Mitodepressive Effect of *Rubia cordifolia* Extract on the Bone Marrow Cells of Mice

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Summary The *Rubia cordifolia* extract showed a mitodepressive effect on the rate of cell division in bone marrow cells of Swiss male mice at all times of exposure and in almost all the concentrations studied compared with corresponding controls. This effect increased as the time of exposure increased. This reduction is attributed to the extract effect of inhibiting protein synthesis, suggesting probable effect of *Rubia* extract on the biosynthesis of certain amino acids as well as RNA synthesis. Following application of the lowest dose (25%), 24 h exposure was regarded the higher limit of time exposure in the experiments. After 24 h exposure and at the lowest dose 250 mg/ml (25%) the effect of *Rubia* extract started to decrease.

Key words *Rubia cordifolia*, Mitodepression, Mitotic index, Mouse bone marrow.

Previously, it has been shown that medicinal plants like *Rubia cordifolia* can function as anti-inflammatory, antidiarrheal, antidesentery and antispasmodic (Karim 1986). It is also currently used as an antitumor natural formulation (Kato *et al.* 1987, Takeya *et al.* 1993, Gupta *et al.* 1999, Wakita *et al.* 2001). Moreover, Rubiadin isolated from *R. cordifolia* possesses potent antioxidant property (Tripathi *et al.* 1997). Furthermore, curde extract preparations of *R. cordifolia* are anecdotally inhibits the platelet aggregation (Tripathi *et al.* 1993). The antiviral activity of roots of *R. cordifolia* has been examined by Ho *et al.* (1996), who found that the extract caused inhibition of hepatitis B-surface antigen secretion in human hepatoma.

The effect of plant extract on various aspects of cytogenetics have been reported (Horn 1973, Omari *et al.* 1996, Abderrahman 1997). Several extracts have the ability to induce various types of mitotic abnormalities (Stenchver *et al.* 1974, Abo El Kheir and Abo El Kheir 1992, Abderrahman 1998).

It is due to the point of the wide existing use of *R. cordifolia* in the folkloric medicine, that the idea arose to investigate the effect of *Rubia* extract upon the process of mitosis in Swiss white mice. However, this work was aimed to produce additional cytogenetic information on the effect of *R. cordifolia* extract upon the process of mitosis in Swiss white mice.

Materials and methods

Roots of *Rubia cordifolia* were collected locally and the outer root's bark has been chosen for extraction. An aqueous extract was prepared by placing 25 g of the dried powder in 100 ml of warm 0.9% NaCl, normal saline. The contents were stirred for at least 3 h. The solution was then filtered twice through Whatman no. 4 filter paper. The filtrate was then brought up to 50 ml with normal saline so that 1 ml was equivalent to 500 mg of the starting material and considered to be the highest concentration.

A series of ascending concentration of this extract *viz.* 25, 50 and 75% with 0.9% NaCl were prepared and tested cytologically on bone marrow cells of Swiss white mice at 6, 24, 36 and 48 h

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after a single intraperitoneal injection. A dose of 0.1 ml/g body weight of the *R. cordifolia* extract was applied. Animals in the control groups received an equivalent volume of normal saline. Five male mice and control groups were used for each dose level.

Cytogenetic studies were performed. Direct preparations were obtained from bone marrow following the technique of Tjio and Whang (1962). Preparations were stained with Giemsa solution. Slides were coded and scored blind for the presence of dividing cells: 3002 to 4305 cells per treatment were scored. The mitotic index was calculated with a minimum of 3002 cells. The mitotic index was calculated as the number of cells in division expressed as a percentage of the total number of cells observed. Mitodepression was calculated using the following equation:

$$\text{MI (control)} - \text{MI (treatment)} \times 100 / \text{MI (control)}$$

Results

The cytological effect of an aqueous extract of *Rubia cordifolia* was estimated on the basis of the mitotic index in bone marrow cells of Swiss male mice following a single i.p. dose of 500, 375, 250 and 125 mg/ml of the extract. The mitotic index was assessed from 6 to 48 h following application. The mitotic index and mitodepression determined in each of the doses are presented in Table 1.

A remarkable decrease in the mitotic index was evident in the bone marrow cells at all 4 concentrations used and at all 4 periods when compared with the control. The mitotic index declined from 4.46 to 3.24. Depression of cell division in these cells increased with increasing time of exposure in almost most cases. Referring to Table 1, the effect of *Rubia* extract started to decrease after 24 h exposure and at the lowest dose (25%).

Effects of *Rubia* extracts upon mitosis of *Allium sepa* are given in Table 2.

Table 1. Mitotic and Mitodepressive indices in bone marrow cells of mice treated with different concentrations of *Rubia cordifolia* extracts

Exposure (h)	Dose		Cells examined	Mitoses examined	Mitotic index (MI)	Mitodepressive index
	mg/ml	%				
6	500	100	3518	136	3.86	16.45
	375	75	3672	144	3.92	15.15
	250	50	3112	134	4.30	6.92
	125	25	3004	134	4.46	3.46
	Control		4305	199	4.62	—
24	500	100	3196	124	3.87	15.13
	375	75	3505	136	3.88	14.91
	250	50	3118	132	4.23	7.23
	125	25	3120	137	4.39	3.72
	Control		3000	137	4.56	—
36	500	100	2945	108	3.66	15.86
	375	75	3110	118	3.79	12.87
	250	50	3033	122	4.02	7.58
	125	25	3108	134	4.31	0.91
	Control		3441	150	4.35	—
48	500	100	4111	—	—	—
	375	75	3175	103	3.24	26.53
	250	50	3002	122	4.06	7.93
	125	25	3031	135	4.45	0.90
	Control		3126	138	4.41	—

Table 2. Frequency of normal and abnormal phases in mitosis after treating *Allium cepa* with various concentrations of *Rubia cordifolia* extract

Conc. (%)	Interphase			Prophase			Metaphase			Anaphase and Telophase									
	Total No.	Normal No.	Abnormal		Total No.	Abnormal No.	Total No.	Abnormal		Total No.	Normal No.	Abnormal							
			Res. nuclei	Micro and multi-nuclei				C-bands	C-met.			%	Desp.	Bridges	Non-cong.	Lagg.	%		
8 h																			
100	7850	7811	36	3	112	102	10	100	66	2	32	34.00	120	64	28	2	4	2	30.00
75	8332	8314	14	4	313	305	8	155	110	2	43	29.03	252	180	59	8	3	2	28.57
50	8411	8388	23		207	203	4	122	90	1	31	26.23	183	135	46			2	26.23
25	7963	7955	2	6	315	310	5	131	111		20	15.27	215	183	18	8		6	14.66
Cont.	8201	8201			405	404	1	163	163				211	207	2	1	1	1	1.90
24 h																			
100	8215	8167	45	3	125	120	5	119	46	4	69	61.34	115	68	38	6	2	1	40.87
75	7518	7432	80	6	128	122	6	85	51	1	33	40.00	116	70	38	3	1	4	39.66
50	7211	7175	33	3	107	103	4	98	58	1	39	40.82	101	62	34	3		2	38.61
25	8009	8000	9		115	112	3	75	60	1	14	20.00	85	71	12	1		1	16.47
Cont.	8112	8112			129	129		83	82		1	1.20	90	88	1		1	1	2.22
48 h																			
100	12215	12129	83	3	14	10	4	175	4	5	166	97.71	60	6	8	12	6	8	56.66
75	10051	9947	96	8	35	30	5	60	4	5	51	93.33	13	6	4	1	1	1	53.85
50	11221	11176	42	3	14	10	4	22	10	1	11	54.55	15	10	2	2		1	33.33
25	10918	10966	15		221	219	2	111	101		10	9.00	174	158	11	2		3	9.20
Cont.	9935	9933	2		231	231		161	160		1	0.62	160	157	3				1.88
72 h																			
100	8570	8434	84	2	121	116	5	175	6	3	166	96.57	112	46	11	36	6	13	58.93
75	8621	8525	88	8	115	112	3	112	8	2	102	92.86	109	48	8	37	5	12	55.96
50	7211	7151	56	4	186	185	1	99	3	2	94	96.97	169	71	9	71		18	57.99
25	9215	9192	23		111	109	2	79	7		72	91.14	117	50	9	48		10	57.26
Cont.	9334	9333	1		132	132		101	100		1	0.99	150	148		1		1	1.33

Discussion

The *Rubia cordifolia* extract showed a marked reduction in the mitotic index at all exposure times and with almost all concentrations compared with the corresponding control. The reduction revealed a mitodepressive effect of the *R. cordifolia* extract on the dividing bone marrow cells of mice. These results were confirmed by the work of Kabarity and Malallah (1980) who found that Khat extract caused a mitotic depression in the meristematic regions of *Allium cepa* root tips. These results are also coincided with the results of Omari *et al.* (1996) who found that the extract of *Catha edulis* (Khat) lowered the mitotic index in bone marrow cells of mice at all times of exposure and in almost all concentrations studied. Moreover, a reduction in the mitotic index following treatment was also demonstrated in maize (Verma 1972, Verma and Lin 1978). In contrast, Abderrahman (1997) found that treatment with *Peganum harmala* extract remarkably increased the mitotic index in *A. cepa* root tips with the treatment of increasing duration at all exposure time used with almost all concentrations. *P. harmala* extract also caused an increase in mitotic index of *Zea mays* treated cells with increasing the time of treatment in all cases (Abderrahman 1998). Furthermore, alkaloids extracted from *P. harmala* caused an increase of mitotic index in *A. cepa* root tips meristems (Abo El-Khier and Abo El-Khier 1992).

In the present study, a drastic reduction in the mitotic index was observed in bone marrow cells treated with the high concentration (50, 100%) at all times of exposure. This reduction may be due to decrease in the number of cells moving into prophase from G2 (Baszczynski *et al.* 1980), or may be attributed to the inhibitory effect *R. cordifolia* extract on DNA, RNA and protein synthesis (Al-Ahdal *et al.* 1988).

It is known that specific proteins are required for the condensation of chromosomes and their movement through mitosis. Many of these proteins play structural roles in mitoses while others are concerned with energy requirements, all of which influence the normal progression of nuclear division (Mitchison 1971). The observation that various doses affected mitotic index differently prompted an investigation of protein synthesis, suggesting the probable effect of *Rubia* extract on the biosynthesis of certain amino acids as well as RNA synthesis, both of which are essential for protein synthesis.

At 6 and 24 h following application of a low dose 125 mg/ml (25%), the mitodepression was lower than at 36 and 48 h. This suggests that 24 h following application may be the threshold time in producing mitodepressive effects. After 24 h exposure the effect of *R. cordifolia* extract started to decrease.

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