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Cytogenetic Effect of *Arum maculatum* Extract on the Bone Marrow Cells of Mice

Modallal[1] Nabeel, Salim Abderrahman[1*] and Alessio Papini[2]

[1] Department of Biological Sciences and Biotechnology. The Hashemite University, P.O. Box 150459. Zarqa 13115, Jordan.
[2] Dipartimento di Biologia Vegetale, Università di Firenze, Via La Pira, 4 - 50121 Firenze, Italy; e-mail alpapini@unifi.it.

Abstract — Treatment with *Arum maculatum* extract remarkably lowered the mitotic index in bone marrow cells of Swiss male mice at all of the exposure times and in almost all concentrations studied compared with corresponding controls. The reduction in the mitotic index revealed a mitodepressive effect of the extract on dividing bone marrow cells of mice. Following application of the lowest dose (125 mg/ml), 24 h was considered the threshold limit of exposure time in the experiment able to provide the highest result. After 24 h exposure at the lowest dose (125 mg/ml), the recorded cytogenetic effect of *Arum maculatum* extract started to decrease (after 36h it was lower than at 24h exposure).
Arum maculatum extract induced a wide range of abnormalities involving all the stages of mitosis in the bone marrow cells of mice. These include restitution, micro and multinuclei, abnormal prophase, C-metaphase, sticky chromosomes, fragments, bridges, non-congression and laggards. The main effect of the extract, however, was found on interphase and both metaphase and anaphase.

Key words: *Arum maculatum*, bone marrow cells, chromosomal abnormalities, mitodepression, mitosis.

INTRODUCTION

The effect of many plant extracts on various aspects of cytogenetics has been widely studied (e. g. Horn 1973; Omari *et al.* 1996; Abderrahman 1997). Moreover, mitotic abnormalities have been observed to be induced by several extracts (e. g. Stencherer *et al.* 1974; Abo El Kheir and Abo El Kheir 1992; Abderrahman 1998 and 2004).
Arum maculatum is a member of family *Araceae*. It is a common plant in north temperate Europe and also known as Lord and Ladies and Cucko pint. In Jordan it is called Arun and was indicated as cause of skin irritation (Al-Qura'n 2005).

The bruised fresh plant has been applied externally in the treatment of rheumatic pain (Stuart 1979). The root is diaphoretic, diuretic, expectorant, strongly purgative and vermifuge (Lust 1983). Alencar *et al.* (2005) investigated the effect of lectin isolated from *Arum maculatum* on the cells of the immune system. They found that agglutinin presents pro-inflammatory activity inducing neutrophil migration.

Antimicrobial activity of *Arum maculatum* has been reported by Uzun *et al.* (2004). They found the MIC value of 39.1 µg/ml against *staphylococcus epidermidis*. Moreover, a novel lectin from roots of *Arum maculatum* agglutinating human ejaculating spermatozoa was discovered (Mladenov *et al.* 1987; Mladenov and Bulanor 1989; Mladenov *et al.* 1993; Mladenov *et al.* 2002).

Leaves of *Arum maculatum* are used by people of Jordan as food and are used by the folkloric medicine as a contraceptive. However, it is necessary to consider that oral contraceptives are intended for prolonged use by a large group of the reproductive portion of the human population. It is important, therefore, to know the possible harmful side effects. Hence the aim of this study was to investigate the cytogenetic effects of *Arum maculatum* by observing its effects on the behavior and structure of chromosomes in bone marrow cells of mice.

* Corresponding author: e-mail: salim1954@yahoo.com

MATERIALS AND METHOD

Arum maculatum plants were collected locally in Jordan. We chose to use dried leaves for extraction. Leaves were powdered mechanically. An aqueous extract was prepared by placing 25 g dried powder in 100 ml warm distilled water. The contents were stirred for 3 hours to dissolve all the water soluble ingredients. The solution was filtered twice through Whatman number 4 filter paper. The filtrate was then adjusted to 50 ml with distilled water, so that 1 ml was equivalent to 500 mg of the starting material (dried powder) and considered as the highest concentration (100%). The lethal dose was estimated to be 1000 mg/ml.

A series of ascending concentrations of this extract *viz* 25, 50 and 75% were prepared and their cytological effects on bone marrow cells were tested for 8, 24, 36 and 48 h., after a single intraperitoneal injection. A dose of 0.1 ml/kg body weight of *Arum maculatum* extract was applied. Animals in the control groups received an equivalent volume of distilled water. Five male mice for both the experimental and the control group were used for each dose level.

Cytogenetic analyses - Metaphase bone marrow cells were prepared for mitotic investigation by the classical methods. The preparations were stained with Giemsa solution. Slides were coded and scored blind for presence of dividing cells: 3755 to 5593 cells were scored from at least five animals per treatment. The mitotic index was calculated on a minimum of 3755 cells, 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:

MI (control)-MI (treatment) X100/MI (control).

Frequency of normal and abnormal phases in mitosis after treating mice cells with different concentrations of *Arum maculatum* extracts was estimated for various periods of times *viz* 8, 24, 48 and 72 h.

The results were analyzed using the t-test statistical method to determine the statistical differences between different treatments. The differences were considered statistically significant at $P \leq 0.05$.

RESULTS

The cytological effect of *Arum maculatum* extract was estimated on the basis of the mitotic index in bone marrow cells of Swiss male mice following a single intraperitoneal dose of 500, 375,

Table 1 — Mitotic and mitodepressive indices in bone marrow cells of mice treated with different concentrations of *Arum maculatum* extracts.

Exposure (h)	Dose: (mg/ml)	%	Cells Examined	Mitosis Examined	MI	Mitodepressive Index
8	500	100	4312	166	3.85	15.94
	375	75	4580	178	3.89	15.10
	250	50	5220	214	4.10	10.48
	125	25	3755	167	4.50	1.75
	Control	-	4305	197	4.58	-
24	500	100	4794	186	3.88	14.73
	375	75	5258	205	3.90	14.29
	250	50	4677	198	4.23	7.03
	125	25	4681	206	4.40	3.30
	Control	-	4488	204	4.55	-
36	500	100	4786	175	3.66	18.12
	375	75	5054	194	3.84	14.09
	250	50	4929	198	4.02	10.07
	125	25	5052	223	4.41	1.34
	Control	-	5593	250	4.47	-
48	500	100	5000	-	-	-
	375	75	5555	181	3.29	25.57
	250	50	5205	209	4.02	9.05
	125	25	5304	233	4.39	0.68
	Control	-	5471	242	4.42	-

Table 2 — Frequency of normal and abnormal phases in mitosis after treating mice cells with different concentrations of *Arum maculatum* extracts.

Conc. (%)	Interphase				Prophase			Metaphase					Anaphase and Telophase						
	Total No.	Normal No.	Abnormal		Total No.	Normal No.	Abnormal	Total No.	Normal No.	Abnormal			Total No.	Normal No.	Abnormal				
			Res. nuclei	Micro- & multinuclei						C-metaphase	Stickiness	%			Frag.	Bridges	Non-cong.	Lagg.	%
									8h										
100	7065	7030	32	3	101	92	9	90	58	30	2	35.60	110	78	28	1	2	1	29.09
75	7499	7483	13	3	282	275	7	139	99	38	2	28.78	227	162	53	8	3	1	28.63
50	7570	7549	21		186	183	3	110	81	28	1	26.40	165	122	41	1		1	26.06
25	7167	7160	2	5	284	279	5	128	100	28		15.63	193	165	18	8		2	14.51
Cont.	7381	7381			365	364	1	147	147				190	186	2	1		1	2.11
									24 h										
100	7394	7350	41	3	113	108	5	107	62	42	3	42.06	116	66	20	10	8	12	43.10
75	6766	6689	72	5	115	110	5	77	46	30	1	40.26	170	98	41	8	8	15	42.35
50	6490	6458	30	2	96	93	3	88	54	33	1	38.64	160	98	38	6	6	12	38.75
25	7208	7200	8		103	101	2	68	54	13	1	20.59	100	84	14	2			16.00
Cont.	7300	7300			116	116		76	74	2		2.63	114	111	3				2.63
									48h										
100	10994	10916	75	3	13	10	3	158	15	140	3	90.51	110	50	15	20	10	15	54.55
75	9045	8952	86	7	32	27	5	55	14	38	3	74.55	140	66	20	35	9	10	52.86
50	10099	10058	39	2	13	9	4	20	10	9	1	50.00	150	100	20	20	4	6	33.33
25	9866	9855	11		201	199	2	100	91	9		9.00	175	160	10	3	1	1	8.57
Cont.	8942	8940	2		230	230		150	149	1		0.67	166	162	3	1			2.41
									72h										
100	7710	7628	76	6	109	104	5	167	15	150	2	91.02	115	48	10	45	4	8	58.26
75	7770	7678	82	10	115	112	3	100	8	90	2	92.00	110	49	10	42		9	55.45
50	6490	6436	50	4	190	189	1	91	3	86	2	96.70	170	70	9	71		20	58.82
25	8294	8273	20	1	112	110	2	72	7	65		90.28	120	50	9	50		11	58.33
Cont.	8400	8399	1		133	133		92	91	1		1.10	160	158		2			1.25

250 and 125 mg/ml of the extract. The mitotic index was detected from 8 to 48 h following application. The determined mitotic and mitodepression indexes for each dose are reported in Table 1. A remarkable decrease in the mitotic index was evident in the bone marrow cells at all concentrations used and at all four periods when compared with the control. The mitotic index declined from 4.50 to 3.29. Depression of divisions in these cells increased with increasing exposure time in almost all cases.

Several chromosomal damages were observed in the treated mice cells at different levels of treatments with *Arum maculatum* extract. These included restitution, micro and multinuclei, abnormal prophase, C-metaphase, sticky chromosomes, fragments, bridges, non-congression and laggards (Table 2). The most important effect exerted on interphase was restitution, while on both metaphase and anaphase it was C-metaphase and fragments respectively.

DISCUSSION

Table 1 shows the mitotic index in bone marrow cells of mice treated with various concentrations of *Arum maculatum* extract. A marked reduction in the mitotic index was observed at all exposure times and with almost all concentrations compared with the corresponding control. The reduction revealed a mitodepressive effect of the *Arum maculatum* extract on the dividing bone marrow cells of mice.

These results were similar to those obtained by KABARITY and MALALLAH (1980) with khat extract, which caused a mitotic depression in *Allium cepa* root tips and to the findings of QURESHI *et al*. (1988) on their study on the effect of khat on bone marrow cells on mice. Similar reduction was also obtained by OMARI *et al*. (1996) again on bone marrow cells of mice treated with khat extract. Moreover, these results are in line with those obtained by ABDERRAHMAN (2004), who found that *Rubia cordifolia* extract caused a mitotic depression in bone marrow cells of mice. On the con-

trary, ABO EL KHIER and ABO EL KHIER (1992) found that alkaloids extracted from *Peganum harmala* caused an increase of the mitotic index in *Allium cepa* root tips. Also ABDERRAHMAN (1997; 1998), observed that the treatment with *Peganum harmala* extract remarkably increased the mitotic index in *Allium cepa* and *Zea mays* root tips.

In the present study, a drastic reduction in the mitotic index was observed in bone marrow cells treated with high concentrations (75 and 100%) at all exposure times. This reduction is due to decrease in number of cells moving into prophase from G2 (BASZCZYNSKI *et a*l. 1980), or may be attributed to the inhibitory effect of *Arum maculatum* extract on DNA, RNA and protein synthesis on cultured mammalian cells, as proposed by AL-AHDAL *et al.* (1988) for Khat.

The decrease in MI or the inhibition of the DNA synthesis might be caused by the decreasing ATP levels (JAIN and ANDSORBHOY 1988).

At 8 and 24 h following application of a low dose (125 mg/ml), the mitodepression was lower than at 36h (1.34 with respect to 3.30 at 24h). This suggests that 24 h following application may be the threshold time in producing mitodepressive effects. Afterwards the effect of *Arum maculatum* extract after a further exposure in the following 24h started to decrease (Table 1).

It is obvious that *Arum maculatum* can alter the frequencies of mitotic stages as well as it can induce a number of mitotic abnormalities such as restitution, micro and multinuclei, abnormal prophase, C-metaphase, sticky chromosomes, fragments, bridges, non-congression and laggards. The main effect of the extract, however, was found on interphase and both metaphase and anaphase (Table 2).

The majority of interphase aberrations were restitution. In higher concentrations and in cells treated for long periods, the frequency of restitution increased. Similar restituted nuclei were obtained by FAHMY and ABDULLA (2001); GEORGE and GHAREEB (2001) and ABDERRAHMAN (2004) on studying the effect of *Rubia cordifolia* extract on the bone marrow cells of mice.

Micro and multinucleated cells were frequently observed in all treatments. The frequency of this type of abnormality fluctuated. The appearance of micronuclei may be produced as a result of the formation of laggards and chromosome fragments, which may be surrounded by a nuclear membrane. These micronuclei may persiste in the following phases (EL-BAYOUMI *et al.* 1979). Such micronuclei were reported also by ABDERRAHMAN (2004).

Abnormal prophase was also observed. It was found that its frequency fluctuated in all treatments. Such abnormal prophases were also reported by (EL-BAYOUMI *et al.* 1979) and ABDERRAHMAN (2004).

In both metaphase and ana-telophase the frequency of abnormal cells increased with the increase of exposure time and the extract concentration almost all cases. The majority of metaphase abnormalities was that of typical C-metaphase. The frequency of C-metaphase increased with the increase of the applied concentration and of the period of treatment in most cases. Such C-mitotic cells were also reported to be induced by treatments with different plants extracts (KAUSHIK 1996; ABDERRAHMAN 2004). The action of *Arum maculatum* extract resembling those of colchicine may be explained by disturbance to protein and nucleic acid synthesis (DEKA 1998).

Stickiness of chromosomes at metaphase fluctuated in treatments. This cytological abnormality caused inability of normal chromosomes movement at anaphase. Such stickiness were also reported by EL-BAYOUMI *et al.* (1979) and DEKA (1998).

The most prominent abnormality in ana-telophase was the formation of fragments. However, such cells with fragments were found less frequently at lower concentrations. The capacity of inducing several fragments or breaks were also reported for other plant extracts (SHARMA and SHARMA 1990; DEKA 1998; BORAH and TALUKDAR 2002).

Another abnormality in anaphase stage was the formation of chromatid bridges. Such bridges persisted during telophase. The frequency of these bridges increased in relation to the extract concentration in most cases. Similar observations were reported by FAHMY and ABDULLA (2001), GEORGE and GHAREEB (2001) and ABDERRAHMAN (2004).

Among anaphase abnormalities there was non-congression of daughter chromosomes in the normal fashion, i.e. disturbed anaphase. The frequency of this abnormality increased in relation to the extract concentration in most cases in all treatments. Similar results were obtained by EL-BAYOUMI *et al.* (1979) and ABDERRAHMAN (2004).

Lagging of chromosomes was frequently noticed during anaphase and telophase. It was found that the frequency of such aberration fluctuated in all treatments. Chromosome fragmentation was induced and was quite clear together with anaphase lagging fragments and in interphase as micronuclei. Lagging chromosomes were considered to depend on the failure of chromosomes to move to either of the poles (BORAH and TALUKDAR

2002). BORAH and TALUKDAR (2002) and ABDERRAHMAN (2004) also reported the induction of laggards following treatments with *Ricinus communis* and *Rubia cordifolia* extract respectively.

CONCLUSION

This investigation revealed a reduction effect in the mitotic index caused by *Arum maculatum* extract on dividing bone marrow cells of mice. Moreover it is noteworthy a wide range of aberrations involving all stages of mitosis.

REFERENCES

ABDERRAHMAN S. M., 1997 — *Effect of Peganum harmala extract on root tips of Allium cepa*. Cytobios, 90: 171-174.

ABDERRAHMAN S. M., 1998 — *Cytogenetic effects of Peganum harmala extract on maize root tips*. Cytologia, 63: 283-291.

ABDERRAHMAN S. M., 2004 — *Mitodepressive effect of Rubia cardifolia extract on the bone marrow of mice*. Cytologia, 69(3): 307-311.

ABO EL KHIER Z. A. and G. M. ABO EL KHIER, 1992 — *Cytological effects of certain active constituents of Peganum harmala on mitosis of Allium cepa*. J. King. Saud. Univ. Science, 4(1): 37-45.

AL-AHDAL M. N., MCGARRY T. J. and M. A. HANNAN, 1988 — *Cytotoxicity of Khat (Catha edulis) extract on cultured mammalian cells*. Mutat. Res., 204: 317-322.

ALENCAR V. B., ALENCAR N. M., ASSREUY A. M., MOTA M. L., BRITO G. A., ARAGAO K. S., BITTENCOURT F. S., PINTO V. P., DEBRAY H., RIBEIRO R. A. and B. S. CAVADA, 2005 — *Pro-inflammatory effect of Arum maculatum lectin and role of resident cells*. Int. J. Biochem. Cell. Biol., 37(9): 1805-1814.

AL-QURA'N S., 2005 — *Ethnobotanical survey of the folk toxic plants in southern parts of Jordan.* Toxicon., 46(2): 119-129.

BASZCZYNSKI C. L., WALDEN D. B. and B. G. ATKINSON, 1980 — *Cyclohexamide induced nuclear alteration in maize root tips*. Canad. J. Genetics and Cytology, 22: 319-331.

BORAH S.P and J. TALUKDAR, 2002 — *Studies on the cytotoxic effect of extract of Castor seed Ricinus communis*. Cytologia, 67: 232-243.

DEKA D.C., 1998 — *Studies on cytotoxic effects of certain organophosphorous insecticides on some plants*. Ph.D. Thesis. Gauhati Uiversity, India.

EL-BAYOUMI A. S., KABARITY A. and A. HABIB, 1979 —*Cytological effects of papaverine hydrochloride on root tips of Allium cepa*. Cytologia, 44: 745-755.

FAHMY M.A and E. F. ABDULLA, 2001 — *Cytogenetic effects induced by the natural pyrethrins and the synthetic lambda cytohalothrin in mice in vivo*. Cytologia, 66: 139-149.

GEORGE N.M and A. GHAREEB, 2001 — *Genotoxicity of the insecticide cytolon on mitosis and seed proteins of Vicia faba*. Cytologia, 66: 77-84.

HORN J. D., 1973 — *Somatic association and an organized nucleus in Zea mays L.*. Ph.D. Thesis, University of Western Ontarion, London, Canada.

JAIN A. K. and R. K. ANDSORBHOY, 1988 — *Cytogenetical studies on the effects of some chlorinated pesticides III. Concluding remarks*. Cytologia, 53: 427-436.

KABARITY A. and G. MALALLAH, 1980 — *Mitodepressive effect of Khat (Catha edulis) in the meristematic region of Allium cepa root tips*. Cytologia, 45: 733-738.

KAUSHIK G.C., 1996 — *Cytological effects of Lantana camara leaf extract on Vicia faba root tip cells*. Advances in Plant Science, 9: 159-164.

LUST J., 1983 — *The Herbal Book*. Bantam books. ISBN 0-553-23827-2.

MLADENOV I. and V. BULANO, 1989 — *Study of the biological properties of isolectin from Arum maculatum.* Eksp. Med. Morfol., 28(4): 33-37.

MLADENOV I., HARALAMBIEVA I. H., IANKOV I. D. and I. G. MITOV, 2002 — *Characterization of 20-kDa lectin-spermagglutinin from Arum maculatum that prevents Chlamydia pneumoniae infection of L-929 fibroblast cells*. FEMS Immunol. Med. Microbiol., 32(3): 249-254.

MLADENOV I., BULANO, V. and M. STAMENOVA, 1993 — *The sperm agglutinating activity of the fractions of saline root extracts from Arum maculatum*. Eksp. Med. Morfol., 31(1-2): 22-28.

MLADENOV I., NAKOR L. and I. BULANOR, 1987 — *Sperm agglutinating, hemagglutinating and precipitating activity of an extract of Arum maculatum roots*. Eksp. Med. Morfol., 26(3): 5-9.

OMARI Y. I., SHRAIDEH Z. A. and S. M. ABDERRAHMAN, 1996 — *Mitodepressive effect of Khat (Catha edulis) on the bone marrow of mice*. Biomedical Lett., 54: 69-72.

QURESHI S., TARIQ M., PARMAR N. S. and I. A. Al-MESHAL, 1988 — *Cytological effects of Khat (Catha edulis) in somatic and male germ cells of mice*. Drug Chem. Toxicol., 11(2):151-156.

SHARMA A.K and A. SHARMA, 1990 — *Chromosome Technique. Theory and Practice*. 3 ed.. Butterworth, London.

STENCHERER M. A., KUNYSZ T. J. and M. A. ALLEN, 1974 — *Chromosome breakage in user of marijuana*. Am. J. Obstet. Gynecol., 118: 106-113.

STUART M., 1979 — *The Encyclopedia of Herbs and Herbalism*. Orbis Publishing, London.

UZUN E., SARIYAR G., ADSERSEN A., KARAKOC B., OTUK G., OKTAYOGLU E. and S. PIRILDAR, 2004 — *Traditional medicine in Sakarya province (Turkey) and antimicrobial activities of selected species*. J. Ethnopharmacol., 95(2-3): 287-296.

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