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Dermal exposure to the herbicide-paraquat results in genotoxic and cytotoxic damage to germ cells in the male rat

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The effects of exposure to low doses of paraquat, a herbicide, via the dermal route were studied on the spermatozoa of Sprague-Dawley rats. Paraquat (1, 1'-dimethyl-4, 4'-bipyridinium dichloride) was administered once a day for five days, at intervals of 24 h at 0, 6, 15 and 30 mg/kg, and the rats were sacrificed on days 7, 14, 28, and 42 after the last exposure. The sperm suspensions were obtained by mincing the caudae epididymes and ductus deferens for the purpose of performing a sperm morphology test, sperm count and analysis of sperm mortality and sperm motility, as per the standard procedures. The sperm count was decreased ($p < 0.05$) only on days 7 and 14 but sperm abnormalities increased on all days ($p < 0.05$). Sperm mortality increased at higher dose-levels ($p < 0.05$) except on day 42, and motility was affected by 30 mg/kg only on day 42. In conclusion, paraquat is a genotoxic and cytotoxic agent to germ cells in the male rat.

Key words: herbicides, pesticides, sperm abnormality, sperm count, sperm mortality, testicular function

INTRODUCTION

Paraquat (Paraquat dichloride; 1, 1'-dimethyl-4, 4'-bipyridinium dichloride) is a non-selective redox-cycling agroherbicide commonly used in weed control. Its herbicidal properties are enhanced by the addition of a single electron to the parent cation, thus generating the free radicals, owing to which it is highly toxic in almost all *in vitro* and *in vivo* systems [2, 8]. Consequently it has been observed as a mutagenic agent under different test conditions involving, for example, micro-organisms and cultured mammalian cells [9, 12]. Increased frequencies of sister-chromatid exchanges and chromosome aberrations have been observed in Chinese hamster pneumocytes exposed to paraquat [15, 16]. Intraperitoneal injection of paraquat to adult male ICR mice induced the formation of micronuclei in the peripheral blood and bone marrow cells [6]. Moreover, a recent study revealed that paraquat increased the incidence of micronuclei in polychromatic erythrocytes in the bone marrow of rats after a single dermal exposure [3]. Gene mutations and chromosomal aberrations have also been reported in several organisms living in a waste water reservoir contaminated by a paraquat manufacturing factory [4] and also in human lymphocytes *in vitro* [14].

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Its germ cell mutagenic potential in terms of increased sperm abnormalities was reported at dose-levels of 0.5, 1.5 and 3 mg/kg, after 1 to 3 weeks of intraperitoneal exposure in BALB/c mice [13]. Occupational or environmental exposure to paraquat or other pesticides is a daily affair for farmers mainly via oral or/and dermal routes. The latter route is a common means of exposure to paraquat and this pesticide is in agricultural use in more than 130 countries. However, there are few studies on its effects on male germ cells. The aim of the present study was to investigate the toxic effects of paraquat via the dermal route on germ cells in the male rat.

MATERIAL AND METHODS

Chemicals

Paraquat was purchased from Sigma Chemicals, USA (Lot No. 092K1359). All other chemicals and reagents used were of analytical grade.

Animals

Adult male Sprague Dawley rats (200–220 g body weight) were procured from the institutional animal house of University Sains Malaysia and housed in polypropylene cages with 3–4 animals per cage on paddy husk bedding under a controlled temperature of 21–25°C. All the animals received standard laboratory chow and water *ad libitum* during the experimental period.

Preparations of animals and paraquat treatment

The animals were allowed to acclimatise for a week and then were segregated into control and treatment groups. The control group consisted of 24 rats, which were further segregated into four groups of six animals each. The treatment group had 72 rats, which were segregated again into 12 groups of 6 rats each. The treatment groups received 6, 15 or 30 mg/kg of paraquat, equivalent to 1/15, 1/6 and 1/3 of $LD_{50,}$ for 5 consecutive days at intervals of 24 h. Approximately 24 h prior to the first application of paraquat the fur on the back of each rat was carefully shaved without causing any skin abrasions. This procedure was performed only once before the first treatment and was not carried out during the treatment. Paraquat was applied uniformly over the shaved area of skin with porous gauze for 4 hours. The area of paraquat application was further covered by an adhesive tape and restrainers were used to prevent the rat from reaching to the area of application. At the end of the fourth hour the skin was cleaned with distilled water. The control groups of rats also underwent similar preparation but were exposed to distilled water. Following the last treatment the rats were sacrificed by cervical dislocation on days 7, 14, 28 and 42.

Sperm count

A laparatomy was conducted and the reproductive system was exposed. The epididymis was separated from the testis. The cauda epididymis was further minced in 1 mL of phosphate buffered saline (pH 7.4) and the suspension thus obtained was filtered through a 80 μ nylon mesh. To the filtrate one drop of 1% eosin Y was added and left for 30 minutes. The stained sperm suspension was sucked slowly into a leukocyte haemocytometer exactly up to the 0.5 mark and then further diluted with phosphate buffered saline up to the 11 mark and mixed thoroughly. The diluted suspension was charged into a Neubauer counting chamber. The sperm count was performed according to the standard procedure [10, 17]. Briefly, the sperm in 8 squares, excluding the central erythrocyte area, were counted and the total count was then multiplied by 5×10^4 to obtain total sperm per epididymis.

Sperm morphology test

For the evaluation of abnormal sperm morphology smears were prepared from the filtrate on clean glass slides and dried. One thousand sperm per animal were screened and classified into normal and different types of abnormal spermatozoa as described earlier [11, 18]. The different types of abnormal spermatozoa were headless, double headed, microcephalous, defective at the cephalocaudal section, hookless, banana-shaped, amorphous, coiled, double tailed and broken tailed. Total sperm abnormality was expressed as percentage incidence per group.

Sperm motility and mortality

In order to estimate the effects on these parameters the ductus deferens was removed and placed in 1 mL of normal saline. A sample of this sperm suspension was charged into Makler's counting chamber, sperm motility and mortality were recorded as per standard procedure, and sperm motility was graded [5]. Dead and immotile sperm were considered equivalent in recording the sperm motility.

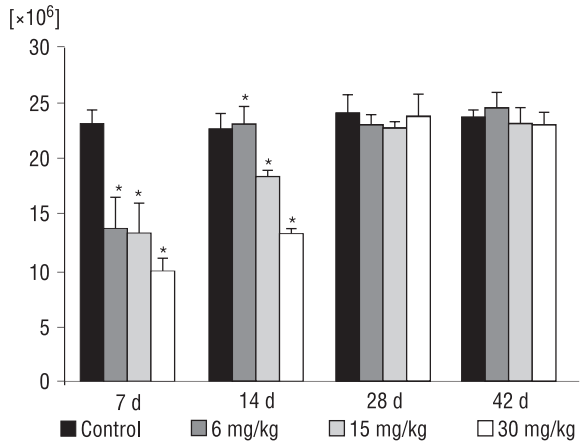

Figure 1. The effect of dermal exposure to paraquat on epididymal sperm count in the rat. Data are expressed as means ±SD. from 6 animals per group; *p < 0.05: vs. control; p < 0.05: 6 mg/kg vs. 15 mg/kg and 30 mg/kg on day 7; p < 0.05: 15 mg/kg vs. 30 mg on day 14; one-way ANOVA followed by Bonferroni's post hoc test.

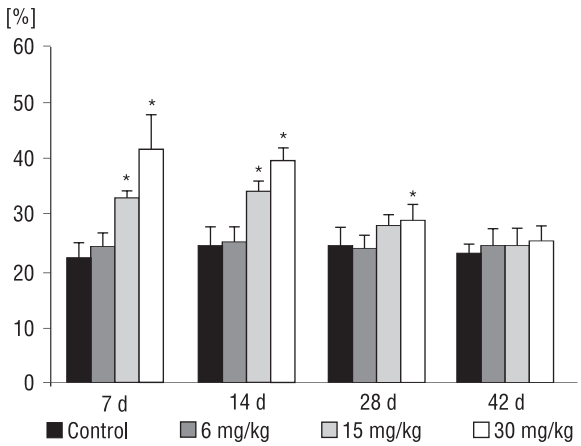

Figure 2. The effect of dermal exposure to paraquat on sperm mortality in the rat. Data are represented as means ±SD from 6 animals per group; *p < 0.05: vs. control; p < 0.05: 15 mg/kg vs. 30 mg/kg on day 7; one-way ANOVA followed by Bonferroni's post hoc test.

Statistical analysis

Data were expressed as means ±SD for each group (six rats per group). Differences were compared for statistical significance by one-way ANOVA followed by Bonferroni's post hoc test. The differences were considered as significant when $p < 0.05$.

RESULTS

The treatment with paraquat via the dermal route caused a decrease in sperm count on days 7 and 14 ($p < 0.05$; Fig. 1). The effect was in a dose-dependent pattern on day 7, whereas only two higher doses showed such an effect on day 14. Paraquat significantly induced the formation of abnormal sperm ($p < 0.05$; Table 1). On day 14 a dose-dependent increase was observed. On other sampling days however, only two lower doses showed a dose-dependent increase and the effect was less marked at a higher than at a lower dose-level, although not significantly so. At 6 mg/kg the abnormal sperm increased in a time-dependent pattern up to day 28 but decreased on day 42, whereas at 15 mg/kg the effect was greater on day 7 and at 30 mg/kg the effect was greater on day 14 and so on, with the effect subsiding in a time-dependent pattern (Table 1). Sperm mortality was increased in the paraquat-treated groups up to day 28. However, the induced mortality was significant only at higher dose-levels ($p < 0.05$) (Fig. 2). Sperm motility was just affected by 30 mg/kg only and unaffected by other doses.

DISCUSSION

For many years occupational or inadvertent exposure to pesticides has been unavoidable, at least for farmers. The main routes of exposure to these treacherous chemicals are the oral and the dermal, although the latter may be the more common. The objective of the present study was to examine the adverse effects on spermatozoa of a commonly used herbicide, namely paraquat. The results showed that paraquat exerted cytotoxic effects on germ cells, especially on epididymal sperm (on day 7) and late spermatids (on day 14), thus decreasing the sperm count. The lack of effect on days 28 and 42 indicates that paraquat did not affect spermatocytes and spermatogonia. The cytotoxicity was further evident in terms of increased incidences of sperm mortality. The cytotoxicity must have been exerted via the free radical generation, as this herbicide is known to have such properties [7]. Paraquat, however, does not seem to affect sperm motility, although 30 mg/kg showed some positive effect, which we believe may not be biologically important.

On the other hand, paraquat affected the morphogenesis of spermatozoa at all dose-levels and on all sampling days, indicating that there was interference in the metamorphosis of germ cells into mature structurally perfect sperm. Sperm abnormality increased in a dose-dependent pattern only on day 14 and not on other days, possibly because of the cytotoxicity of this herbicide. The formation of abnormal

Table 1. The effect of dermal exposure to paraquat on sperm morphology in the rat. Data are represented as means ±SD

Sample time (days)	Dose PQ [mg/kg]	Head deformities						Tail deformaties			
		Headless	Double headed	Micro-cephalous	Cephalo-caudally defective	Hookless	Banana shaped	Amorphous	Coiled tailed	Double tailed	Broken tailed	Total abnormalities (%)
	Control	3.64 ± 0.33	0.00 ± 0.00	4.00 ± 0.58	2.66 ± 0.67	13.00 ± 1.52	7.00 ± 1.54	0.67 ± 0.33	1.00 ± 0.54	0.00 ± 0.00	2.33 ± 0.34	3.22 ± 2.94
7	6	5.35 ± 0.38	0.00 ± 0.00	3.67 ± 0.89	8.03 ± 1.52	12.02 ± 1.15	3.76 ± 0.66	2.34 ± 0.82	3.43 ± 0.58	0.30 ± 0.12	4.36 ± 1.46	4.34 ± 2.66*
	15	7.64 ± 1.21	2.24 ± 0.36	0.68 ± 1.20	10.88 ± 0.38	22.46 ± 1.14	6.68 ± 0.62	3.68 ± 1.28	2.06 ± 0.18	2.48 ± 0.58	4.78 ± 1.28	6.35 ± 1.66*
	30	5.88 ± 0.68	1.66 ± 0.12	3.33 ± 1.22	9.08 ± 2.08	17.66 ± 0.89	6.28 ± 0.34	2.44 ± 0.26	1.36 ± 0.34	1.33 ± 1.20	4.36 ± 0.88	5.64 ± 2.98*
14	6	4.42 ± 0.58	0.00 ± 0.00	3.68 ± 0.62	6.36 ± 1.21	17.27 ± 0.42	7.12 ± 0.51	1.32 ± 0.42	1.66 ± 0.34	0.00 ± 0.00	4.00 ± 0.54	4.66 ± 2.50*
	15	4.36 ± 0.35	2.08 ± 0.83	5.68 ± 0.84	6.48 ± 0.32	21.82 ± 0.66	9.38 ± 2.80	4.44 ± 1.14	2.66 ± 1.48	2.88 ± 0.28	6.38 ± 0.94	6.38 ± 1.92*
	30	4.62 ± 0.36	3.34 ± 1.16	4.88 ± 0.82	9.68 ± 1.34	24.22 ± 0.52	6.22 ± 0.98	3.34 ± 0.88	3.68 ± 0.68	3.08 ± 0.54	4.28 ± 1.86	6.68 ± 1.68*
28	6	4.76 ± 0.34	0.00 ± 0.00	4.42 ± 0.58	7.73 ± 1.20	14.02 ± 1.47	4.00 ± 0.50	1.22 ± 0.14	2.68 ± 0.53	0.00 ± 0.00	4.68 ± 1.53	4.84 ± 2.38*
	15	7.66 ± 0.35	1.48 ± 0.58	4.06 ± 0.60	5.38 ± 0.58	16.88 ± 1.52	2.36 ± 0.54	3.66 ± 0.34	2.63 ± 0.38	1.68 ± 0.22	6.06 ± 1.16	5.54 ± 2.48*
	30	4.66 ± 0.33	0.00 ± 0.00	4.48 ± 0.82	4.24 ± 0.58	17.04 ± 1.58	3.66 ± 0.82	4.33 ± 0.58	2.68 ± 0.66	0.68 ± 1.02	4.66 ± 1.15	4.68 ± 2.34*
42	6	3.64 ± 0.32	0.68 ± 0.16	3.68 ± 0.21	4.76 ± 0.26	18.36 ± 0.36	3.48 ± 1.21	5.44 ± 1.08	2.34 ± 0.35	0.00 ± 0.00	6.23 ± 0.56	4.08 ± 3.68*
	15	6.02 ± 0.51	1.34 ± 0.58	5.12 ± 0.23	5.02 ± 0.54	19.66 ± 1.45	5.00 ± 1.75	3.00 ± 0.34	2.54 ± 0.32	3.34 ± 0.62	7.02 ± 0.89	5.02 ± 2.32*
	30	9.92 ± 3.78	5.58 ± 0.36	2.02 ± 0.26	5.52 ± 1.74	6.78 ± 0.58	21.22 ± 0.54	2.68 ± 0.36	4.48 ± 0.78	3.08 ± 0.34	3.36 ± 0.14	4.52 ± 1.59*

*$p < 0.05$: vs. control; one-way ANOVA followed by Bonferroni's post hoc test

sperm indicates the ability of paraquat to generate point mutations in the germ cells [10, 19]. The results also support the mutagenic activities of paraquat visualised in other test systems [1].

Previous studies have reported that paraquat was capable of generating free radicals [1, 7], which might have caused oxidative damage in the testis. This oxidative stress must have affected the genetic material of the germ cells, and this phenomenon is known to have some relation to abnormal sperm morphology [11, 18, 19]. The present results of the sperm morphology test are in consensus with that of an earlier report [13], in which paraquat affected the morphology of sperm in mice. Another study has shown positive genotoxic effects in *Drosophila melanogaster*, in terms of increased sex-linked recessive lethality [14]. In conclusion, the results of this study indicate that paraquat is cytotoxic and genotoxic to male germ cells in the rat.

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