Cytologia **51**: 375–380, 1986

Antagonistic Activity of Herbal Drug (*Phyllanthus emblica*) on Cytological Effects of Environmental Chemicals on Mammalian Cells

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Accepted March 22, 1985

With increasing industrialisation there is generally a corresponding increase in environmental pollution. Numerous publications are available on the mutagenic effects of different environmental agents (Sugimura *et al.* 1981). Mammalian tissues form the most widely used test system both *in vivo* and *in vitro* (Goyer and Mehlman 1977, Venugopal and Luckey 1978, Giri *et al.* 1978, 1980, 1983, Hsu 1982).

A variety of compounds have been identified as inhibitor of chemical carcinogenesis in animal models. These include synthetic and naturally occurring compounds namely butylated hydroxytoluene (Weisberger *et al.* 1977), ethoxyquin (Wattenberg 1972), selenium (Jacobs *et al.* 1977), vitamin E (Cook and Mc Namara 1980) and vitamin C (Brin 1982); acting through different mechanisms (Fiala *et al.* 1977, Wattenberg 1979). The evidences of a strong association between carcinogenicity and mutagenicity suggest that these inhibitors may act on mutagens as well (Kawachi *et al.* 1979).

The fruit of *Phyllanthus emblica* Linn., a rich source of vitamin C (commonly known as 'Amla' in India) is used in many medicinal preparations of the indigenous 'Ayurvedic' and 'Unani' systems of medicine. The dried fruit in combination with iron is used for anaemia and jaundice (Kirtikar and Basu 1975). In 'Harit Sanghita' (an Ayurvedic treatise) it was referred as inhibiting ulceration (Gupta 1908). It has strong antibacterial, antifungal as well as antioxidative properties (Rao and Siddique 1964). The fruit has been successfully used in the treatment of human scurvy (Chopra *et al.* 1956).

Vitamin C has been shown to antagonise the toxic activity of certain metallic salts in mammalian systems (Chakraborty *et al.* 1977). However, no information is available on the property of vitamin C to reduce clastogenic action of genotoxic agents. The present investigation was undertaken to compare the activity of the extract of *Phyllanthus emblica* fruit and equivalent amount of synthetic ascorbic acid in counteracting the toxicity of three known genotoxic agents of three different groups i.e. zinc chloride (metallic salts), ethyl parathion (insecticide) and metanil yellow (food additives) (Giri *et al.* 1981, 1984a, b).

Materials and methods

a) Decoction of the dried fruit

24 gms of dried *Phyllanthus emblica* (purchased from the local market) cut in small bits were soaked overnight in one litre of boiled water. The decoction was filtered and the filtrate concentrated in a earthenware pot under low flame to 50 ml. The vitamin C content of the extract was determined (0.4 mg/ml) by 2,6-dichloro-indophenol method (Pearson 1976).

b) Male mice (*Mus musculus*) of a laboratory bred strain were treated in groups of five with a series of oral doses. Metanil yellow was dissolved in distilled water and administered by gavage in a daily dose of 2 mg/kg body weight given at a fixed time each day for 30 successive days. Two similar sets of eaeh of five mice were gavaged, in addition to metanil yellow as above, simultaneously with vitamin C (2 mg/kg) and with decoction of *Phyllanthus emblica* Linn. (5 ml/kg).

Aqueous zinc chloride was gavaged to five mice at the rate of 16.5 mg/kg (1/20 LD_{50}) and ethyl parathion dissolved in olive oil to another set at the rate of 1 mg/kg for 21 days. Combination of both the chemicals with vitamin C and fruit extract respectively were gavaged in the similar manner for 21 days as described earlier. Controls were fed with 0.05 ml of distilled water and 0.05 ml of olive oil as the cases are. The animals were sacrificed at the interval of 24 hours of receiving the final (30th or 21st) dose. Each experiment was carried out in duplicate. Bone marrow preparations were made following the usual colchicine-hypotonic flame drying Giemsa procedure (Sharma and Sharma 1980). The mitotic indices were obtained from scanning 2000 cells from each animal of each set and compared with the control. From the five animals in each set 300 metaphases were scanned. The results of the duplicate experiments were pooled, and were analysed by student's *t*-test.

Observations

The chronic study of the action of metanil yellow showed a significant decrease in the mitotic index in the series treated with only metanil yellow and increased in that with metanil yellow plus vitamin C and plus the extract (Tables 1, 2). A significant decrease in the mitotic index was observed in zinc chloride when compared with control (Table 1). Mitotic index in vitamin C treated series was increased when compare to only zinc chloride treated series (Table 2). In parathion treated series no changes were observed in the mitotic index compared to controls.

Chromosomal abnormalities were classified into three groups (Giri *et al.* 1984a, c, Banerjee *et al.* 1984).

Group I. a disturbance in spindle formation, indicated by stickiness, C-mitosis and diplochromosomes in the late metaphase;

Group II. chromosomal abonormalities (breaks, gaps, deletion and others) but only one or two per cell;

Group III. gross abnormalities, in which a number of chromosomal abnormalities could be observed in the same metaphase plate together with spindle disturbances.

Chronic treatment with vitamin C and fruit extract antagonised the effects of

the chemicals examined to a significant extent in bone marrow chromosomes of mice *in vivo*. Metanil yellow treated series gave a significant increase in the groups I and III abnormalities (Table 1). The vitamin C or the extract could not alter the group I type aberrations but showed a significant decrease in group III type (Table 2).

Zinc chloride treated series showed a significant increase in abnormalities for all the three groups (Table 1). Vitamin C and the extract reduced the chromosomal abnormalities of the groups II and III (Table 2).

Table 1. Percentage of mitotic index and chromosomal abnormalities induced by different chemicals and their comparison with control

	Control	Metanil yellow	Zinc chloride	Ethyl parathion
Normal metaphase percentage	90.67	67.97	63.08	54.00
Mitotic index	1.42 ±0.15	0.61 ±0.25****	0.76 ±0.13****	1.53 ±0.23
Group I Abnormalities	3.68±1.24	11.43 ±1.86****	14.22 ±1.76****	21.05 ±2.42****
Group II Abnormalities	2.50±1.20	4.56 ±2.01	7.02 ±1.83***	9.25 ±1.29****
Group III Abnormalities	3.15±0.92	18.04± ±4.90****	15.68 ±1.62****	15.70 ±2.61****

Figures are the means±S.D. and are the results of two sets of experiments in which 300 metaphases from each set of five mice in each test and control group were scanned.
Degrees of freedom=8 *** $P<0.01$; **** $P<0.001$

A remarkable change was observed in ethyl parathion treated series where a decrease in all the three groups was found.

Discussion

The reason of selecting the three different types of ehemicals was to find out whether the extract of *P. emblica* L. has any effect on all the three different chemicals. Although metanil yellow is a non-permitted dye it is included because it is often used in food in India (Khanna *et al.* 1973). It is interesting to note that it minimizes the mitostatic properties of metanil yellow but with zinc chloride and ethyl parathion it has no remarkable effect on the mitotic index. Regarding chromosomal abnormalities it has no effect on the group I abnormalities, i.e., on the spindle apparatus, but has strong effect on minimizing the structural abnormalities of metanil yellow and zinc chloride. On the other hand, it minimizes both spindle disturbances and structural abnormalities in case of ethyl parathion. The anticlastogenic effects of the extract and vitamin C are almost same in case of metanil yellow and zinc chloride but it is significantly more effective in case of ethyl parathion (Table 2). The greater efficacy of the herbal extract may be attributed to other ingredients present in the extract, which can act directly or synergistically in reducing the genotoxic action of the different chemicals. It was observed that the extract acts more rapidly on

Table 2. Percentage of mitotic index and chromosomal abnormalities induced by chemicals plus Vitamin C and chemicals plus extract and their comparison with chemicals alone

	Metanil yellow	Metanil yellow+ Vit. C	Metanil yellow+ extract	Zinc chloride	Zinc chloride +Vit. C	Zinc chloride +extract	Ethyl parathion	Ethyl parathion +Vit. C	Ethyl parathion +extract
Mitotic index	0.61 ±0.25	1.52 ±0.15****	1.75 ±0.20****	0.76 ±0.13	1.26 ±0.14***	0.71 ±0.15	1.53 ±0.23	1.55 ±0.18	1.45 ±0.14
Group I Abnormalities	11.43 ±1.86	9.07 ±1.36	9.87 ±1.74	14.22 ±1.76	12.98 ±2.74	12.74 ±1.69	21.05 ±2.42	15.66 ±2.02***	10.52 ±1.47****
Group II Abnormalities	4.56 ±2.01	3.53 ±1.25	4.03 ±1.30	7.02 ±1.83	4.56 ±0.84*	4.38 ±1.31*	9.25 ±1.29	6.43 ±1.43**	3.55 ±2.31***
Group III Abnormalities	18.04 ±4.90	9.43 ±1.05***	8.91 ±2.04***	15.68 ±1.62	10.65 ±2.15***	11.02 ±1.63***	15.70 ±2.61	11.33 ±1.68**	8.49 ±1.65****

Figures are the means±S.D. and are the results of two sets of experiments in which 300 metaphases from each set of five mice in each test and control group were scanned.

Degrees of freedom=8 *$P<0.05$; **$P<0.02$; ***$P<0.01$; ****$P<0.001$.

patients suffering from pulmonary tuberculosis than the vitamin C alone (The Wealth of India, 1952). Chromosome aberrations and breakages have been in many instances related to carcinogenic risk to human (Purchase *et al.* 1978, Cairns 1981). The herbal extract is reported to have a strong anticarcinogenic nature. Therefore, it should be assumed that this extract is effective as a therapeutic drug of cancer.

Summary

Phyllanthus emblica Linn., a rich source of vitamin C, has been shown to reduce the clastogenic and mitostatic activities of different known mutagens. It reduces the chromosomal abnormalities induced by metanil yellow and zinc chloride. Further, it antagonises both the spindle disturbance and chromosomal abnormalities caused by ethyl parathion. The antagonistic action of the extract is greater than the dose of synthetic vitamin C equivalent in amount of vitamin C present in the extract. This is presumably due to the presence of the other natural ingredients in the herbal extract.

Acknowledgements

The authors are grateful to Prof. A. K. Sharma, Programme Co-ordinator, Centre for Advanced Study in Cell and Chromosome Research, Department of Botany, University of Calcutta, for encouragement and facilities provided and Prof. (Mrs.) Archana Sharma and Dr. (Mrs.) Geeta Talukder for their guidance and help.

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