EVALUATION OF COMBINED HERBALEXTRACTOF *Withania somnifera*, *Ocimum sanctum* AND *Tinospora cordifolia* AS AN CHEMOPROTECTIVEIN CANCER

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Summary

Chemoprotective effect of herbal extract

To study the chemo protective effect of combined herbal extract of *Withania somnifera*, *Ocimum sanctum* and *Tinospora cordifolia* in the chemotherapy of cancer.
The drugs were extracted with 50% ethanol. The equal proportion of these three drugs was mixed to get a homogenous mixture. This ethanolic extract was treated on the blood sample (taken from a healthy volunteer). In-vitro cytogenic analysis was performed by arresting metaphase using colchicine.
The chromosomal aberrations were observed under the microscope. The types of aberrations were found out and their percentage was calculated.
It was concluded that combination of plant extract of *Ocimum sanctum, Withania somnifera* and *Tinospora cordifolia* when given along with chemotherapy, it showed chemoprotective properties via decrease in chromosomal aberrations impressively.

Key words: *Ocimum sanctum, Withania somnifera* and *Tinospora cordifolia,* Chemoprotective effect, Cancerous patient and Cytogenic analysis.

Introduction:

Many human malignant tumors exhibit abnormal chromosomal segregation at cell division. It is believed that these anomalies play a role in tumorigenesis by increasing the rate of chromosome mutations, including deletion and amplification of genes involved in cellular proliferation and/or survival. *In vitro* experiments have also shown that mitotic instability may be a mechanism for developing resistance to cytotoxic drugs. Abnormal mitotic mechanisms may result in numerical or structural aberrations in the daughter cells. Numerical aberrations can be caused either by the loss of chromosomes at metaphase/anaphase or by multipolar divisions associated with abnormal number or structure of centrosomes. Structural rearrangements have been associated with chromosomal breakage-fusion-bridge (BFB) cycles that can be initiated by telomeric dysfunction, giving rise to unstable dicentric or ring chromosomes.

At progression towards higher malignancy in these tumours, complex structural and numerical aberrations become more frequent. This may be explained by a process initiated by telomeric dysfunction and anaphase bridging, which, in turn, may give rise to an increased frequency of multinucleated cells through failure of cytokinesis. These cells will contain an abnormal number of centrosomes leading to multipolar mitotic figures at the next cell division.

The frequency of cells with structural chromosomal aberrations (CAs) in peripheral blood lymphocytes is the first genotoxicity biomarker that has shown an association with cancer risk. CAs is usually divided into chromosome-type (CSAs) and chromatid-type aberrations (CTAs), with different mechanisms of formation[1].

Chemotherapy has been in use for decades to cure the cancer[2]. But during the course of therapy patient suffers a number of side-effect. The major cause of these side effects of this chemotherapeutic agent is chromosomal aberrations in healthy cells which lead to genotoxicity[3]. A successful anticancer drug should kill or incapacitate cancer cells without causing damage to healthy cell. So the motto of this research work is to reduce this extent of chromosomal aberrations and provide chemo protective role in chemotherapy of cancer. Recently investigations are going on for screening of compounds derived from plant origin that can be used as an adjuvant to chemotherapy having better chemo protective profile

As the literature survey reveals that these individual plants have potent antioxidant properties, which in turn increases the life of cell. By taking this view in mind, chemoprotective behavior of herbal extract were analyzed in chemotherapy of cancer.

So the literature survey was done on plant extract having chemoprotevtive behavior. This suggested that *Ocimum sanctum* [4-12], *Withania somnifera* [13-18] and *Tinospora cordifolia* [19-22] have potent chemoprotective properties individually. Finally these three plants extracts were selected and their combined effect was taken to investigate their chmoprotective role in chemotherapy of cancer.

Material and Methods:

For the present research work aseptic condition was a prerequisite for a tissue culture laboratory. So the culture room was regularly fumigated once in a month with 3% potassium permagnate in 1:1 N-butanol:formic acid. All the equipments and glasswares were sterilized before and after use.

Plant of *W. somnifera, O.samctum* and *T. cordifolia* were collected dried, powdered and extracted with 50% hot ethanol using the soxhlet apparatus for about 48 hours. Ethanolic extract was filtered through whatmann filter paper no.1. The filtrate was dried in vacuum distillation and than in desiccators. An equal amount of

W. somnifera, O.samctum and *T. cordifolia* extract were weighed and mixed and stored.

Chromosomal aberration studies in human blood sample:

5-10 ml of venous blood from healthy volunteer (JLNCRH, Bhopal, India) was drawn with a sterile disposable needle (1.10x 38mm) and syringe (10ml) aseptically in to a sterile Bisou bottle containg 30 units of heparin (1000IU/MI).

The blood was allowed to settle by gravity sedimentation. Roswell Park Memorila Institute, USA (RPMI) 1640 (Himedia, Bombay) was prepared and sterilized and transferred in to the cultured bottle. Phytohaemagglutin (PHA) was added in sufficient quantity along with media into the autoclaved bottles.

Five sample bottles were prepared for analysis which contains:

a. Blood sample
b. Blood sample + plant extract
c. Blood sample + chemotherapy
d. Blood sample + plant extract + chemotherapy (after 1 hr)
e. Blood sample + chemotherapy + drug (after 1 hr)

They were kept for seventy two hours for developing of culture in carbon di-oxide (CO_2) incubator. In seventy two hr colchicines was added in each bottle to arrest the metaphase and again kept in incubator for two to three hours. Finally blood samples were taken out and transferred in to separate test tubes. Blood samples were centrifuged at 500 rpm/10 min. Cells were settled down in tubes and supernatant was discarded and pellets were fixed in freshly prepared chilled cornoys fixative for 30 min (methanol and acetic acid). After fixation cells were centrifuged and the supernatant was discarded. Fresh cornoys fixative was added and the process was repeated accordingly to debris in the cell sample so as to get clear solution. Finally the cell suspension was dropped on clean, pre chilled slides using Pasteur pipettes, from a height of 6 inches. The excess of fixatives was allowed to run off by fitting the slides which were gently heated to help in the spreading of chromosomes and dried at 40-60 ^{0}C. The slides were stained with Giemsa (0.1% Qualigens) for 10-15 min and rinsed in tap water. The slides were dried and mounted in DPX and observed under light microscope. For these 6 slides of different group were selected. The remainders were stored at -10^{o}C. The aberrations scored were breaks, (chromatid breaks and chromosomes breaks), asymmetrical exchanges (dicentrics, rings and fragments), severally damaged cells, polyploidy and anaphase aberrations. 100 metaphaes per groups were counted and percentage of chromosomal aberrations was calculated.

Result and discussion

Natural compounds including flavonoids and alkaloids may play major role in scavenging free radicals, such as hydroxyl radical generated by chemotherapeutic in cells. Chemotherapy generates free radical damage DNA and induced genotoxic effects and event cell death there is possibility that pre treatment with flavonoids and alkaloids can induce protection against oxidative stress. The herbal extract selected for the present work contains a large number of flavonoids and alkaloids and other active compounds in a good concentration.

The present research work explained the mutagenic behavior of Cyclophosphamide and cellular chemo protective role of herbal extracts. In-vitro lymphocyte has been analyzed and 100 metaphase counted per group. In group one where there is only blood as a sample (control) 98 metaphases are normal and aberrant metaphase are 2. In second group (plant extract + blood sample) the number normal metaphses are 97 and abrrated were 3. In third group, cyclophosphamide has been administered with dose of 200 mg/body surface with blood sample. The results showed 66 normal metaphases and 34 aberrant chromosomes.

In fourth group when the combined herbal extract was given 1 hr before the chemotherapy the results were found to be out standing as normal metaphase were 92 and 8 were aberrant. In last group where plant extracts were given after one hr of cyclophosmamide, the results were as 80 and 20 normal and aberrant metaphases respectively. The results showed in fourth group that the number of abbarant metaphases reduces from 34 to 8 and in group fifth it reduces from 34 to 20. Overall it was concluded that chemoprotective treatment is more beneficial when given 1 hour before the chemotherapy.The greatest chemoprotective effect was observed in group two. It directly indicates that combined plant extract have potent chemoprotective effect. It also represents that there are 34% aberrant chromosomes in the group treated with cyclophosmamide alone. It was noticed that double minutes and dicentric morphology has been increased due to chemotherapy drug alone. When herbal extract of combine drug administered as an adjuvant has shown chemo protective properties, dicentric and double minutes almost get negligible. (Graph: 1 and Table: 1). The results of this study demonstrated the chemo protective effects of 50% ethanolic extract of combined plant drugs against genotoxicity and toxicity induced by chemotherapy by in-vitro method in human peripheral lymphocyte culture.

It was notice that double minutes and dicentric morphology has been increased due to chemotherapy drug alone. When herbal extract of combine drug administered as an adjuvant has shown chemo preventive properties, dicentric and double minutes almost get negligible. Flavonoids and alkaloids and other active compounds which are present in herbal extract had shown excellent scavenging property to scavenge free radicals due to high reactivity of hydroxyl substituents. Hence it is possible that the extract may protect chromosomal and genomic damages with its antioxidant property. Never the less extract may have certain different other mechanism probably to contribute its chemo preventive effects. So it was concluded that combination of crude extract of *Ocimum sanctum, Withania somnifera* and *Tinospora cordifolia* has been fruitfully presented as chemo preventive as supported by chromosomal morphology. Further study is essential with reference to different doses to understand the better chemo preventive effect of combined extract.

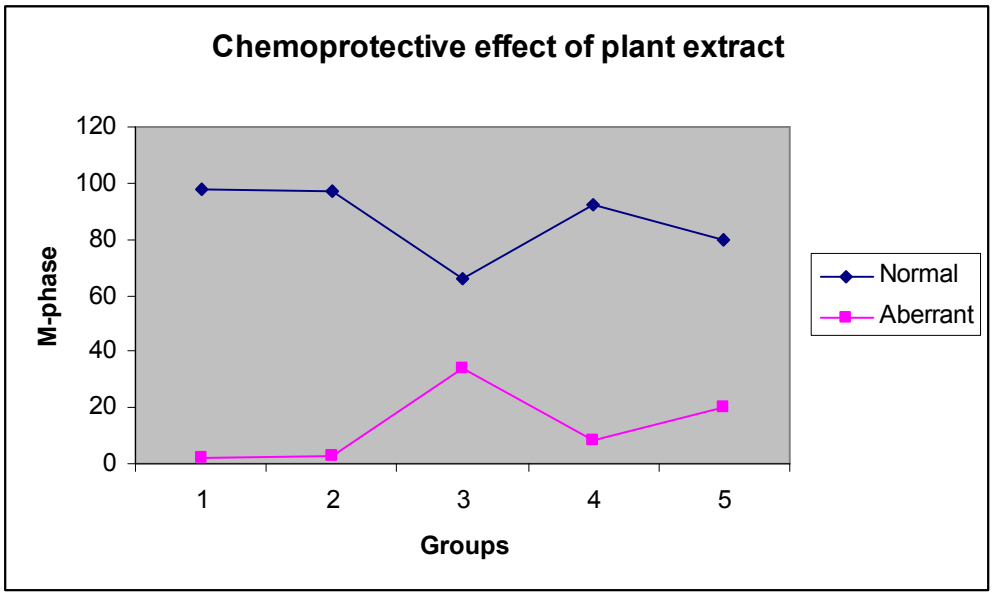

Fig: 1 Chemoprotective effect of plant extract: Normal M-phase Vs Aberrant M-phase

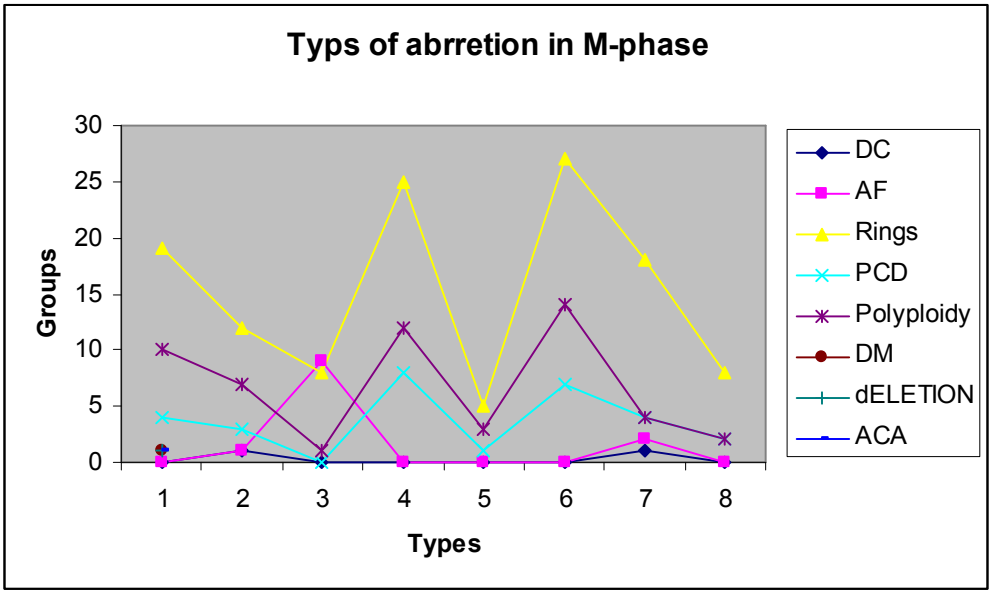

Fig: 1 Number of typs of Aberration in M-phase

Table l: Chemoprotective effect of plant extract: Normal M-phase Vs Aberrant M-phase

Group	Total M-phase	Normal M-phage	Aberrant M-phase
Control	100	98	2
Extract control	100	97	3
Chemo T control	100	66	34
Extract than Chemo T	100	92	8
Chemo T then Extract	100	80	20

Table 2: Number of typs of Aberration in M-phase

Types of aberrations							
DC	AF	Rings	PCD	Polyploidy	DM	Deletion	ACA
0	1	0	0	0	0	1	0
0	1	9	0	0	0	2	0
19	12	8	25	5	27	18	8
4	3	0	8	1	7	4	2
10	7	1	12	3	14	4	2

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