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ORIGINAL ARTICLE

Cytogenetic effects of sildenafil citrate (Viagra) on SWR/J mouse bone marrow cells

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Mitotic index

Abstract The present study was conducted to investigate the cytogenetic effects of sildenafil citrate in SWR/J mouse bone marrow cells. Thirty-six males and 36 females were used and divided into four groups. Each group contained 18 animals (9 males and 9 females), weighing 30–35 g. These animals were orally administered with a single dose of 13, 26 or 40 mg/kg sildenafil citrate solution. A control group received normal saline in an identical condition. The animals were sacrificed at 12, 24 or 48 h, after the treatment. Chromosome aberrations were investigated in 50 metaphases per animal.

No significant differences in the percentages of mitotic indices or in the frequencies of chromosome aberrations were observed between treated male and female mice at any doses or at any time intervals used, therefore, data from the two sexes were pooled when analyzed statistically.

No significant ($p < 0.05$) differences in the percentages of mitotic indices or in the frequencies of chromosome aberrations were observed between sildenafil citrate-treated groups and the control group at any doses or at any time intervals used. However, the percentages of centromeric adhesions increased significantly ($p < 0.01$) in treated groups as compared with the control group at all doses and at all time intervals used.

In conclusion, the results of the present study suggest that sildenafil citrate does not have cytogenetic effects on mouse bone marrow cells, but the centromeric adhesions induced by this drug need further studies to confirm them and to investigate the possible mechanism(s) responsible for such effect.

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1. Introduction

Sildenafil citrate (Viagra) is an oral medication used to treat male erectile dysfunction. It is a cyclic guanosine-specific phosphodiesterase (PDE) type 5 inhibitor that prevents the metabolism of cGMP which produces arterial smooth muscle relaxation within the corpora cavernosa of the penis and ultimately enhances penile tumescence (Krenzelok, 2000; Ji et al., 2005; Padma-Nathan, 2006). Sildenafil citrate has

demonstrated effectiveness in men with erectile dysfunction associated with prostatectomy, radiation therapy, diabetes mellitus, certain neurologic disorders and drug therapy (Boyce and Umland, 2001). It is also used in the treatment of pulmonary arterial hypertension and it could be an alternative in the treatment of intrauterine growth retardation and premature delivery (Derchi and Forni, 2005; Villanueva-Garcia et al., 2007). Moreover, there is now robust evidence that its use in recreation has gained credence in young healthy males as a sexual enhancer as well in older men requiring it for importance problems (Vatansever et al., 2003; Smith and Romanelli, 2005; Glenn et al., 2009).

The widespread use of sildenafil citrate is of concern, because it is a selective type 5 phosphodiesterase inhibitor and the phosphodiesterase inhibitors have been shown to affect sperm function and embryo development (McKinney et al., 1994; Scott and Smith, 1995; Glenn et al., 2009). Furthermore, to the best of our knowledge, there is only one report concerning with the cytogenetic effect of this drug on experimental animals (Lemus-Varela et al., 2006).

It is therefore imperative that the cytogenetic effects of sildenafil citrate be investigated before it is incorporated without

Table 1 Effect of various doses of sildenafil citrate (Viagra) on the mitotic index (MI) in bone marrow cells of SWR/J mice after 12 h of treatment.

Dose (mg/kg)	No. of females used	No. of cells examined	No. of dividing cells	Mitotic index (%)
Control	6	6000	244	4.07
13	6	6000	233	3.88
26	6	6000	240	4.00
40	6	6000	232	3.87

Table 2 Effect of various doses of sildenafil citrate (Viagra) on the mitotic index (MI) in bone marrow cells of SWR/J mice after 24 h of treatment.

Dose (mg/kg)	No. of females used	No. of cells examined	No. of dividing cells	Mitotic index (%)
Control	6	6000	253	4.22
13	6	6000	259	4.32
26	6	6000	251	4.18
40	6	6000	241	4.02

Table 3 Effect of various doses of sildenafil citrate (Viagra) on the mitotic index (MI) in bone marrow cells of SWR/J mice after 48 h of treatment.

Dose (mg/kg)	No. of females used	No. of cells examined	No. of dividing cells	Mitotic index (%)
Control	6	6000	258	4.30
13	6	6000	238	3.97
26	6	6000	242	4.03
40	6	6000	244	4.07

Table 4 Effect of various doses of sildenafil citrate (Viagra) on the chromosomal aberrations in bone marrow cells of SWR/J mice after 12 h of treatment.

Dose (mg/kg)	No. of animals used	No. of cells examined	No. and types of numerical chromosomal aberrations					No. and types of structural chromosomal aberrations										
			Hypoploidy (2n−)		Hyperploidy (2n+)		Total	B		CA		Cad		G		F		Total	
			No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Control	6	300	–	–	2	0.7	2	0.7	1	0.3	1	0.3	2	0.7	–	–	1	0.3	5	1.7
13	6	300	–	–	2	0.7	2	0.7	3	1.0	3	1.0	11	3.7*	–	–	2	0.7	19	6.3**
26	6	300	–	–	6	2.0	6	2.0	–	–	3	1.0	14	4.7**	–	–	1	0.3	18	6.0**
40	6	300	3	1.0	6	2.0	9	3.0	3	1.0	2	0.7	17	5.7**	1	0.3	2	0.7	25	8.3**

B = break, CA = centromeric attenuation Cad = centromeric adhesion, G = gap, F = fragment.
* Differences are statistically significant from the control group at $p < 0.05$.
** Differences are statistically significant from the control group at $p < 0.01$.

Table 5 Effect of various doses of sildenafil citrate (Viagra) on the chromosomal aberrations in bone marrow cells of SWR/J mice after 24 h of treatment.

Dose (mg/kg)	No. of animals used	No. of cells examined	No. and types of numerical chromosomal aberrations						No. and types of structural chromosomal aberrations											
			Hypoploidy ($2n-$)		Hyperploidy ($2n+$)		Total		B		CA		Cad		G		F		Total	
			No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Control	6	300	–	–	2	0.7	2	0.7	1	0.3	1	0.3	2	0.7	–	–	1	0.3	5	1.7
13	6	300	–	–	5	1.7	5	1.7	–	–	3	1.0	14	4.7**	–	–	1	0.3	18	6.0**
26	6	300	3	1.0	1	0.3	4	1.3	–	–	4	1.3	18	6.0**	–	–	–	–	22	7.3**
40	6	300	2	0.7	3	1.0	5	1.7	2	0.7	5	1.7	22	7.3**	–	–	2	0.7	31	10.3**

B = break, CA = centromeric attenuation, Cad = centromeric adhesion G = gap F = fragment.
** Differences are statistically significant from the control group at $p < 0.01$.

Table 6 Effect of various doses of sildenafil citrate (Viagra) on the chromosomal aberrations in bone marrow cells of SWR/J mice after 48 h of treatment.

Dose (mg/kg)	No. of animals used	No. of cells examined	No. and types of numerical chromosomal aberrations						No. and types of structural chromosomal aberrations											
			Hypoploidy ($2n-$)		Hyperploidy ($2n+$)		Total		B		CA		Cad		G		F		Total	
			No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Control	6	300	–	–	2	0.7	2	0.7	1	0.3	1	0.3	2	0.7	–	–	1	0.3	5	1.7
13	6	300	2	0.7	5	1.7	7	2.3	–	–	6	2.0	14	4.7**	–	–	1	0.3	21	7.0**
26	6	300	–	–	4	1.3	4	1.3	2	0.7	8	2.7	20	6.7**	–	–	2	0.7	32	10.7**
40	6	300	–	–	5	1.7	5	1.7	4	1.3	3	1.0	24	8.0**	2	0.7	2	0.7	35	11.7**

B = break, CA = centromeric attenuation, Cad = centromeric adhesion G = gap F = Fragment.
** Differences are statistically significant from the control group at $p < 0.01$.

caution into routine clinical use. So, the aim of the present study was to investigate the possible cytogenetic effects of this drug on SWR/J mouse bone marrow cells.

2. Materials and methods

Inbred SWR/J mice, 10–12 weeks old and weighing 30–35 g were used throughout the study. Animals were kept and bred in an environmentally controlled room at a temperature of 22 ± 1 °C, a relative humidity of 45 ± 5% and a light/dark cycle of 10/14 h. Rodent chow (commercially available in Saudi Arabia) and water were offered *ad libitum*. A total of 36 males and 36 females were used and divided into 4 groups, each group contained 18 animals (9 males and 9 females). Animals of groups II–IV were orally treated with a single dose of 13, 26 or 40 mg/kg body weight of sildenafil citrate (Plizer Inc., New York, USA), which correspond to 2, 4 or 6 times human equivalent 50 mg, dissolved in sterile normal saline. Animals of group I were similarly treated with the vehicle only (0.4 ml sterile normal saline) and served as a control group. The animals were killed by cervical dislocation 12, 24 or 48 h following the treatment and the cytogenetic effects of the drug on those animals were evaluated using *in vivo* bone marrow cells.

The methods of Adler (1984) and Preston et al. (1987) were used for chromosome preparations. A minimum of 10 slides were prepared and 50 well spread and distinctly identifiable metaphases from each mouse were selected. Each selected metaphase was examined using the 100× oil immersion objective of a Zeiss microscope for detecting possible chromosome aberrations. Prior to scoring the drug effect on the chromosomes, the slides were covered and coded. The chromosome aberrations scanned were: chromatid gaps (G), chromatid breaks (B), fragments (F), centromeric attenuations and adhesions (Cad), hypoploidy and hyperploidy. Photomicrographs of selected metaphases were taken under bright illumination using 100× oil immersion objective and 10× eyepiece.

The data obtained were statistically analyzed using a 2×2 contingency table (X^2) (Sokal and Rohlf, 1981).

3. Results

In the present study, no significant differences in the percentages of mitotic indices or in the frequencies of chromosome aberrations were observed between sildenafil citrate-treated male and female mice at any dose levels or at any time intervals used. Accordingly, the data obtained from the two sexes were pooled together and statistically analyzed.

Data in Tables 1–3 show no significant differences ($p > 0.05$) between sildenafil citrate-treated groups and the control group in the percentages of mitotic indices of bone marrow cells at any dose levels or at any time intervals used

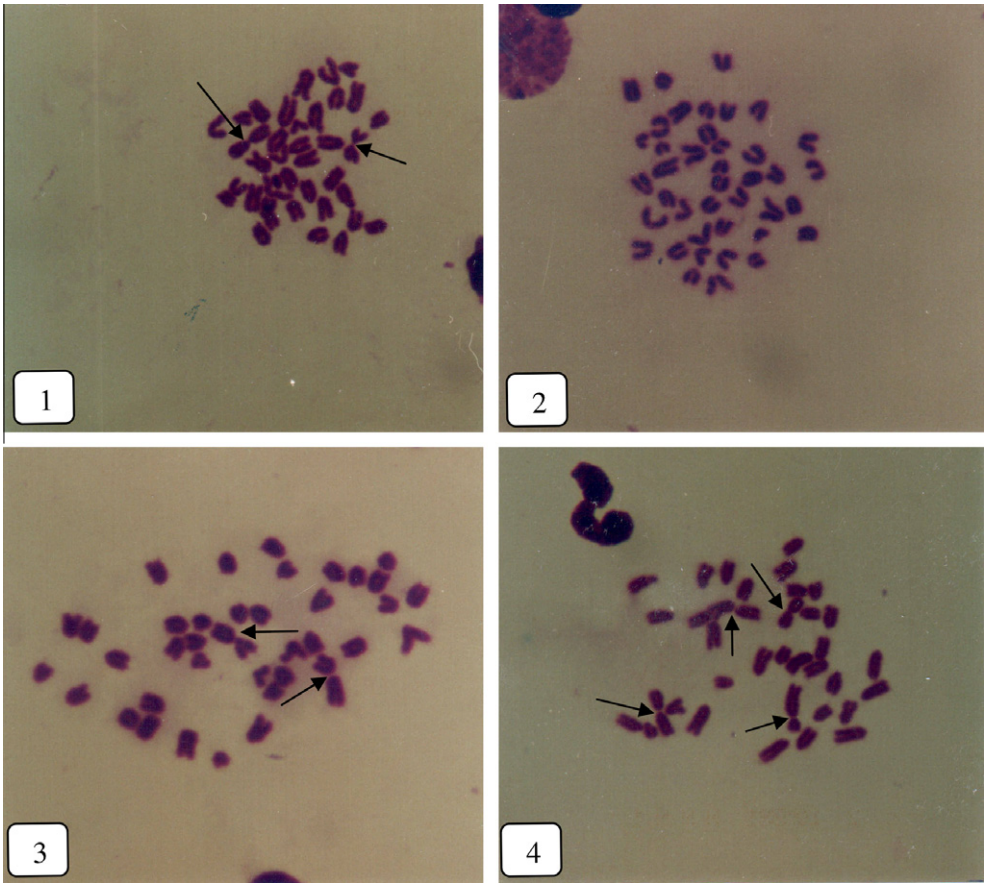

Figure 1 Photomicrograph of mouse bone marrow cells at normal metaphase stage, (2). In metaphase images, (1), (3) and (4) arrows showing the centromeric adhesions (Cad), observed in sildenafil-treated animals.

in the present study. Moreover, data in Tables 4–6 do not show any significant differences in the percentages of polyploidy and fragments in bone marrow cells between sildenafil citrate-treated groups and the control group at any doses or at any time intervals used.

However, data in Tables 4–6 and Fig. 1 show that the percentages of centromeric adhesions in bone marrow cells are highly significantly increased ($p < 0.01$) in sildenafil citrate-treated groups as compared with the control group at all doses and at all time intervals used in the present study.

4. Discussion

The present results clearly demonstrate that a single oral administration of 13, 26 or 40 mg/kg body weight of sildenafil citrate does not have any significant effects on the mitotic indices or on the induction of chromosomal aberrations (both numerically and structurally) in proliferative cells of the bone marrow of SWR/J mice. Our results are in an agreement with the reported non-effective role of sildenafil citrate in the induction of significant micronuclei in *Callithrix jacchus*, a primate model (Lemus-Varela et al., 2006). But, to the best of our knowledge, no other studies have been conducted to evaluate the cytogenetic effects of this drug in other experimental animals.

However, all dose levels of sildenafil citrate significantly increased the percentages of centromeric adhesions in bone marrow cells of sildenafil citrate-treated groups at all time intervals used in the present study. And again, to the best of our knowledge, no other reports have been documented concerning with such effect for this drug. Therefore, further studies are needed to confirm our results and to investigate the mechanism(s) responsible for the induction of centromeric adhesions. However, centromeric adhesions could well be related to a specific affinity of sildenafil citrate for A–T base pairs in the A–T rich repetitive DNA heterochromatin of the pericentromeric regions of the chromosomes (Kusyk and Hsu, 1976). Such affinity could render these regions to be more susceptible to adhere to each other giving rise to such effect. However, further studies are needed to elucidate the real mechanism(s) underlie this effect.

5. Conclusions

The present results suggest that sildenafil citrate does not have cytogenetic effects on mouse bone marrow cells. However, the centromeric adhesions induced by this drug need further studies to confirm them and to investigate the possible mechanism(s) responsible for this effect.

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