

Mutagenicity of nicotine in *Schistosoma mansoni* - infected mice

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ABSTRACT

Schistosomiasis is a widespread and serious infection in Egypt and many parts of the world, and tobacco smoking by infected patients is not uncommon. Evidence for association of either smoking or *Schistosoma* infection with carcinogenicity and/or mutagenicity has been reported. This study deals with the induction of chromosomal damage assessed by bone marrow micronucleus (MN) and chromosome aberration (CA) tests, as well as the analysis of chromosomal aberrations in diakinesis-metaphase I spermatocytes in male mice infected with *Schistosoma mansoni* cercariae and treated with nicotine. *Schistosoma* infection increased the percentage of micronucleated erythrocytes and total CAs in bone marrow cells. Analysis of meiotic chromosomes showed significant elevation in the *Schistosoma*-infected mice. Administration of nicotine to infected mice substantially increased the percentages of micronucleated cells and total CAs. The percentage of chromosomal abnormalities in spermatocyte metaphase-I cells increased significantly in *Schistosoma*-infected mice. The incidence of chromosomal aberrations of metaphase I spermatocytes showed a highly significant increase after nicotine treatment; the effect on infected mice was significantly higher than that noted on the non-infected group. The data obtained in this study suggest that animals afflicted with schistosomiasis with synergistic nicotine administration suffer from a higher genetic risk, and may serve as a warning to schistosomiasis patients.

KEYWORDS: chromosome aberrations, micronucleus, carcinogenicity, spermatocyte diakinesis-I, mice.

INTRODUCTION

Schistosomiasis constitutes a major health problem in Egypt and many parts of the world (WHO 1987). There is ample evidence indicating that *Schistosoma* infection is involved in the incidence of several cancers (El-Sebai 1978). Urothelial cells collected from patients infected with *S. haematobium* were shown to have an increased frequency of micronuclei suggesting the induction of chromosome injury (Raafat *et al.* 1984). Schubber and Saleh (1987) presented data indicating that infection with *S. haematobium* elevated the values of sister chromatid exchanges (SCEs) and chromosomal aberrations in blood lymphocytes. Infection of mice with *S. mansoni* for 12 weeks resulted in a marked increase in the incidence of chromosomal aberrations in bone marrow cells and sperm head abnormalities (Hamada *et al.* 1992).

Cigarette smoking constitutes a major risk to health. Tobacco smoke is a complex mixture of chemicals that possesses cytotoxic, genotoxic and carcinogenic activities (San *et al.* 1989). The association between cigarette smoking and human cancer is well documented (Hoffmann *et al.* 1991). Three nitrosation products of processed tobacco: N'-nitrosonornicotine (NNN), 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanal (NNA) were reported to be powerful carcinogens (Hoffmann *et al.* 1993), and possibly mutagenic in the hepatocyte culture / DNA repair test (Williams & Laspia 1979). Moreover, the use of cigarette smoke in both *in vitro* and *in vivo* studies resulted in elevation of incidences of chromosome aberration, micronuclei and SCEs (Lee *et al.* 1987).

Nicotine (methylpyrrolidin-pyridine) a liquid alkaloid, is the chief pharmacologically active ingredient that causes addiction in tobacco and its smoke (Rang & Dale 1993). Toth (1982) concluded that neither nicotine nor its primary metabolites are carcinogenic. However,

other reports indicated a tumorigenic action of nicotine (Habs & Schmahl 1984). *In vitro* studies with Chinese hamster ovary (CHO) cells showed that nicotine induce chromosomal aberrations and SCEs in a dose and duration dependent manner (Trivedi *et al.* 1990). Similarly, the percentage of micronucleated bone marrow erythrocytes of mice exposed to mainstream cigarette smoke was directly correlated with the content of nicotine and tar in the smoke (Nersessian & Arutyunyan 1994). Rosin and Anwar (1992) demonstrated that the micronucleus frequencies were significantly higher in urothelial cells from *Schistosoma*-infected group than among control, with a trend towards an increase in micronucleus frequencies in infected individuals who smoke compared with those were non-smokers.

The prevalence of current smokers among Egyptians in the age group of 30-39 years was reported to be 20.5% (Ministry of Health, ARE 1987). Cigarette smoking is the most frequent type of smoking among males and females in urban and rural areas in Egypt. The problem of the combined adverse effects of smoking and schistosomiasis among Egyptian farmers in particular has been addressed in this report. In the present study, the mutagenic potential of nicotine, infection with the cercariae of *S. mansoni*, and their combined effect has been investigated. Three cytogenetic assay tests were used: the micronucleus test, chromosomal aberration assay, and chromosomal abnormalities in diakinesis metaphase-I spermatocytes. The design of the experiment provides a model that mimics the human situation where smoking and infection prevail among Egyptian farmers.

MATERIALS AND METHODS

Adult male Swiss albino mice [CD1], 10-12 weeks old were obtained from Theodor Bilharz Institute, Giza, Cairo. Four groups of mice, each of 26 animals, were used in this study; *Schistosoma*-infected group, nicotine group, nicotine/infected group and a control group. *Schistosoma*-infected group (Group 1): Infection of mice with *S. mansoni* cercariae was carried out according to the method of Peter & Warren (1969). Each mouse was injected subcutaneously with about 100 cercariae obtained from infected *Biomphalaria alexandrina* snails. The infected animals were left for 8 weeks after infection before being subjected to nicotine treatment. *Nicotine group (Group 2)*: Nicotine (Merck, Germany) was diluted in saline (0.9%) and used immediately after dilution. Nicotine was injected intraperitoneally (i. p.) at a dose of 0.8 mg/kg body weight (b. wt.) for 5 consecutive days. Each animal received the assigned dose in a total volume of 0.25 ml. *Nicotine/Infected group (Group 3)*: A group of *Schistosoma* infected mice were injected daily after 8 weeks with the same dose of nicotine for 5 consecutive days. *Control group (Group 4)*: The same volume of saline was administered both to infected and non-infected mice.

Three cytogenetic assays were used for assessment of chromosomal damage, metaphase chromosomes and micronuclei in bone marrow cells and spermatocyte diakinesis metaphase-I.

Micronucleus assay followed the procedure described by Schmid (1969). Half of the mice (10 animals) were sacrificed after 24h and the other half were sacrificed after 7 days. Both femurs were removed and the bone marrow was flushed into foetal calf serum. After centrifugation, slides were prepared, coded, stained with Giemsa-May Gruenwald and scored blind for the presence of MN in polychromatic erythrocytes (PCEs) and normochromatic erythrocytes (NCEs). The number of micronucleated PCEs was recorded in 500 PCEs for each mouse. The number of NCEs was also counted in the same fields for PCEs and the incidence of MNNCEs was determined.

Metaphase chromosome analysis was conducted according to Adler (1984). Animals were injected (i.p.) with colchicine solution in a dose of 4 mg/kg b. wt., 2-hours before sacrifice. Assessment of CAs was carried out 24h and 7days after cessation of treatment. One hundred metaphase spreads were analysed per animal and different types of chromosomal aberrations were recorded.

Cytogenetic analysis of spermatocyte diakinesis-metaphase-I was carried out as described by Adler (1984). Sampling from testes of 6 animals in each group was taken 14 days after the last treatment in both infected and non-infected mice. For each mouse, 50 meiotic metaphases were analysed and abnormal configurations were scored.

Statistical analysis: The data were analysed by the Student's t-test to determine the differences between groups. Analysis of variance (2-way ANOVA) was also used to test differences between and within groups. Chi square test was used to compare the observed and expected MN and CA frequencies in different treated groups.

RESULTS

Micronucleus Test

Effect of *S. mansoni* infection: The frequencies of MNPCEs and MNNCEs in infected mice and in the non-infected control group at the two sampled times are summarized in Table 1. No significant differences were obtained between the two sampling times (24h and 7 days), in either group, for any of the measured parameters. The infected group showed a highly significant increase in the percentage of MNPCEs with one micronucleus (0.80%) compared to the non-infected control (0.04%). PCEs with 2 micronuclei were observed only in infected groups and showed a frequency of up to 0.14%. MNNCEs were also higher in the infected group than the control group, however, there was no difference between the time intervals. The ratio PCE/NCE decreased strongly in the infected mice and the difference was highly significant compared to the non-infected ones. This indicates that bone marrow depression was evident in the infected animals. In the infected group, the ratio increased significantly toward the control level at 7 days (Table 1).

Effect of nicotine: The incidences of MNPCEs and MNNCEs (Figure 1) in infected and non-infected mice treated with nicotine are given in Table 1. Treatment of mice with nicotine significantly elevated the incidence of total MNPCEs by 20 and 14 times above the control values at 24h and 7 days, respectively. The percentage of MNNCEs showed a highly significant increase after nicotine treatment (0.31%) compared to the control (0.02%) after 24h but decreased significantly after a further 7 days. The percentages of total MNPCEs showed 5-fold and 2-fold increases in the infected/nicotine group compared to the infected group assessed at 24h and 7 days, respectively. These differences were highly significant. PCEs with 3 micronuclei were observed only in the infected-nicotine group; 0.22% after 24h and 0.09% at 7 days. These results suggest that nicotine induced a more dramatic increase in chromosomal damage when given to infected mice than when given to non-infected animals.

The percentage of MNNCEs assessed after 24h showed a highly significant increase in the infected/nicotine group compared to the infected group. At the 7th day the incidence significantly decreased but was still higher than the infected and non-infected nicotine-treated groups. The PCE/NCE ratio of non-infected nicotine-treated mice showed a highly significant decrease below the control at 24h, indicating the myelotoxicity of nicotine. The ratio increased significantly 7 days post-treatment compared to that at 24h and reaching the control level. However, in the infected-nicotine group, the rate of decrease in the ratio was much higher at 24h compared to that of non-infected nicotine treated animals. At the 7th day, the ratio of infected/nicotine group increased toward the infected group level.

Chromosomal Aberration Test

Effect of *S. mansoni* infection: The frequencies of cells with different types of CAs were significantly higher (100-fold) in infected groups compared to that of the non-infected control (Table 2). Chromosome breaks, fragments, exchanges and polyploidy were observed only in the infected group (Figure 2). The frequency of cells with CA showed a significant increase in the 7 days-interval group compared to the 24h group.

Effect of nicotine: Administration of nicotine to the non-infected group increased the frequency of cells assessed after 24h and 7 days with chromosomal aberrations over 200 times the control. Chromatid and chromosome breaks, fragments, ring chromosomes, asymmetric

exchanges and polyploidy were observed in the nicotine-treated group at both assay intervals (Figure 2). Types of CA (with the exception of breaks and fragments which increased) decreased significantly 7 days after nicotine treatment compared to the frequencies scored at 24h.

Treatment of the *Schistosoma*-infected group with nicotine caused a highly significant increase in the percentages of aberrant cells, total CA, and all types of structural CA assayed at both 24h and 7-days. However, the incidences of several types of chromosomal abnormalities in the infected/nicotine group decreased significantly at 7 days.

Chromosomal Abnormalities in Diakinesis Metaphase-I Spermatocytes

Table 3 shows different types of chromosomal aberrations in dividing spermatocytes at diakinesis metaphase-I stages in different groups of mice. The types of chromosomal abnormalities in diakinesis metaphase-I spermatocytes observed in this study are mainly: X-Y univalents, autosomal univalents, ring quadrivalents (R-IV), chain quadrivalents (C-IV), chain hexavalents (C-VI), chain octavalents (C-VIII), chain trivalents plus one univalent (C-III+I) and polyploidy (Figure 3).

Effect of *S. mansoni* infection: Mice of the infected group had higher percentages of cells carrying chromosomal aberrations, total chromosomal aberrations, autosomal univalents and polyploidy compared to the non-infected control; these differences are all statistically significant (Student *t*-test). X-Y univalents show higher percentages in the infected groups compared to the non-infected control but the differences are not significant. R-IV configurations were observed only in the infected group.

Effect of nicotine: Nicotine treatment significantly increased the percentage of aberrant cells, total chromosomal aberrations, X-Y univalents, and autosomal univalents compared to the control (Table 3). R-IV, C-IV and fragments were observed only in the treated group and not in the control. Polyploid cells were more frequent in the nicotine-treated group but the difference was not significant compared to the control. The percentages of aberrant cells, total chromosomal aberrations, X-Y and autosomal univalents, C-IV configurations and fragments were elevated in the infected-nicotine mice and were significantly higher than that of the infected control. C-III+I figures were observed only in the infected/nicotine group. Polyploidy was higher in the infected-nicotine group but the difference was not significant compared to the infected control. The percentages of aberrant cells, total chromosomal aberrations and all observed types of chromosomal aberrations (Figure 3) showed a significant increase in the infected/nicotine group compared to the non-infected/nicotine group, except for X-Y univalents and R-IV configurations.

Evaluation of the Synergistic Interaction Between

Nicotine and *Schistosoma* infection

The results of infection and nicotine, singly or in combination, on the induction of chromosomal aberrations were further analysed to assess the interaction between the two causative agents. Tables 4, 5 and 6 give the calculated summation (expected) of the effect of single treatments compared with the (observed) combined effect.

Table 4 and Figure 4 show higher observed frequencies of total MNPCs compared with the expected frequencies. These differences, however, are not statistically significant.

There are highly significant differences between the observed and expected frequencies of different types of CA, suggesting a very strong synergistic effect of nicotine and infection with *S. mansoni* in mice (Table 5 and Figure 5).

Meiotic CAs in diakinesis metaphase-I spermatocytes do not show any synergistic effect of nicotine and infection: all types except for chain configurations have higher expected frequencies than were observed (Table 6 and Figure 6).

Table 1. Effect of Nicotine treatment on MNPCEs and MNNCEs in control non- infected and infected groups assayed 24 h and 7 days post treatment.

Animal Groups	Assay Time	M N P C E s (% ± S. E.)			MNNCEs (% ± S. E.)	PCE / NCE ± S. E.	
		Total MNPCEs	PCEs with 1 MN	PCEs with 2 MN			PCEs with 3 MN
Non-infected (Control) (Saline)	24 h	0.04 ± 0.03	0.04 ± 0.03	0	0	0.02 ± 0.02	1.15 ± 0.01
	7 days	0.04 ± 0.03 ***	0.04 ± 0.03 ***	0 **	0	0.05 ± 0.03 ***	1.15 ± 0.01 ***
Non-infected & Nicotine-treated	24 h	0.82 ± 0.05 *** ++	0.66 ± 0.05 *** ++	0.16 ± 0.05 *	0	0.31 ± 0.05 +	0.98 ± 0.03 ++
	7 days	0.56 ± 0.07 †††	0.42 ± 0.06 †††	0.14 ± 0.06 †	0	0.13 ± 0.05 ††	1.12 ± 0.01 †††
Infected	24 h	0.94 ± 0.08 †††	0.80 ± 0.05 †††	0.14 ± 0.05	0	0.18 ± 0.04	0.63 ± 0.01 ††† †††
	7 days	0.88 ± 0.04 *** ψψψ	0.82 ± 0.04 *** ψψψ	0.06 ± 0.03 *** ψψψ	0	0.15 ± 0.07 **	1.38 ± 0.01 *** ψψψ
Infected & Nicotine-treated	24 h	4.56 ± 0.10 *** ψψψ †††	3.10 ± 0.05 *** ψψψ †††	1.24 ± 0.11 *** ψψψ †††	0.22 ± 0.05 * ψ	0.44 ± 0.06 ψ +	0.44 ± 0.01 *** ψψψ †††
	7 days	2.07 ± 0.08	1.49 ± 0.04	0.49 ± 0.04	0.09 ± 0.04	0.25 ± 0.02	0.60 ± 0.01

*, ** and *** : Significant differences between each treated group and the control at each sampling time at p<0.05, 0.01 and 0.001 respectively.
 +, ++ and +++ : Significant differences between each treated group at 7 days compared to that at 24 h interval at p<0.05, 0.01 and 0.001 respectively.
 †, †† and ††† : Significant differences between each infected group and the corresponding non-infected one at p<0.05, 0.01 and 0.001 respectively.
 ψ, ψψ and ψψψ : Significant differences between each treated-infected group compared to treated non-infected group at p<0.05, 0.01 and 0.001 respectively.

MNPCEs : Micronucleated Polychromatic Erythrocytes
 MNNCEs : Micronucleated Normochromatic Erythrocytes
 MN : Micronucleus
 PCEs : Polychromatic Erythrocytes
 NCE : Normochromatic Erythrocyte

(Student t - test)

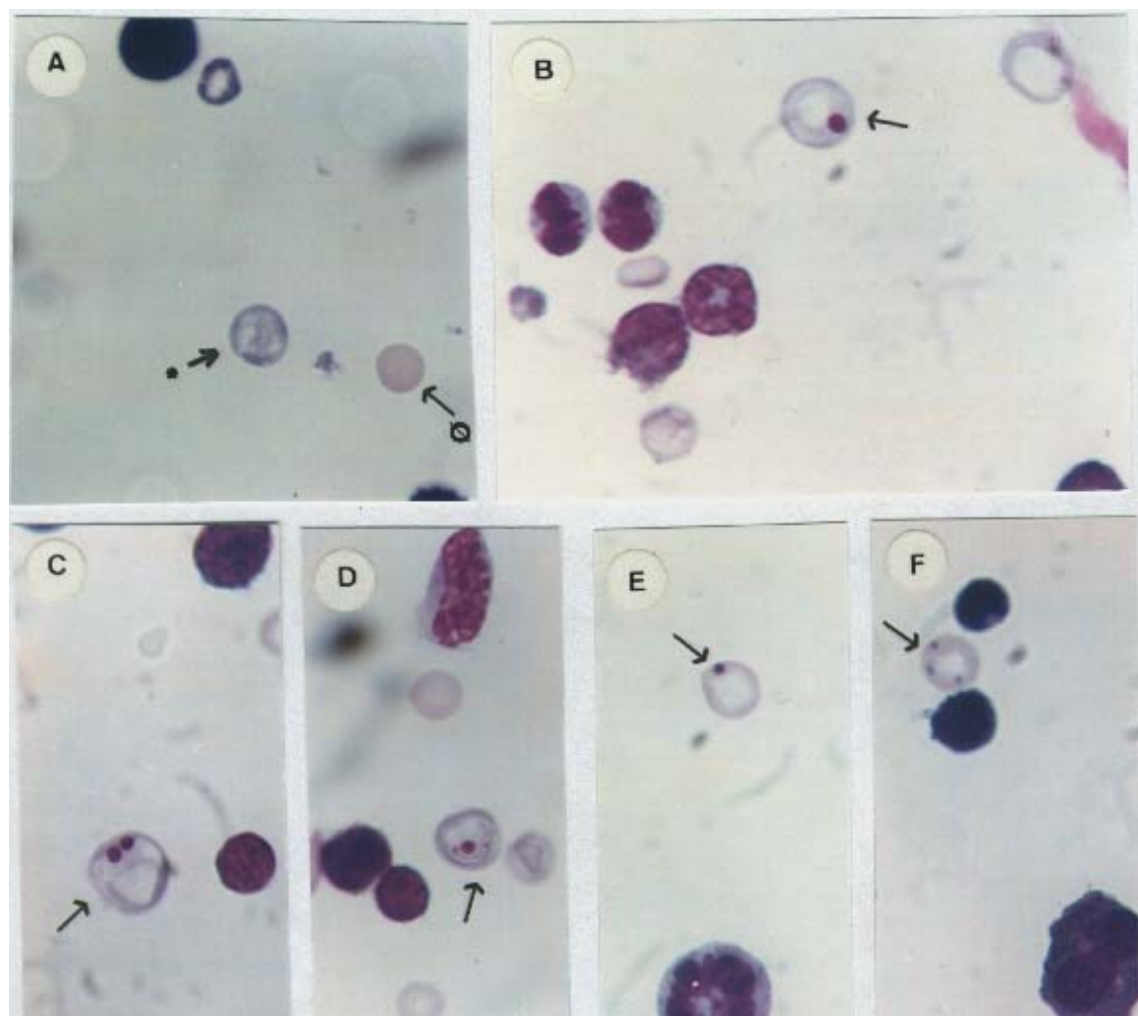


Figure 1. Bone marrow from a control and infected nicotine treated mice: (A) a normal PCE (•) and NCE (∅); (B) PCE with single MN; (C) PCE with 2 MN; (D) PCE with 3MN; (E) NCE with single MN and (F) NCE with 3MN. PCE, polychromatic erythrocyte; NCE, normochromatic erythrocyte; MN, micronucleus.

Table 2. Effect of Nicotine on the incidences of chromosomal aberrations in bone marrow cells of infected and non-infected mice assayed 24 h and 7 days after treatment.

Animal Groups	Assay time	Aberrant cells (% ± s. E.)	Types of chromosomal aberrations (% ± S. E.)							Polyploidy
			Total aberrations	Gaps		Breaks & Fragments	Exchanges			
				chromatid gaps	chromosome gaps		Centric fusions	Chromosome rings	Asymmetrical interchange	
Non-infected (Control) (Saline)	24 h	00.20 ± 0.13	0.20 ± 0.13	0	0	0	0.20 ± 0.13	0	0	0
	7 days	00.20 ± 0.13 ***	0.30 ± 0.20 ***	0.10 ± 0.10 ***	0.10 ± 0.10 ***	0 ***	0.10 ± 0.10 ***	0 *	0 ***	0
Non-infected & Nicotine-treated	24 h	44.50 ± 3.01 *** +++	53.00 ± 4.17 *** ++	32.00 ± 2.06 *** +++	9.40 ± 0.56 *** ++	4.20 ± 0.64 *** +++	3.70 ± 0.45 *** +++	0.70 ± 0.26 +	2.80 ± 0.44 * ++	0.20 ± 0.13
	7 days	27.00 ± 0.78 †††	29.33 ± 0.72 †††	13.22 ± 0.62 †††	6.15 ± 0.55 †††	7.56 ± 0.55 †††	1.20 ± 0.15 †††	0	0.44 ± 0.18 †	0.33 ± 0.24
Infected	24 h	20.30 ± 1.94 †††	22.50 ± 1.28 †††	15.40 ± 1.16 †††	2.90 ± 0.28 ††† +	1.50 ± 0.43 +++	1.80 ± 0.33 ††† +++	0	0.60 ± 0.27 ††	0.30 ± 0.21
	7 days	24.50 ± 1.22 *** ΨΨΨ	26.60 ± 1.99 *** ΨΨΨ	12.30 ± 1.67 *** ΨΨΨ	6.00 ± 1.32 *** ΨΨΨ	4.20 ± 0.33 *** ΨΨΨ	3.50 ± 0.22 *** ΨΨΨ	0	0.70 ± 0.21 *** Ψ	0
Infected & Nicotine-treated	24 h	60.20 ± 1.64 *** ΨΨΨ +++	110.10 ± 2.96 *** ΨΨΨ +++	66.10 ± 2.69 *** ΨΨΨ +++	21.10 ± 1.31 *** ΨΨΨ +++	6.90 ± 0.39 *** ΨΨΨ	9.10 ± 0.66 *** ΨΨΨ +++	1.40 ± 0.16 ** ΨΨ +	5.20 ± 0.47 ** ΨΨ +++	0.30 ± 0.15
	7 days	53.40 ± 0.52	80.80 ± 0.74	44.60 ± 0.72	13.10 ± 0.31	7.90 ± 0.26	12.50 ± 0.27	0.70 ± 0.16	2.00 ± 0.37	0

*, ** and *** : Significant differences between each treated group and its control in both infected and non-infected group and at each time. (p<0.05, 0.01 and 0.001, respectively)

+, ++ and +++ : Significant differences between the two sampling times for each treated group in both infected and non-infected mice. (p<0.05, 0.01 and 0.001, respectively)

†, †† and ††† : Significant differences between each infected group and the corresponding non-infected one. (p<0.05, 0.01 and 0.001, respectively)

Ψ, ΨΨ and ΨΨΨ : Significant differences between treated-infected group and the corresponding non-infected one. (p<0.05, 0.01 and 0.001, respectively)

(Student t - test)

Table 3. Effect of Nicotine on the frequencies of different chromosomal aberrations observed in diakinesis-metaphase I spermatocytes of infected and non-infected mice.

Animal Groups	Aberrant Cells (% ± S.E.)	Different types of chromosomal aberrations (% ± S.E.)									Polyploidy
		Total Aberrations	X-Y Univalents	Autosomal Univalents	R-IV	C-IV	C-VI	C-VIII	C-III+1	Fragments	
Non-infected Control (Saline)	8.50 ± 1.00	12.00 ± 1.73	2.00 ± 0.70	7.00 ± 1.68	0	0	0	0	0	0	3.00 ± 0.54
Non-infected & Nicotine-treated	28.33 ± 2.03 ***	38.00 ± 2.01 ***	8.30 ± 0.76 ***	16.67 ± 2.78 **	2.33 ± 0.76 **	5.67 ± 0.46 ***	0	0	0	1.00 ± 0.19 ***	4.00 ± 0.96
Infected	22.66 ± 1.55 +	31.00 ± 2.60 +++	4.33 ± 2.74	16.67 ± 2.93 ++	0.34 ± 0.21	1.32 ± 0.53 +	0	0	0	0.67 ± 0.46	7.67 ± 1.68 +
Infected & Nicotine-treated	40.33 ± 3.13 *** Ψ	59.38 ± 3.34 *** ΨΨΨ	9.33 ± 1.31 *	27.33 ± 1.99 ** ΨΨ	0.34 ± 0.28	11.33 ± 0.76 *** ΨΨΨ	0	0	0.38 ± 0.09 *** ΨΨΨ	1.67 ± 0.13 * Ψ	9.00 ± 1.18 ΨΨ

*, ** and *** : Significant difference between each treated group and its control in both infected and non-infected animals. (P<0.05, 0.01 and 0.001, respectively)

+, ++ and +++ : Significant difference between each infected group and the corresponding non-infected one. (P<0.05, 0.01 and 0.001, respectively)

Ψ, ΨΨ and ΨΨΨ : Significant difference between the infected-treated group and the non-infected one. (P<0.05, 0.01 and 0.001, respectively)

(Student t - test)

300 spermatocyte-I cells were counted per each group

Table 4. Expected and observed frequencies of MNPCEs after treatment of *S. mansoni* infected mice with nicotine.

Animal Groups	% of MNPCEs			
	Total MNPCEs	PCEs with 1 MN	PCEs with 2 MN	
24 h	Expected	1.76	1.46	0.30
	Observed	4.56	3.10	1.24
7 days	Expected	1.44	1.24	0.20
	Observed	2.07	1.49	0.49

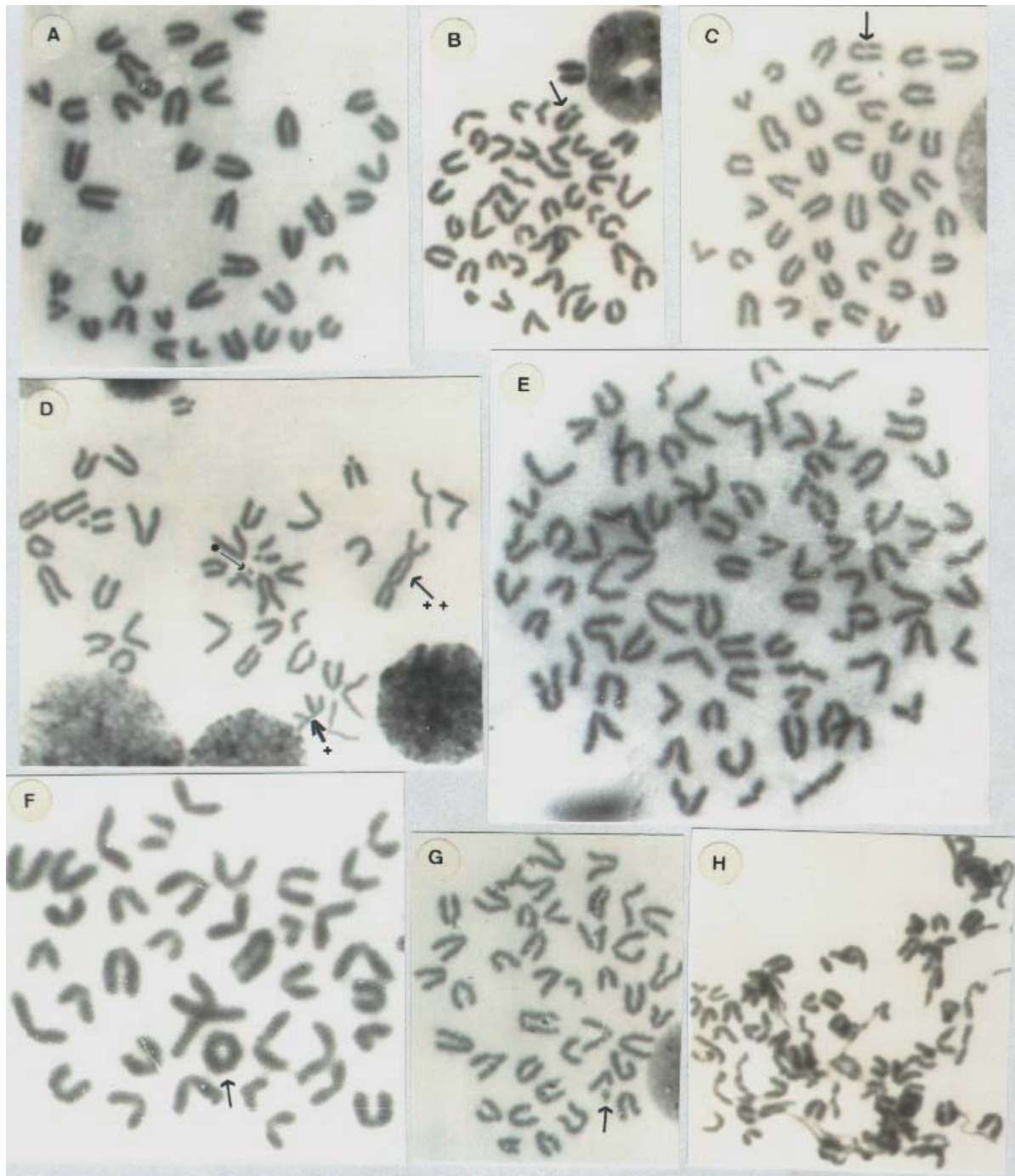


Figure 2. Bone marrow metaphase spreads showing: (A) normal chromosomes; (B) chromatid gap; (C) chromosome gap; (D) monocentric (+), dicentric fusions (++) and fragments (*); (E) polyploidy; (F) chromosome ring; (G) chromatid break and (H) stickiness from control (A) and infected, nicotine-treated mice (B, C, D, E, F, G, H).

Table 5. Comparison between the expected and observed frequencies of CAs in bone marrow cells after treatment of infected mice with nicotine.

Animal Groups		% of different types of Chromosomal Aberrations			
		Total Aberrations	Total Gaps	Breaks & Fragments	Total exchanges
24 h	Expected	75.50	59.70	5.70	9.60
	Observed	110.10 ^{***}	87.20 ^{**}	6.90	15.70 [*]
7 days	Expected	95.93	37.67	11.76	5.84
	Observed	80.80	57.70 [*]	7.90	15.20 [*]

*: Significant at $P < 0.05$; **: Significant at $P < 0.01$; ***: Significant at $P < 0.001$; (Chi-squared test)

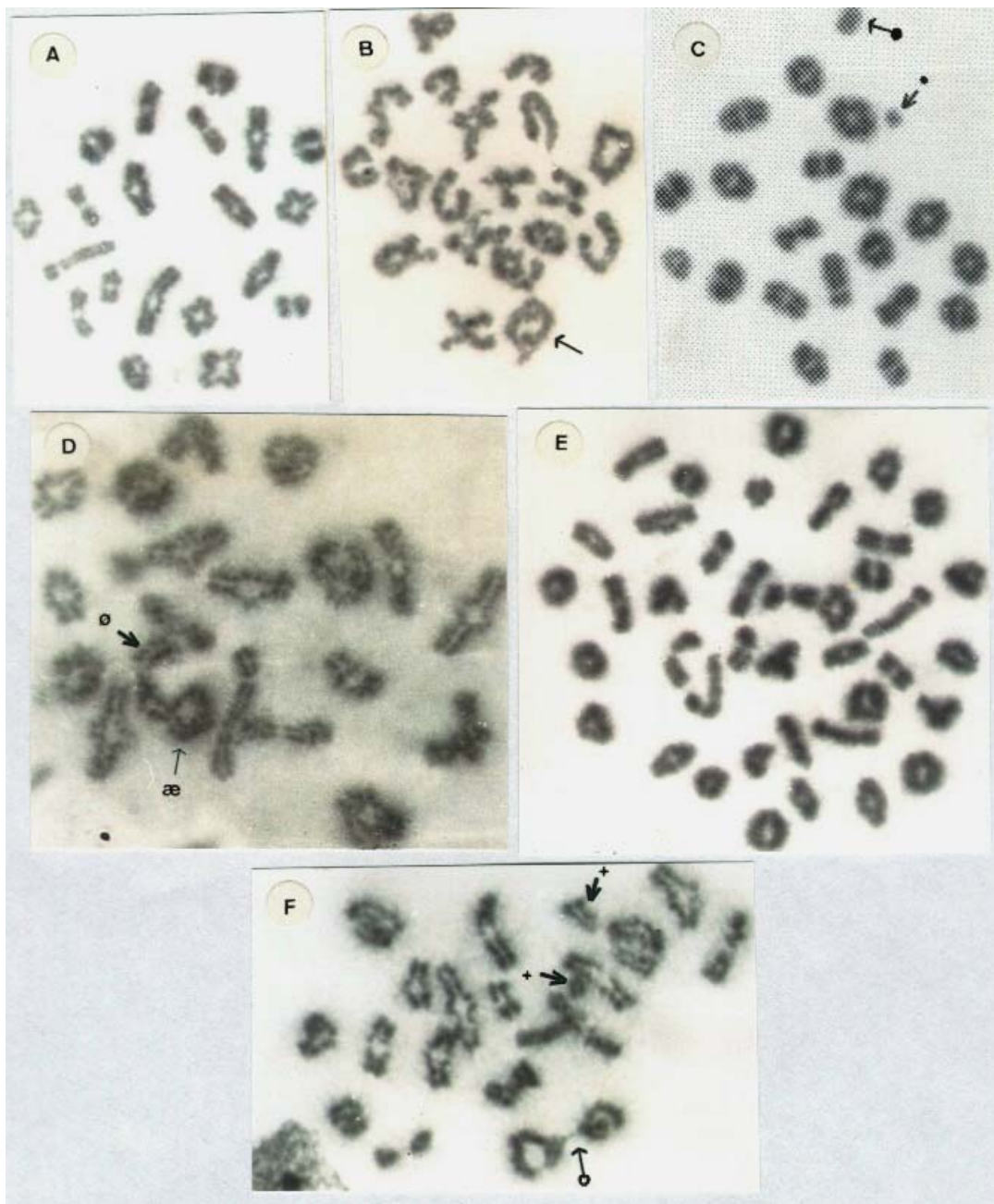


Figure 3. Spermatocyte diakinesis-I spreads showing: (A) normal spermatocyte diakinesis metaphase-I; (B) ring quadrivalent (R-IV); (C) autosomal univalent (*) and chromosome fragment (\uparrow); (D) chain trivalent (æ) plus one univalent (ø) (CIII-I); (E) polyploidy and (F) X-Y univalents (+) and chain quadrivalent (C-IV) (\uparrow) from control (A) and infected, nicotine-treated mice (B, C, D, E, F).

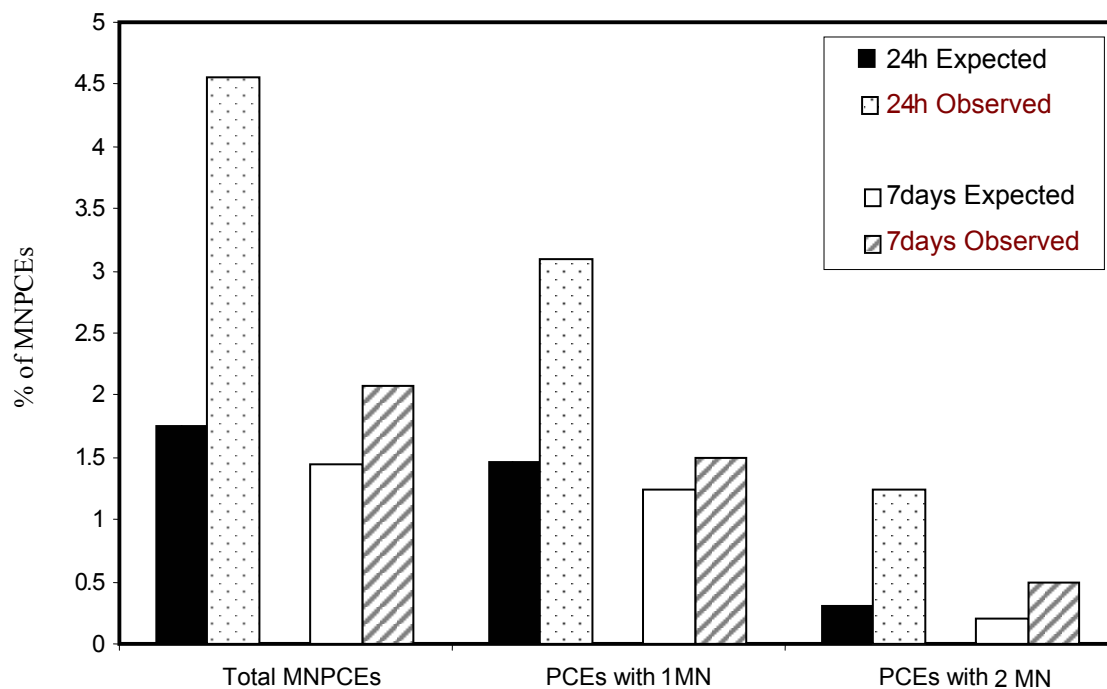


Figure 4. Expected and observed frequencies of MNPCEs after treatment of infected mice with nicotine. Exp., expected; Obs., observed; MNPCEs, micronucleated polychromatic erythrocytes; PCEs, polychromatic erythrocytes; MN, micronucleus.

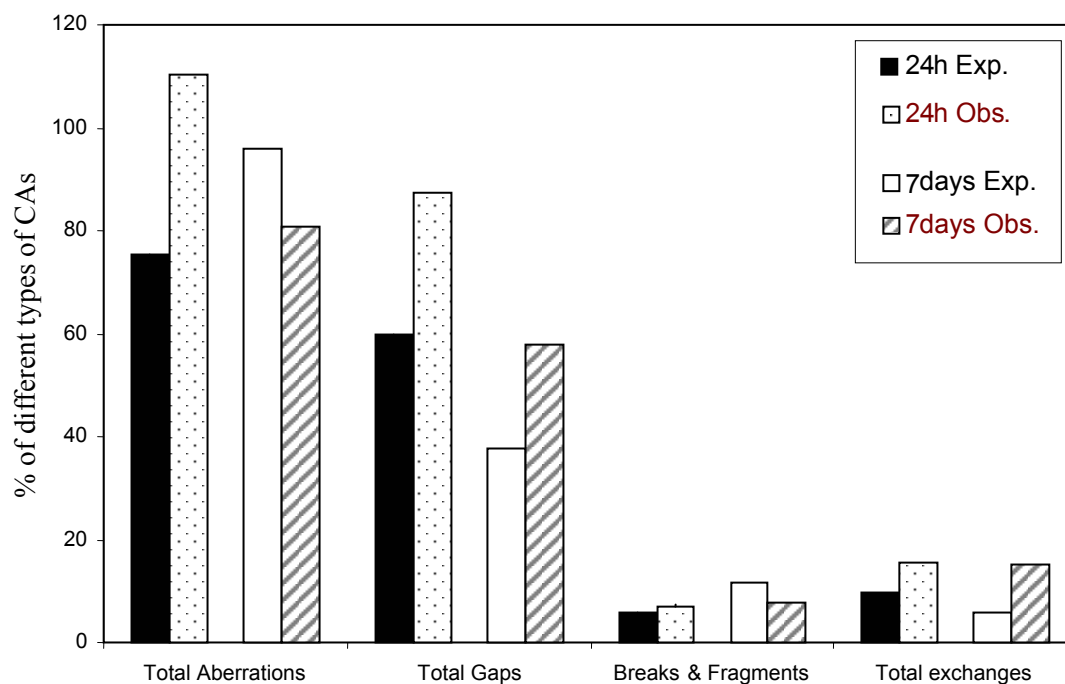


Figure 5. Comparison between the expected and observed frequencies of CAs in bone marrow cells after treatment of infected mice with nicotine. Exp., expected; Obs., observed; CAs, chromosomal aberrations.

Table 6. Comparison between the expected and observed frequencies of chromosomal aberrations in diakinesis-metaphase I spermatocytes after treatment of infected mice with nicotine.

	% of different types of chromosomal aberrations						
	Total aberrations	X-Y univalents	Autosomal univalents	R-IV Configurations	Total chain Configurations	Fragments	Polyploidy
Expected	69.00	12.63	33.34	2.67	6.99	1.67	11.67
Observed	59.38	9.33	27.33	0.34	11.71	1.67	9.00

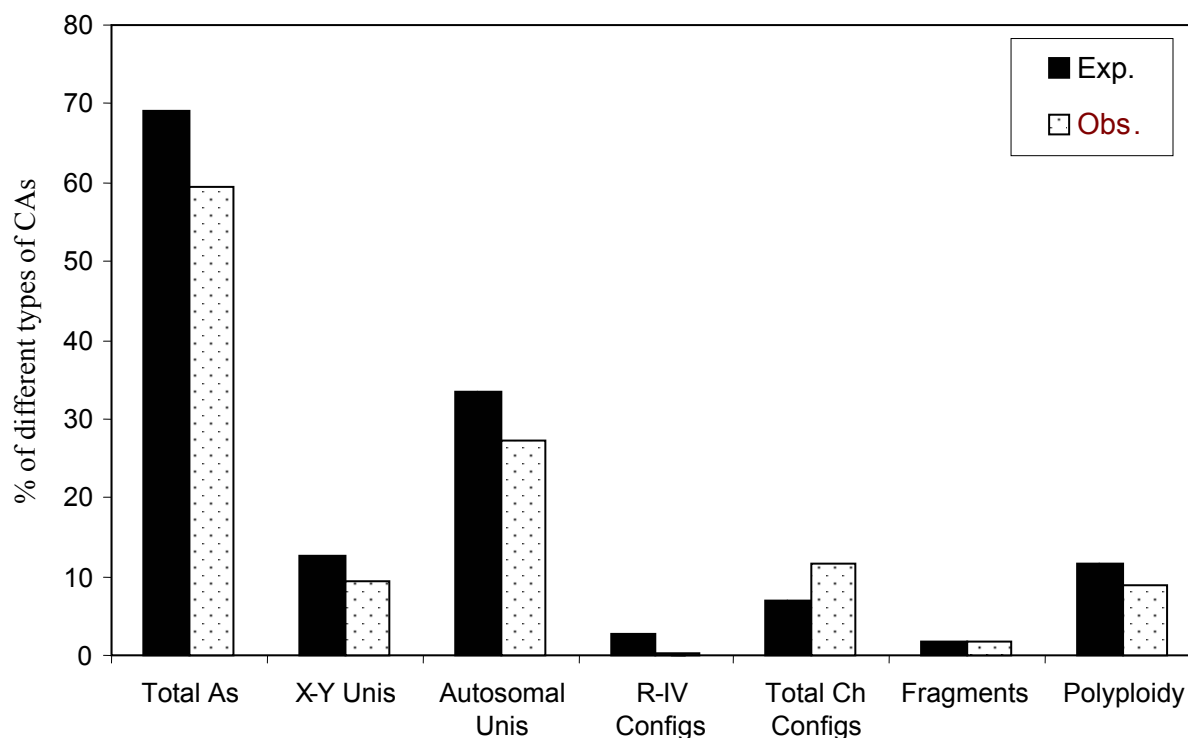


Figure 6. Expected and observed frequencies of chromosomal aberrations in diakinesis-metaphase I spermatocytes after nicotine treatment of infected mice. CAs, chromosomal aberrations; Exp., expected; Obs., observed; As, aberrations; Unis, univalents; Ch, chain; Configs, configurations.

DISCUSSION

The data obtained in this study demonstrate the potential mutagenicity of *S. mansoni* infection *per se* in mice. It has been suggested that *Schistosoma* infection results in the release of certain toxins, some of which are mutagens (Raafat *et al.* 1984). Such mutagens have been correlated with many chromosomal abnormalities (Hamada *et al.* 1992). The absence of differences between the two sampling times in the infected groups with respect to the incidence of micronuclei suggests the persistence of the induced genotoxic damage and/or delayed DNA repair.

Three possible mechanisms have been put forward to account for the observed CAs: The release of an array of mutagenic metabolites by activated inflammatory cells in response to *Schistosoma* infection (Schuber 1985); Increased nitrosation capacity involving bacteria present as secondary infections in schistosomiasis (Hicks 1982); Rapidly proliferating tissues sustain greater genotoxic damage, increased sensitivity to carcinogenic-induced DNA damage and decreased repair (Preston-Martin *et al.* 1990).

Schistosoma haematobium patients represent a high-risk group in which such factors may interact. Infected individuals are characterized by chronic stimulation of cell

proliferation in the bladder epithelium. This stimulation results from the release of ova by mature worms into the veins of the perivesical plexus and the penetration of these eggs through the urothelium into urine, provoking ulceration, inflammation and regenerative cell proliferation (Tawfik 1988). Reactive oxygen species produced by inflamed leucocytes and macrophages during *Schistosoma* infection induce DNA strand breakage, chromosomal damage and malignant transformation in mammalian cells cultured with such activated leucocytes (Weitzman & Gordon 1990).

The present study showed that nicotine treatment causes a highly significant increase in the incidence of MNPCs 24h post-treatment compared to the control. Such incidence decreased at 7 days but remained significantly higher than that of the control. This may be attributed to the lingering genotoxic effect of nicotine, or continued production of micronucleated cells in bone marrow as a result of damage in the stem cell populations (Au *et al.* 1990). Elevated frequencies of micronuclei in the oral mucosa, lung cells and lymphocytes were reported in individuals with high risk for oral and lung cancer due to cigarette smoking and chewing tobacco (Cheng *et al.* 1996).

Treatment of *Schistosoma*-infected mice with nicotine exhibited a remarkably high incidence of MNPCs compared to either infected mice or to the nicotine-treated/non-infected group. The presence of PCs with 3 micronuclei only in the infected/nicotine group illustrates the synergistic damage effect of nicotine and infection.

The PCE/NCE ratio was significantly lower in the nicotine treated group compared to the control group and this decrease was more prominent in the infected/nicotine group. It is well known that the ratio of PCEs/NCE decreases after treatment with chemicals that inhibit proliferation of bone marrow cells (Fujie *et al.* 1990). This finding suggests that both nicotine and infection induce toxicity in bone marrow cells. The increase in the PCE/NCE ratio at 7 days indicates increased proliferation of bone marrow.

Treatment of infected and non-infected mice with nicotine significantly increased the frequencies and types of chromosomal aberrations in bone marrow cells. Many carcinogens were reported to induce more exchange-type aberrations in addition to break-type aberrations (Ischidate *et al.* 1981). Machemer and Lorke (1981) considered that 10 chromosomal exchanges among 807 metaphases represent a marked mutagenic effect of cyclophosphamide. In the present study, nicotine induced 35/1000 exchanges in the control group and 66/1000 in the *Schistosoma*-infected ones. The presence of different types of chromosome exchange, including rings and centric fusions, provides evidence of the mutagenic activity of both nicotine and *Schistosoma* infection. These results strongly confirm those obtained by the micronucleus test, which demonstrate the potential mutagenicity of nicotine and *Schistosoma* infection.

Analysis of translocations and other chromosomal abnormalities in spermatocyte diakinesis metaphase-I stages gives an evaluation of the genetic hazard of the tested chemical and possible heritable abnormalities (Leonard 1977). Infection of mice with *Schistosoma* cercariae induced significantly high frequencies of CA in spermatocyte I-metaphases. Gametes carrying unbalanced translocations usually result in lethality of the zygote or impaired fertility and, in other cases, cause sterility (Leonard 1977).

While the mechanisms involved in the mutagenicity of nicotine are not yet clear, tobacco smoke contains precursors that upon nitrosation cause mutagenicity and carcinogenicity via oxidative damage (Cerutti 1988). Tobacco users were reported to exhibit increased endogenous nitrosation (Nair *et al.* 1986). The possibility of endogenous formation of carcinogenic and mutagenic nitrosamines from nicotine and its metabolites has been suggested (Hoffmann 1989). Free radical-induced oxidative damage has been suggested to play a role in the pathogenesis of smoking-related disorders (Kodama *et al.* 1997); many oxidants and free radicals are found in cigarette smoke (Church and Pryor 1985). In addition to smoke-borne radicals, cigarette smoking can also induce endogenous reactive oxygen species that enhance recruitment of phagocytes, which in turn stimulate further the production

of reactive oxygen species (Kalra *et al.* 1991). Reactive oxygen species are capable of reacting with and inducing lesions in biological materials, including single-strand breaks in DNA (Max 1987). The presence of increased level of 8-hydroxydeoxyguanosine (8-OH-dG) has been proposed as a non-invasive biomarker of oxidative damage in humans (Loft *et al.* 1993); smokers were shown to excrete in their urine 50% more 8-OH-dG than non-smokers.

Our study also suggests that infected individual mice administered with nicotine are prone to have more chromosomal damage in their cells. Similar results were reported in the bladder mucosa of *Schistosoma*-infected individuals who are cigarette smokers (Rosin & Anwar 1992). Data from epidemiological studies suggest that interaction does occur in individuals with bladder *Schistosoma* infection and smoking, and this effect may be additive or multiplicative with respect to cancer risk (La Vecchia *et al.* 1991).

The synergistic interaction of nicotine and toxins released by the parasite *S. mansoni* in infected mice can be brought about by similar mechanisms, which operate independently. The increased susceptibility of infected mice to further mutagenic or carcinogenic action by nicotine or similar agents is likely to suppress dramatically the initial activation of the inherent chromosome repair enzyme systems in treated animals. Similarly, the reactions triggered by the released toxins will be substantiated more easily in the presence of nicotine. Furthermore, Trush *et al.* (1990) suggested that cigarette smoking increased chromosome damage in cases of parasitic infection by conversion of promutagens to mutagens via oxidant- and peroxidase-dependent reactions.

It can be concluded from the present study that *S. mansoni* infection and nicotine, independently, have pronounced clastogenic effects on the mouse somatic and meiotic chromosomes. The results suggest that tobacco smoking by schistosomiasis individuals would substantially increase a genetic risk and, possibly, carcinogenic risk due to a synergistic interaction between nicotine and infection. Extrapolation of data obtained using experimental models to man has to be verified through further experimentation on humans; however, the results obtained in this study justify issuing a warning to schistosomiasis patients to refrain from smoking till they are cured from the infection.

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