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Genotoxic Effects of Crude Extract of Neem (*Azadirachta indica*) in Bone Marrow Cells of Mice

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Chemicals from natural source, i.e., from the plants specially from neem, *Azadirachta indica*; family Meliaceae, act as antifeedant (Mathur and Nigam 1993), anti-attractant or repellent (Sharma *et al.* 1993a, b), anti-ecdysone (Warbrick *et al.* 1993), oviposition deterrent (Yadav 1993) and sterilant (Schmidt and Pesel 1987) in different genera of insects and pests. In contrast to the synthetic pesticides they are considered more safe and ecofriendly because they have no associated problems of resistance (Rice 1993) and resurgence and are non-pollutant also (Johri *et al.* 1993).

The products of neem in different formulations are used not only for integrated insect-pest management (Dhaliwal *et al.* 1993) but also to cure various diseases in human beings like viral (see Rawat 1994 for review), microbial, malarial, pyretic, ulcer (Van Der Nat *et al.* 1991), helminthiasis (Saxena *et al.* 1993), inflammatory (Wali *et al.* 1993) and possibly immune-deficiency syndrome (Upadhyay *et al.* 1993). Several antifertility drugs (Upadhyay *et al.* 1993) and contraceptives (Riar *et al.* 1993, Paranjape *et al.* 1993) have also recently been prepared from neem.

A large section of human beings is thus constantly exposed to the neem-based products. On the lines of residue toxicity estimation of synthetic pesticides, it is highly desirable to test the toxicity of neem extract in non-target organisms. Among the various toxic effects of these products, the histopathological changes (Badri *et al.* 1993) have been reported very widely, but their cytogenetic toxicity is poorly known (Abraham *et al.* 1993, Awasthy and Chaurasia 1994). The present work is an attempt to fill in this gap in the information and has been made to assess genotoxic effects, if any, of crude leaf extract of neem in laboratory mice, *Mus musculus*.

Materials and methods

The soxhlated extract (80% v/v ethanol) from oven dried (at 60°C) leaves of mature neem plant was further dried in vacuum-rotatory evaporator. The latex form of the extract so obtained was mixed with the equal amount (by weight) of gum-acacia (distilled water dissolved, Loyds Pharma, Delhi) for having a homogeneous suspension of the former. This suspension was fed orally at the rate of 0.5 or 1.0 or 2.0 g/kg body weight (bw)/day to three different groups of animals for seven consecutive days. The control group of animals received only the gum-acacia solution in the same volume. There were 10 mice in each group—control as well as treated.

The animals were sacrificed on the eighth day and slides were prepared by following the standard technique of Preston *et al.* (1987). The cytogenetic effect was assessed in mitotically dividing bone marrow cells by randomly screening 300 Giemsa-stained well-spread metaphase plates selected at the rate of 30 metaphases/animal in each group.

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Results and discussion

The vehicle of the leaf extract, gum-acacia, was not genotoxic. However, the leaf extract did induce both gross and individual types of abnormalities. Aneuploidy, polyploidy and c-mitosis were most common among the gross type, while chromatid breaks and gaps, acentric

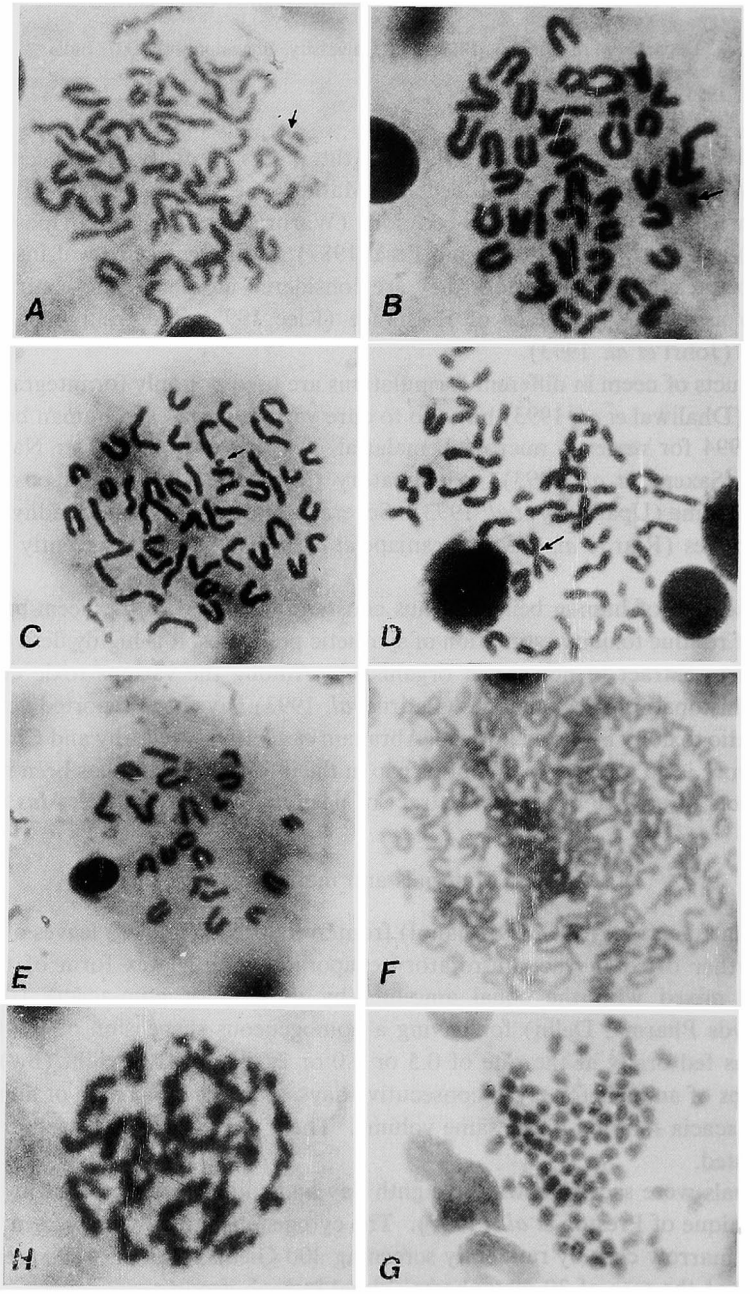

Fig. 1. Chromosomal abnormalities in bone marrow cells of albino Swiss mice treated with neem-extract. A, chromatid break with acentric fragment. B, acentric fragment. C, minute fragment. D, metacentric chromosome. E, aneuploidy. F, polyploidy. G, C-mitosis. H, stickiness.

Table 1. Number of abnormal cells and the frequencies of different types of abnormalities scored in the bone marrow cells of mice fed with leaf extract of neem (*A. indica*)

Amount†	Abnormal cells‡		Individual type abnormality								Gross type abnormality									Grand Total	
	No.	%±SE	Ctb	Ictb	Ctg	Del	Acf	Hypc.	Total No.	Total %±SE	Aneu	Hyp	Poly	CF	c-mit	Pcs	St	Total No.	Total %±SE	No.	%±SE
Cont. A	14	4.6±1.2	3	0	2	0	3	0	8	2.6±0.9	0	1	1	0	0	0	4	6	2.0±0.8	14	4.6±1.2
Cont. B	13	4.3±1.2	6	0	1	0	1	0	8	2.6±0.9	2	0	2	0	1	0	0	5	1.6±0.7	13	4.3±1.2
0.5 gm	35	11.6±1.8*	3	0	4	0	1	0	8	2.6±0.9	15	3	2	5	2	2	0	29	9.6±1.7*	37	12.3±1.8*
1.0 gm	42	14.0±1.9*	7	0	1	1	2	1	12	4.0±1.1	14	4	4	6	4	0	1	33	11.0±1.8*	45	15.0±2.1*
2.0 gm	51	17.0±2.2*	16	2	6	1	2	0	27	9.0±1.7*	8	3	6	6	10	2	0	35	11.6±1.8*	62	20.6±2.3*

Cont. A (Control A)=No treatment. Cont. B (Control B)=Gum-acacia only. †=gm/kg b.wt. of extract administered per animal (10 animals in each group). ‡=total no. of metaphases screened in each group=300. *=Indicates significant difference (at 0.1% level) with the corresponding value in the control. Key to abbreviations: Ctb=Chromatid break; Ictb=Isochromatid break; Ctg=Chromatid gap; Del=Deletion; Acf=Acentric fragments; Hypc=Heteropycnosis; Aneu=Aneuploidy; Hyp=Hyperploidy; Poly=Polyploidy; CF=Centric fusion; c-mit=c-mitosis; Pcs=Precocious separation; St=Stickiness.

fragments of known and unknown origin were most common among the individual type (Fig. 1). Though most of the cells contained only one type of abnormality in them, some were, no doubt, observed with two different types of abnormalities.

The frequency of total abnormal cells increased in comparison to the control group of animals (4.3%) by about three-fold (11.6%); three and half-fold (14.0%) and four-fold (17.0%) after the treatment with the lowest (0.5 g/kg bw), the middle (1.0 g/kg bw), and the highest (2.0 g/kg bw) doses respectively of the neem extract (Table 1). The increase in the frequency of abnormal cells is thus dose-dependent.

The gross and individual types of abnormalities, however, differed in their incidence-pattern at different doses of the extract. Almost uniform and significantly higher rates (~10%) of the gross type damages were noticed with respect to the control at all the concentrations, but the individual type damages got significantly so increased only at the highest (2.0 g/kg bw) dose.

Presence of gross type changes even in the groups treated with lower doses of the extract and incidences of structural damages only in the groups treated with the highest dose of the extract might be the result of the damages occurring at two different levels; by affecting the internal milieu of the cell at the lower doses, causing mitotic poisoning in it and leading to the induction of gross type of changes, and then by affecting the chromosome morphology at higher doses to produce individual type damages. In both the eventualities, the likely damage is at the protein level—either of the spindle proteins or of the chromosome packaging ones. Production of electrophilic ions and radicals during the metabolism of the leaf-extract can also be there, that might be interacting with the nucleophilic sites in DNA, leading to break and other related damages in the latter (Klopman *et al.* 1985).

Using bio-mutagens like ochratoxin and aflatoxin (secondary fungal metabolites), Bose and Sinha (1994) and Dharmshila and Sinha (1994) could find that the incidence of gross type damages was significantly more than that of the individual type of damages. This result is in conformity with our present findings. On the other hand, Kumar (1990), while studying the dose-rate dependence of synthetic-pesticide-induced abnormalities in the bone marrow cells of the same strain of the mice, observed that the individual type of damages were more frequent than the gross type of damages at various lower and higher concentrations of the pesticides. It is, therefore, clear that biomutagens, whether they are of fungal origin or obtained from neem extract, cause more of mitotic poisoning leading to gross type changes and comparatively less of cell-killing individual type of damages. By virtue of this property, the neem extract can be considered as less genotoxic than the synthetic pesticides. Its use should therefore, be preferred to avoid injuries to the non-target organisms.

Summary

Oral administration for one week of 0.5, 1.0 or 2.0 g/kg body weight/day of crude leaf extract of neem (*Azadirachta indica*) to laboratory inbred albino Swiss-mice induced both individual and gross types of mitotic chromosome abnormalities in the bone marrow cells. The frequency of total abnormalities was dose-dependent. Gross type abnormalities appeared even at the lowest dose and remained unchanged in their frequency at the higher doses; the individual type abnormalities were induced only at the highest dose.

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