research, 47: 2990-2995 (1987).
57. Unverferth DV, Unverferth BJ, Balcerzak SP, Bashore TA, Niedhart JA, Cardiac evaluation of mitoxantrone. Cancer Treatment Reports, 67: 343-350 (1983).
58. Vorobiof DA, Iturralde M, Falkson G, Amsacrine cardiotoxicity: Assessment of ventricular function by radionuclide angiography. Cancer Treatment Reports. 67: 1115-1117 (1983).
59. Satoskar RR, Shah SJ, Shenoy SG, Evaluation of anti-inflammatory property of curcumin (diferuloylmethane) in patients with post-operative inflammation. Int. J. Clin. Pharmacol, 24: 651-654 (1986).
60. Brouet I, Ohshima ZH, Curcumin, an anti-tumor promoter and anti inflammatory agent, inhibits induction of nitric oxide synthase in activated macrophages. Biochem Biophys Res Commun, 206: 533-540 (1995).
61. Huang MT, Wang ZY, Georgiadis CA, Laskin JD, Conney AH, Inhibitory effects of curcumin on tumor initiation by benzo(a)pyrene and 7,12-dimethylbenz(a)anthracene. Carcinogenesis, 13: 2183-2186 (1992).
62. Donatus IA, Sardjoko A, Vermenlen NPE, Cytotoxic and cytoprotective activities of curcumin: effects onparacetamol-induced cytotoxicity, lipid peroxidation and glutathione depletion in rat hepatocytes. Biochem. Pharmacol, 39: 1869-1875 (1990).
63. Unnikrishnan MK, Rao MNA, Curcumin inhibits nitrite-induced methamoglobin formation. FEBS Lett, 301: 195-196 (1992).
64. Rajakumar DV, Rao MNA, Antioxidant properties of dehydrozingerone and curcumin in rat brain homogenates. Mol. Cell. Biochem, 140: 73-79 (1994).
65. Shankar TNB, Shanta NV, Ramesh HP, Murthy IAS, Murthy VS, Toxicity studies on turmeric *Curcuma longa*; acute toxicity studies in rats, guinea pigs and monkeys. Ind J Exp Biol, 18: 73-75 (1980).
66. Soni KB, Kuttan R, Effect of oral curcumin administration on serum peroxides and cholesterol levels in human volunteers. Ind J Physiol Pharmacol, 36: 273-275 (1992).

67. Deshpande SS, Maru GB, Effects of curcumin on the formation of benzo(x) pyrene derived DNA adducts *in vitro*. Cancer Lett, 96: 71-80 (1995).
68. Mukundan MA, Chaco MC, Annapurna VV, Krishnaswamy K, Effects of turmeric and curcumin on BP-DNA adduct. Carcinogenesis, 14: 493-496 (1993).
69. Subramanian M, Sreejayan N, Rao MNA, Devasagayam TPA, Singh BB, Minimization of singlet oxygen induced DNA damage by curcumin and related antioxidants. Mutat Res, 311: 249- 255 (1994).
70. Tonnesen HH, Greenhill JV, Studies on curcumin and curcuminoids. XXII. Curcumin as a reducing agent and as a radical scavenger. Int J Phar. 87: 79-87 (1992).
71. Chan MM, Inhibition of tumor necrosis factor by curcumin, a phytochemical. Biochem. Pharmacol, 26: 1551-1558 (1995).