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Effect of Boric Acid on Fertility, Aggressiveness and Sex Behaviour in Male Rats

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The aim of this research was to investigate the effects of long-term ingestion of boric acid on fertility, aggressiveness and sexual behaviour in Sprague-Dawley male rats.

The study was conducted in the Animal House Unit at Jordan University of Science and Technology, School of Medicine during the period September 2002 to January 2003. The control group (n = 10) received powdered feed with no additives and the treated group (n = 10) received powdered feed containing 9000 ppm (w/w) boric acid for 60 days. After 24 h of the last dose, the animals were weighed and autopsied under light ether anesthesia. The blood was collected for serum studies. Fertility was estimated in both groups. Sperm was tested for motility and counted. Initial and final body weights were recorded. Reproductive tract organs were also weighed. Histological and histometry studies were performed on reproductive organs (testes, epididymides, seminal vesicle, ventral prostate and vas deferens) as well as on liver, kidney and heart muscle. Testicular cell population was counted. Total protein, cholesterol, triglycerides, serum aspartate aminotranferase, serum alanine aminotranferase, plasma FSH and testosterone concentrations were measured. Aggressiveness and sex behaviorus were investigated. Data was expressed as mean ± standard deviation.

Key Words: Boric acid, Aggressive behaviour, Sex behaviour, Fertility, Reproduction, Male rat.

INTRODUCTION

Boric acid (H_3BO_3) a white crystalline powder, is a simple inorganic acid with widespread commercial use and consumer exposure. Generally, boric acid is not recognized as a poisonous substance. However, boric acid has potentially fatal actions such as hypotension, metabolic acidosis and oliguria. Death may result from circulation collapse and shock[1, 2]. Boric acid is used as a food and wood preservative, water softener, emulsifier, viscosifier for cosmetics, antiseptic, irritant solutions, fungicide and pesticide[1]. Boron compounds in general possess

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a wide variety of industrial applications such as fuel additives, abrasives, fireproofing agents and in textile and glass manufacturing. In the microelectronics industry, boron is the principal dopant for silicon wafers[3]. Thus, in addition to occupational exposure hazards, the potential exists for widespread environmental contamination of boron in air and surface water sources[4–7].

In animals, testicular atrophy was observed in dogs, rats and mice on chronic diet of borax or boric acid at 1000 to 2000 ppm boric acid equivalents[8, 9]. The National Toxicology Program's Reproductive Assessment[10] by Continuous Breeding study in mice exposed to boric acid showed multiple sites of action, with male fertility being the most sensitive[11, 12]. The testicular lesion in adult rats fed 9000 ppm boric acid was characterized by an initial inhibition of spermiation followed by epithelial disorganization, germ cell loss and atrophy[13–15].

The mechanism for the testicular toxicity of boric acid is unknown. One mechanism could be decreased testosterone levels. Rats fed boric acid displayed slightly reduced basal serum testosterone levels; this reduction appears to be central nervous system-mediated[13, 16–18]. However, it is unlikely that hormone changes can explain the atrophy, since it has been shown that spermatogenesis can be maintained in the presence of significantly reduced intratesticular testosterone[19–22]. Data on boric acid tissue disposition suggested that neither the testicular toxicity nor the slight CNS hormonal effect can be explained on the basis of selective accumulation of boric acid in the testes or brain/hypothalamus, respectively[23, 24]. Thus, these data suggested that other possible mechanisms should be considered and raised the issue of the reproductive toxicity of high-level boron exposure. The previous studies left a need for an adequate evaluation of male fertility, as well as a correlation of testicular boron levels to the levels of lesion development.

In this study, the effects of long-term ingestion of boric acid on fertility, on aggressive behaviour and on sexual behaviour were investigated in adult male rats fed 9000 ppm boric acid.

EXPERIMENTAL

During the period September 2002 to January 2003, adult male and female albino rats of Sprague Dawley strain, weighing about 300 g were raised in the Animal House Unit at Jordan University of Science and Technology School of Medicine under controlled temperature of 21 ± 1°C and 12 : 12 h light/dark cycles. Food and deionized water were available *ad libitum*. The control group (n = 10) received powdered feed with no additives. The treated group (n = 10) received powdered feed containing 9000 ppm (w/w) boric acid (1575 ppm boron) *ad libitum* for 60 days. Feed consumption was monitored gravimetrically on daily basis. Feed spillage was negligible. After 24 h of the last dose, the animals were weighed and autopsied under light ether anesthesia. The blood was collected through cardiac puncture using a dry clean syringe for serum studies.

Fertility Test: Fertility was estimated in adult male rats treated with boric acid and in control male counterparts. Each male rat was placed in an individual cage with two virgin untreated females of the same strain; they were left together

for ten days, during which two estrous cycles should have elapsed[25]. One week after the removal of the exposed males, females were killed by cervical dislocation under light ether anesthesia and the number of pregnant females, number of implantation sites, number of viable fetuses and number of resorptions were recorded.

Sperm Motility and Count: To determine sperm motility and sperm counts, 100 mg of cauda epididymides was minced in 2 mL of physiological saline. One drop of an evenly mixed sample was applied to a Neubauer's counting chamber under a cover slip. Quantitative motility expressed as a percentage was determined by counting both motile and immotile spermatozoa per unit area. Cauda epididymal and testicular sperm counts were made by routine procedure and expressed as million/mL of suspension[26, 27].

Body and Organ Weights: Initial and final body weights of the animals were recorded. The reproductive tract was taken out trimmed free of fat and each organ was weighed separately on an electronic balance. The male reproductive organs used for the study included testes, epididymides, ventral prostrate, seminal vesicle and vas deferens. Some vital organs such as liver, kidney, adrenal, heart and thyroid were also taken out and weighed. Reproductive organs along with a small piece of liver, heart and kidney were fixed in Bouin's fixative for histological studies.

Histological Studies: The Bouin's fixed reproductive organs (testes, epididymides, seminal vesicle, ventral prostate and vas deferens) along with liver, kidney and heart muscles were cut into small pieces and processed. The paraffin embedding was followed by section cutting (5 μm) and staining (Harris haematoxyline and eosin).

Histometry: With the help of Camera Lucida hundred circular appearing seminiferous tubules were traced at x80 and the diameter of each tubule was measured separately. The measurement was expressed as the mean of all the traced tubules. Similarly, Leydig cell nuclei were traced at x800. The epithelial cell height of cauda epididymides, caput epididymides and seminal vesicle were also traced at x360.

Testicular Cell Population Counting: Spermatogenic elements, *i.e.,* spermatogonia, spermatocytes and spermatids were counted in 5 μm thick cross-sections of 10 seminiferous tubules in 10 animals of each group. All raw counts were transformed to true counts by an adaptation of Abercrombie formula[28] from the germ cell diameter measurement.

Interstitial cell types such as fibroblast, immature and mature Leydig cells and degenerating cells were estimated, applying a differential count of over 200 cell population and statistically verified by the binomial distribution[29].

Serum Biochemistry: Total protein, cholesterol, triglycerides, serum aspartate aminotranferase (AST) and serum alanine aminotranferase (ALT) were obtained using commercial kits from Cis BIO International) Gif sur Yvette, France).

Hormonal Assays: Plasma FSH and testosterone concentrations were measured by radioimmunoassay using commercial kits from Cis BIO International (Gif sur Yvette, France).

Aggressive Behaviour Testing: A rectangular observation cage with a plexiglass front (45 × 27 × 40 cm) was used for aggression testing. A stud male rat was placed in the testing arena for 10 days. A second male either control or treated was then placed in the testing arena with the stud male rat for 5 min and the following parameters were recorded: (1) Lateralization by the stud male (2) Boxing bouts with the stud male (3) Fights with the stud male (4) Ventral presenting postures.

Sex Behavior Testing: Animals were observed in a rectangular cage with a plexiglass front (45 × 27 × 40 cm), lightened by a 15 watt bulb above the arena. Observations were performed between 09.00 and 15.00 h. All behavioural measures were monitored by a single observer unfamiliar with the male rat exposed group. Male rats were present with a female of the same strain, brought into estrus by sequential subcutaneous treatment with 12.5 mg animal estradiol benzoate (Sigma Chemical Co., St Louis, MO, USA) 54 h before testing and 0.5 mg animal progesterone (Gift from Roussel Uclaf, Paris, France) 6 h before testing. The hormones were dissolved in corn oil (ALFCO, Arab International Food and Oil Processing Co.) in a total volume of 0.1 mL.

Males were placed in the mating arena 5 min before the receptive females were introduced. Mating performance of male rats and number of ejaculations were classified as follows:

Number of mounts: Number of mounts without penile intromission and the time to the first mount.

Intromission latency: Time in minutes from the presentation of the female to the first intromission.

Intromissions: Number of mounts with penile intromissions.

Ejaculation latency: Time from the first intromission until ejaculation.

Post-ejaculatory interval (latency period): Time from the end of ejaculation until the next intromission.

The observations were terminated when no intromission had occurred within 15 min after presentation of the female, or if the male had not ejaculated within 30 min after the first intromission, or at the first intromission following ejaculation, or if no intromission had occurred within 15 min after ejaculation[30–32].

Statistical Calculation: Data was expressed as mean ± standard deviation (SD). The differences between boric acid exposed groups and control were analyzed using either Chi-square test or Student "t" test. P values less than 0.05 were considered significant.

RESULTS AND DISCUSSION

Effect of boric acid on the body and organs weight

Table-1 shows that ingestion of boric acid caused a slight decrease in body weight, when initial and final body weights were compared in the experimental group. On the contrary, an increase in the body weight was observed in the control group. However, the weight of the testes, epididymides, seminal vesicle, ventral

prostate and vas deferens were significantly ($P < 0.01$) decreased in the treated male rats when compared to the control group.

TABLE-1
EFFECT OF LONG-TERM INGESTION OF 9000 ppm OF BORIC ACID ON BODY AND ORGAN WEIGHTS IN ADULT MALE RAT

Treatment	Body weight (g)		Testes	Body weight (mg/100 g)			
	Initial	Final		Epididymides	Seminal vesicle	Ventral prostate	Vas deferens
Control	324 ±12.80	339 ±12.65	895 ±25.21	387 ±21.61	434 ±14.38	246 ±4.1	81.2 ±4.36
Boric acid	332 ±14.55	312 ±17.6	767† ±22.1	315.2† ±19.3	358† ±18.1	203† ±8.35	58.75* ±6.20

Results are expressed as mean ± S.D.
Ten rats were included per group.
*$p < 0.05$. †$p < 0.01$ significantly different from control group (Student's "t" test).

Effect of boric acid on Sperm Dynamics and Histometrical Parameters

While sperm motility in cauda epididymis was significantly ($P < 0.01$) decreased in treated animals in comparison to the control, sperm density, seminiferous tubule diameter and Leydig cell nuclear diameter in treated male rats were significantly ($P < 0.01$) increased. Epithelial cell height in epididymides (caput, cauda and seminal vesicle) were also significantly ($P < 0.01$) increased (Table-2).

TABLE-2
EFFECT OF LONG-TERM INGESTION OF 9000 ppm OF BORIC ACID ON HISTOMETERICAL PARAMETERS AND SPERM DYNAMICS IN ADULT MALE RAT

Treatment	Sperm density million/mL		Sperm motility %	Seminiferous tubule	Leydig cell nuclear	Epithelial cell eight		
	Testes	Cauda	Cauda	Diameter	Diameter	Caput	Cauda	Seminal vesicle
						µm		
Control	4.75 ±0.47	56.0 ±1.94	74.1 ±1.94	290.6 ±3.2	6.45 ±0.96	38.8 ±0.4	26.08 ±0.32	17.32 ±0.17
Boric acid	5.55* ±0.14	61.185† ±1.08	43.26† ±1.08	301.27† ±21.35	8.79† ±0.762	44.68† ±2.66	33.4† ±2.68	28.45† ±0.27

Results are expressed as mean ± S.D.
Ten rats were included per group.
*$p < 0.05$, †$p < 0.01$ significantly different from control group (Student's "t" test).

Effect of Boric acid on Testicular Cell Population Dynamics

Table-3 demonstrates that the administration of boric acid caused a significant decrease in the germinal cell population: spermatocytes (primary ($P < 0.01$) and

secondary ($P < 0.01$)) and spermatids were also decreased to a significant level ($P < 0.001$). Similarly the immature and mature Leydig cell numbers were also considerably decreased ($P < 0.01$). However the degenerating cell number was greatly increased ($P < 0.001$). The number of fibroblasts was also notably decreased ($P < 0.01$). On the other hand, spermatogonia were not extensively altered.

TABLE-3
EFFECT OF LONG-TERM INGESTION OF 9000 ppm OF BORIC ACID ON TESTICULAR CELL POPULATION DYNAMICS IN ADULT MALE RAT

Treatment	Germinal cell types				Interstitial cell type			
	Spermatogonia	Spermatocyte (primary)	Spermatocyte (secondary)	Spermatids	Fibroblast	Immature Leydig cell	Mature Leydig cell	Degenerating cell
Control	23.99 ±1.93	18.85 ±0.80	64.126 ±3.51	147.71 ±4.87	63.83 ±1.64	65.195 ±3.47	70.64 ±1.03	18.34 ±1.67
Boric acid	22.05 ±2.44	12.96† ±2.41	17.97† ±3.73	9.32‡ ±6.82	38.66† ±1.33	41.66† ±1.65	46.66† ±0.78	69.6‡ ±0.76

Results are expressed as mean ± S.D.
Ten rats were included per group.
*$p < 0.05$, †$p < 0.01$, ‡$p < 0.001$ significantly different from control group (Student's "t" test).

Effect of Boric Acid on Serum Biochemical Markers

Results presented in Table-4 show that glucose, bilirubin, total cholesterol and triglyceride levels were within the normal range. SGOT and SGPT were found to be significantly increased in the treated group when compared to the control. On the contrary, levels of plasma testosterone were extensively decreased in the treated group when compared to the control group. However, FSH levels were not notably changed.

TABLE-4
EFFECT OF LONG-TERM INGESTION OF 9000 ppm OF BORIC ACID ON SERUM BIOCHEMISTRY IN ADULT MALE RAT

Treatment	Glucose	Cholesterol	Triglycerides	Bilirubin	SGOT	SGPT	Testosterone	FSH
	mmol			(μmol)	U/L		(nmol/L)	(IU/L)
Control	7.3 ±0.212	1.4 ±0.147	0.8 ±0.07	3.175 ±0.142	36.7 ±1.98	41.7 ±7.14	14.4 ±2.53	18.47 ±1.87
Boric acid	7.9 ±1.03	1.56 ±0.07	1 ±0.05	3.3 ±0.22	40.33 ±2.22	45.3 ±8.87	6.64† ±1.88	22.18 ±1.26

Results are expressed as mean ± S.D.
Ten rats were included per group.
*$p < 0.05$, †$p < 0.01$ significantly different from control group (Student's "t" test).

Effect of Boric Acid on Male Rat Fertility

Table-5 demonstrates a significant decrease in the number of females impregnated by boric acid treated male rats. The number of implantations and

number of viable fetuses were also considerably decreased in female rats impregnated by those male rats ingested boric acid. On the other hand, the number of resorptions was significantly increased in females impregnated by male rats ingested boric acid.

TABLE-5
EFFECT OF LONG-TERM INGESTION OF 9000 ppm OF BORIC ACID ON MALE RATS FERTILITY

Treatment	No. of males	No. of females	No. of pregnant females	No. of implantation sites	No. of viable fetuses	Total No. of resorption	No. of resorption/ total No. of implantation
Control	10	20	18/20 (90%)	9.62 ±2.66	9.37 ±1.16	8	8/173 (4.6%)
Boric acid	10	20	15/20 (75%)	7.2† ±3.31	6.63† ±1.54	19	19/123 (15.5%)

Results are expressed as mean ± S.D.
*$p < 0.05$, †$p < 0.01$, ‡$p < 0.001$ significantly different from control group (Student's "t" test).

Effect of Boric Acid on Sex Behavior

In the control group, shortly after introducing the female rat into the observation arena, exploratory activities conducted by the male rat including sniffing, nose-to-nose contact, genital exploring and grooming were observed. These activities were followed by mounting and copulation. Exploratory activities which were not quantitatively recorded seem to be reduced in those male rats ingested boric acid.

Results presented in Table-6 show the effect of boric acid on the parameters of sex-behavior in male rats. The number of mounts by males ingested boric acid was significantly decreased. Conversely, the time to ejaculation increased significantly. Exposure of male rats to boric acid had significantly increased the post-ejaculatory interval. Boric acid ingestion had significantly reduced the number of ejaculating males.

TABLE-6
EFFECT OF LONG-TERM INGESTION OF 9000 ppm OF BORIC ACID ON SEXUAL BEHAVIOUR IN ADULT MALE RAT

Treatments	No. of animals	Time to the first mount (min)	Number of mounts	Intromission latency (min)	Number of intromissions	Ejaculatory latency (min)	Post-ejaculatory interval (min)	% of males ejaculation
Control	10	1.05 ±0.59	15.94 ±2.75	2.22 ±0.64	8.72 ±1.96	15.21 ±1.57[14]	4.72 ±0.95[14]	(80%)
Boric acid	10	1.84* ±1.34	16.42† ±1.98	3.78‡ ±1.65	4.42‡ ±2.54	14.88 ±0.78	25.11‡ ±1.26	(40%)

Results are expressed as mean ± S.D.
*$p < 0.05$, †$p < 0.01$, ‡$p < 0.001$ significantly different from control group (Student's "t" test).

Effect of Boric Acid on Aggressive Behavior

Administration of boric acid had an effect on the parameters of territorial aggression in male rats (Table-7). Male rats ingested boric acid had significantly less lateralizations. Boxing bouts were also drastically reduced. Fighting with stud male rats was also reduced in male rats ingested boric acid. Treated male rats had a significant decrease in the number of ventral presentation.

TABLE-7
EFFECT OF LONG-TERM INGESTION OF 9000 ppm OF BORIC ACID ON AGGRESSIVE BEHAVIOUR IN ADULT MALE RATS

Treatments	No. of animals	Laterialization by stud male	Boxing bouts with stud male	Fights with stud male	Ventral presenting
Control	18	14.11 ± 1.99	5.88 ± 1.52	1.94 ± 0.63	1.77 ± 0.54
Boric acid	22	4.95‡ ± 1.49	2.31‡± 0.83	1.63 ± 0.499	1.31* ± 0.47 (16)

Results are expressed as mean ± S.D.
Ten rats were included per group.
*$p < 0.05$, †$p < 0.01$, ‡$p < 0.001$ significantly different from control group (Student's "t" test).

The effects of long-term exposure of an adult male rat to 9000 ppm concentration of boric acid, on aggression, sex behaviour, fertility and reproductive system were investigated.

Up to date, there is a shortage of data on the effects of long-term ingestion of boric acid on various parameters of biological behaviours and reproductive capacity in adult male rats. These facts prompted the authors of this article to initiate this study. The animal model used in this work has been previously used to assess the adverse effects of metal saltus ingestion on behaviour and fertility in small laboratory animals[31, 32] without compromising the health of the experimental animals.

The dose of 9000 ppm of boric acid in drinking water used in this study was selected because of the reported toxicity potentials of higher doses of this compound including decreased body weight and water consumption and clinical signs of toxicity such as dehydration, lethargy and hunched posture[11]. This dose level was also selected to obtain broader range of information on the effects of boric acid on behavior parameters and reproduction.

In rats, the whole spermatogenic process requires 53 days out of which spermatozoa spend the last 6 to 7 days in the final transit through epididymides[33]. Boric acid was administrated for one complete spermatogenic cycle.

The present investigation shows that oral administration of boric acid promoted decreased fertility in male albino rats. The weights of reproductive organs were markedly decreased (Table-1). The weight, size and secretory function of testes, epididymes, seminal vesicles, ventral, prostate, vasa and deferentia are closely regulated by androgens[34–36]. The drug may act on pituitary gland and increase the main hormone of spermatogenesis. The process of spermatogenesis and accessory reproductive organs function are androgen dependent. Increased androgen production is reflecting a decrease in the number of mature Leydig cells and their functional status. In the present study the number of degenerating Leydig cells was significantly decreased, this reflects the decrease of androgen level. It is further confirmed by decreased number of spermatocytes (both primary and secondary)

and spermatids as these stages are completely androgen dependent[37–39]. The decrease in weight and histometry of reproductive organs further confirmed androgen increase. Significant decrease in the sperm motility of cauda epididymis was observed in the treated group. This may be due to the effects of boric acid on the enzymes of oxidative phosphorylation.

The results presented in this paper show that ingestion of boric acid by adult male rats decreases the number of females impregnated by the exposed males (Table-5). Also, the number of implantations and the number of viable fetuses were decreased. This decrease appearing to this effect may be due to decrease in sperm motility and sperm density.

One of the main findings of the present work is that the ingestion of boric acid abolished aggressive behaviour postures exhibited by adult male rats. Male rats exposed to boric acid showed low aggression that is evident by significantly less lateralization, boxing bouts and reduction in number of ventral presenting postures (Table-6). Male rats ingested boric acid used in this work withdrew from the field and remained away from the resident stud male and only if challenged by the resident male would engage in any interactive behaviour. Conversely, control males would engage with the resident male in some aggressive behaviour and displayed greater level of aggression than did the boric acid exposed males. The other main finding of the present work is that the ingestion of boric acid by adult male rats resulted in marked suppression of sexual performance. Prolongation of the intromission latency, decrease in the number of intromissions and marked increase in post-ejaculatory interval are facts testifying the suppressive effects upon mating capabilities. It would appear from the data presented in this research that aggressive and sexual behaviours are very susceptible to intervention by general toxicity action produced by the ingestion of boric acid.

The suppressive effect of boric acid on both aggression and sex behaviour could be explained by the fact that this chemical compound acts directly or indirectly on the testes, influencing androgen biosynthesis pathway and producing effects on these two types of behaviour. An agent acting directly on the brain, hypothalamus or anterior pituitary gland will in turn affect the testes and will possibly affect sexual behaviour.

In conclusion, these results confirm that the long-term boric acid ingestion produces adverse effects on aggression, sexual behaviour, fertility and reproductive system in adult male rats. However, the exact mode of action requires further studies. Moreover, these findings underline the importance of examining a number of parameters concerning fertility and etiology to monitor the toxic potentials of various xenobiotics.

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