

FUNDAMENTAL RESEARCH

Alterations of Cytogenetical Effects by the Oral Administration of a Homoeopathic Drug, *Ruta graveolens*, in Mice Exposed to Sub-lethal X-irradiation

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Abstract

The 30th and 200th potencies of the homoeopathic drug, *Ruta Graveolens*, commonly used in treatment of bone injuries, have been tested for their possible radio-protective action, using several protocols like bone marrow chromosome aberration, micronuclei testing (MNT) and sperm-head anomaly, against sub-lethal x-irradiation at 100 rad and 200 rad doses in the Swiss albino mice, *Mus musculus* at four different fixation intervals, viz., 24 hr, 48 hr, 72 hr and 96 hr maintaining suitable controls. While the frequencies of chromosome aberrations, micronucleated cells and abnormal sperm-heads were found to be appreciably decreased in all the fixation intervals in the Ruto-30 and Ruta-200 fed mice in both the 100 rad and 200 rad treated series when compared to their respective controls, interestingly enough, an apparent increase in the mitotic index was noted at all the intervals in the Ruta-30 as well as Ruta-200 fed series.

Introduction

Various agents, organic and inorganic compounds, hormones, vitamins, antibiotics etc. have been extensively tested for their possible radio-protective action (see Thomson, 1962; Evans, 1962 for review), but homoeopathic drugs have been relatively less explored for their anti-radiation activity (Khuda-Bukhsh et. al., 1982, 1983; Khuda-Bukhsh, 1986), for which the present work was undertaken. *Ruta graveolens*, a homoeopathic drug produced from the whole plant extract of *Ruta graveolens* (Fam : *Ruta-ceae*), is generally used in the treatment of bone injury and the 30th and 200th potencies of this drug have been tested for their possible radio-

protective activity using several protocols in the present study.

Materials and Methods

i) Protocols used : Chromosome aberration study, micronucleus testing (MNT), sperm-head abnormality study and mitotic index study were made as per the standard procedure in Swiss albino mice, *Mus musculus*, exposed to whole-body X-irradiation at the single dose of 100 rad and 200 rad and fed with Ruta-30 and Ruta-200 separately maintaining suitable controls (fed with alcohol-30 and alcohol-200, respectively).

ii) Experimental design and detailed procedure of feeding : The details of X-irradiation procedure, feeding of the homoeopathic drug from the stock solution, scoring of data as well as preparations of slides etc. were similar to what have been described in the Ginseng-200 fed experiment (Khuda-Bukhsh, 1992 the fixation intervals being 24 hr, 48 hr, 72 hr and 96 hr. The routine procedure was followed in the preparation of sperm smears subsequently stained with Giemsa.

Results and Discussion

i) Chromosome aberration study : The data of the chromosome aberration study in X-irradiated mice fed with Ruta-30 and Ruta-200 as well as their respective controls (fed with alcohol-30 and alcohol-200, respectively) and a few representative aberration types have been furnished in Figs 1-4. Among the various types of aberrations noted, the individual type included fragments (Fr.), breads (Br.), constrictions (Cons.), gaps, the exchange type included rings (R.), centromeric fusions (Cf.), translocations (Trans.) while the physiological types including centromeric dissociation (Cd.), chromosome stretching (St.) pycnosis and pulverizations (Pulv.). The data in Table 1 would

Reprint of article published in " Perspectives in Cytology and Genetics"(eds. G.K. Manna and S.C. Roy) 7 (1992) 727-734 with the prior permission of authors.

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Table - 2

Frequency distribution of Sperms with abnormal heads in 15000 sperms examined (3000 per individual) at different fixation intervals in X-irradiated (100 rad & 200 rad) mice fed with Ruta (30 & 200 potencies) and their respective alcohol (30 & 200 potencies) controls (in parentheses)

Fix. hr.	Radiation dose	Potency of Ruta	Sperms with abnormal heads		Protection in %
			Total No.	% ± SE	
24	100	30	113 (142)	0.75 ± 0.05 (0.95 ± 0.05)	0.20
		200	73 (159)	0.49 ± 0.06 (1.06 ± 0.06)	0.57***
	200	30	163 (93)	1.09 ± 0.05 (0.62 ± 0.06)	0.34**
		200	236 (262)	1.57 ± 0.05 (1.57 ± 0.07)	0.18
48	100	30	102 (192)	0.68 ± 0.04 (1.28 ± 0.03)	0.60***
		200	140 (204)	0.93 ± 0.05 (1.36 ± 0.04)	0.43***
	200	30	80 (285)	0.53 ± 0.05 (1.72 ± 0.06)	1.19***
		200	149 (272)	0.99 ± 0.05 (1.81 ± 0.05)	0.82***
72	100	30	203 (288)	1.35 ± 0.06 (1.92 ± 0.04)	0.57***
		200	158 (315)	1.05 ± 0.05 (2.10 ± 0.05)	1.05***
	200	30	45 (287)	0.30 ± 0.02 (1.91 ± 0.05)	1.61***
		200	298 (372)	1.99 ± 0.05 (2.48 ± 0.06)	0.49***
96	100	30	117 (292)	0.78 ± 0.04 (1.95 ± 0.06)	1.17***
		200	139 (284)	0.93 ± 0.05 (1.89 ± 0.06)	0.96***
	200	30	125 (308)	0.83 ± 0.05 (2.05 ± 0.04)	1.22***
		200	349 (374)	2.33 ± 0.04 (2.49 ± 0.05)	0.16

Asterisk marks for denoting the level of statistical significance,
 ** = P < .01; *** = P < .001

reveal that at all intervals in both Ruta-30 and Ruta-200 fed mice, there was reduction in the frequencies of total aberrations as compared to the alcohol-30 and alcohol-200 fed controls. The differences in aberration frequencies of the drug-fed and unfed mice were in most cases statistically significant at various levels. Further, Ruta-30 appeared to show greater degree of protection at 24 hr than Ruta-200, but at 48 hr Ruta-200 more or less caught up with Ruta-30 in rendering protection and at 72 hr, Ruta-200 showed slightly more protection than Ruta-30. The protective action more or less appeared to be similar in extent at 96 hr. Further, Ruta-30 appeared to show more protection against 100 rad at 24 hr and against 200 rad at 48 hr. However, Ruta-200 did not show as much protective action at 24 hr as it showed at 48 hr and 72 hr against both 100 rad and 200 rad treatment.

ii) Micronucleus test : The data of both drug-fed and alcohol-fed control mice have been noted and some representative photographs of micronucleated bone marrow cells presented in Figs. 5-8. The data would reveal that the occurrence of MN in the drug-fed series was less than in the alcohol-fed mice at all intervals of fixation. The differences in the occurrence of MN in the drug-fed and controls were statistically significant at various levels except for Ruta-200 at 96 hr where the difference was not statistically significant.

iii) Mitotic index : The results of the mitotic index study in the drug-fed and alcohol-fed mice have been furnished in Table 1. It is interesting to note that there was an increase mitotic index at all intervals differences were statistically significant at various levels except for Ruta-30 fed mice against 200 rad treatment where the difference was not statistically significant.

iv) Sperm-head anomaly : The data of the Sperm-head morphology in the drug-fed and control mice have been furnished in Table 2 and a few representative photographs of normal and abnormal sperms presented in Figs. 9-14. It would be revealed from the data that at all intervals, there was a reduction of sperms with abnormal head-morphology in both Ruta-30 and Ruta-200 fed mice as compared to their respective controls. The differences in the occurrence of abnormal sperms

in the drug-fed series were in most cases statistically significant. However, the dose-potency relationship could not be clearly made out from the data although Ruta-30 appeared to show a greater degree of protection/repair than Ruta-200.

Therefore, all the three protocols of chromosome aberration study; MNT and sperm-head morphology study revealed that the homoeopathic drug Ruta and a good potential for being considered as a radio-protective agent. The increase of the mitotic index was also a significant finding as the drug is claimed to have a profound regenerative activity in the case of any bone injury. However, the exact mechanism or pathway of action of this drug which brought in so spectacular protective effect on these cytogenetical parameters in X-irradiated mice could not be properly understood.

Acknowledgement

The authors are grateful to Chittaranjan Cancer Research Institute for some of the specimens of mice used in the present study; Sincere thanks are due to Head, Department of Zoology, Kalyani University, for the laboratory facilities.

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