



# Curcumin palliative effects on sexual behavior, fertility and reproductive hormones disorders in mercuric chloride intoxicated mice offspring

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## ABSTRACT

The present study was designed to investigate the impacts of mercuric chloride on offspring during pregnancy and lactation. Pregnant mice was selected and divided into various groups. To the experimental animal mercuric chloride and curcumin were incorporated. Only tap water was provided to the control group of animals. To the Group II and III animals, curcumin was administered at 150 and 300 ppm. Group IV animals received mercury chloride (10 ppm) and curcumin at two different doses (150 and 300 ppm). Experiment was started at the first day of pregnancy and treatment was continued up to PD 15 stage further, only water was provided. Male and female pups were separated and allowed for mating. Then, viability of sperm, sperm count and motility was tested. The hormonal level was analyzed from the serum sample. In the case of males, testosterone and FSH was analysed. In the case of female, progesterone and LH were detected from the serum sample. The present findings revealed decreased body weight, sexual behavior, viability of sperm and mating performance. Exposure of  $HgCl_2$  during lactation and gestation is highly dangerous. Exposure of  $HgCl_2$  affected fertility and social behavior during lactation and at the time of gestation. In this investigation, curcumin was used to minimize the toxic effect of  $HgCl_2$ .

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## 1. Introduction

Mercury is one of the toxic heavy metals in the world and has been discovery very early (Parsons and Percival, 2005). The symbol “Hg” was derived from Latin. In earth, mercury occurs as Hg-bearing minerals and Hg, the most important form is cinnabar (Huang et al., 2007; Antonisamy et al., 2015). Mercury is used in various chemical industries. In chlorine production plants, it is widely used for the synthesis of chlorinated compounds and also used in the production of sodium hydroxide. Mercury has various applications including to control weeds, fungi, bacteria and insects.

Curcumin (Cur) is a phytochemical present in the rhizome of *Curcuma longa* L. Cur is a phenol rich compound, diferuloylmethane (Balamurugan, 2015; Kannan and Agastian, 2015; Rath et al., 2015). *Curcuma longa* L. comes under the family Zingiberaceae and widely distributed in Asian countries (Altintas et al., 2016). Cur has various benefits and is very much effective to treat various

diseases and disorders including, coryza, anorexia, cough, sinusitis and hepatic diseases (Maheshwari et al., 2006). It is also used to control various diseases and also acts as antifungal, antiviral, anti-infectious, anti-inflammatory, anticarcinogenic, antimutagen, antiparasitic, and antioxidants (Ciftci et al., 2010; Valsalam et al., 2019a; Valsalam et al., 2019b). Curcumin has the potential to treat neurological disorders (Rajkumari et al., 2019; Gurusamy et al., 2019; Arokiyaraj et al., 2015). The neuroprotective effects of curcumin have been reported and this compound was found to be effective to treat various neurodegenerative diseases and disorders (Cole et al., 2007). The phytochemicals of curcumin showed various antioxidant properties (Ciftci et al., 2012). Abu-Taweel et al. (2013) analyzed the composition of commercial curcumin. Curcumin contains 77% curcumin, 17% de-methoxycurcumin and 3% bisdemethoxycurcumin. In this study, mercury chloride was treated to experimental animal and the protective role of curcumin on mercury chloride induced toxicity in mice was analyzed.

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## 2. Materials and methods

### 2.1. Experimental animals

In this study, male and female Swiss-Webster mice with the age of about 12 weeks were selected. All animals are maintained

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in plastic cages (30 × 12 × 11 cm). The selected experimental animal was maintained at Saud University, Riyadh, Saudi Arabia. Ethical committee approval has been obtained.

## 2.2. Mercury and curcumin administration and experimental design

In this study, pregnant mice were divided into six groups. To the control group curcumin was not provided. The experimental group II and III was supplemented with Curcumin (150 and 300 ppm) and group IV was supplemented with only mercury chloride. In group V, curcumin was administered at two different doses (150 and 300 ppm). Experimental and control animal were fed with healthy diet and water. All experimental animals were further divided on the day of pregnancy. Analysis such as, sperm motility, sperm count and viability was analyzed. Male hormones were analyzed from the serum of male rats and the female hormone was tested in the female rats.

## 2.3. Total body weight analysis

Body weight is an important indicator to analyze metabolism. During experiments, the pups were frequently weighed up to PD40. The relative weight of liver, brain, prostate gland, seminal vesicle, vas deferens and epididymidis was analyzed.

## 2.4. Sexual behavior

The sexual activity tests carried out by introducing the experimental male mice in the home cage of the age matched estrous virgin females. The sexual activities of each male mouse had observed directly for three hours. The parameters recorded for sexual behavior were:

- Latency of copulation (mounting): the time taken by the male mice to make the first attempt of mounting the female mice and making copulation.
- The frequency of copulation: the number of copulation trials that will occur within a unit time scale will be frequency of copulation.
- Duration of copulation: the total time spent by the males to copulate within the stipulated three hours observation will give the duration of copulation.
- Approach and following: The time spent by the male near the female or follow her.
- Threat and bite: The time spent by male in the process of the threat or biting the female.
- The number of times the male sniffs the nose or vagina of the female.
- Number of rears and wall rears: The number of times the male stands on its hind legs and hold its hands in the air or on the wall.
- Number of the squat: The number of male lies on the floor of the cage away from the female.
- Number of wash and digging: The number of times the animal cleans its body and scatters in the cage floor.

## 2.5. Fertility parameters

### 2.5.1. Sperm count, motility, and viability

In this study, sperm was counted from the epididymal region.

### 2.5.2. Mating test

Mating experiment was conducted using male and female mice treated at 2:1 ratio based on the instructions provided in WHO protocol (1983).

### 2.5.3. Analysis of hormones

The level of serum FSH and LG was analyzed as suggested by Peege et al. (2005) and Pidoux et al. (2007) by double antibody radioimmunoassay method. The level of testosterone was analyzed by the method of radioimmunoassay according to the manufacture instructions.

### 2.5.4. Data analysis

The results were analyzed statistically using one-way analysis of variance (ANOVA) between control and experimental groups. Also Student-Newman-Keuls multiple comparison test was performed as suggested by Yamane (1973). The significance of sexual activity of the animal was analyzed using Mann-Whitney *U* test as described by Sokal and Rohlf (1981).

## 3. Results

### 3.1. Body weight analysis

In the present study, body weight of the animal has decreased significantly due to the exposure of mercuric chloride. Table 1 and Fig. 1(A–H) showed marked depletion of absolute and relative body weight of the experimental animal. Also the organ such as, liver, brain, epididymidis, seminal vesicle, testis, vas deferens, and prostate gland weight was decreased considerably in the experimental animals treated with mercuric chloride than curcumin groups. Absolute and relative body weight of curcumin administered animal was increased than mercuric chloride experimental group. Body weight and other organs weight considerably reduced in mice offspring treated with mercuric chloride (Fig. 2A–D) (Table 1). The attenuating effect of curcumin on female animals were described and the variation was statistically significant (Fig. 2A–D).

### 3.2. Sexual behavior

Mercuric chloride significantly affected sexual behavior in experimental animal and the behavior analysis was statistically significant. Naso-Nasal and Naso-Genital contacts, pelvic thrust and bite decreased, however the bite was increased and was statistically significant (Table 2A). The behavior such as, rears, wall rears, digging, wash were decreased considerably and was statistically significant (Table 2B). However, squat increased in experimental animal than curcumin treated animals. The protective role of curcumin on Hg toxicity in experimental animal is tabulated (Table 2B).

### 3.3. Fertility of male and female offspring of mice

Analyses was performed to explore the impact of sperm count, mating index and viable sperm in mercuric chloride exposed animal and control group. The hormone level such as, testosterone and FSH and sperm mortality were significantly reduced in mercuric chloride exposed group than curcumin groups (Table 3, Fig. 3A–F). The impact of curcumin on resisting mercuric chloride toxicity is dose dependent and was statistically significant. Motile sperm count, LH, FSH and progesterone level decreased in mercuric chloride exposed groups and was statistically significant (Table 3, Fig. 4A–D).

## 4. Discussion

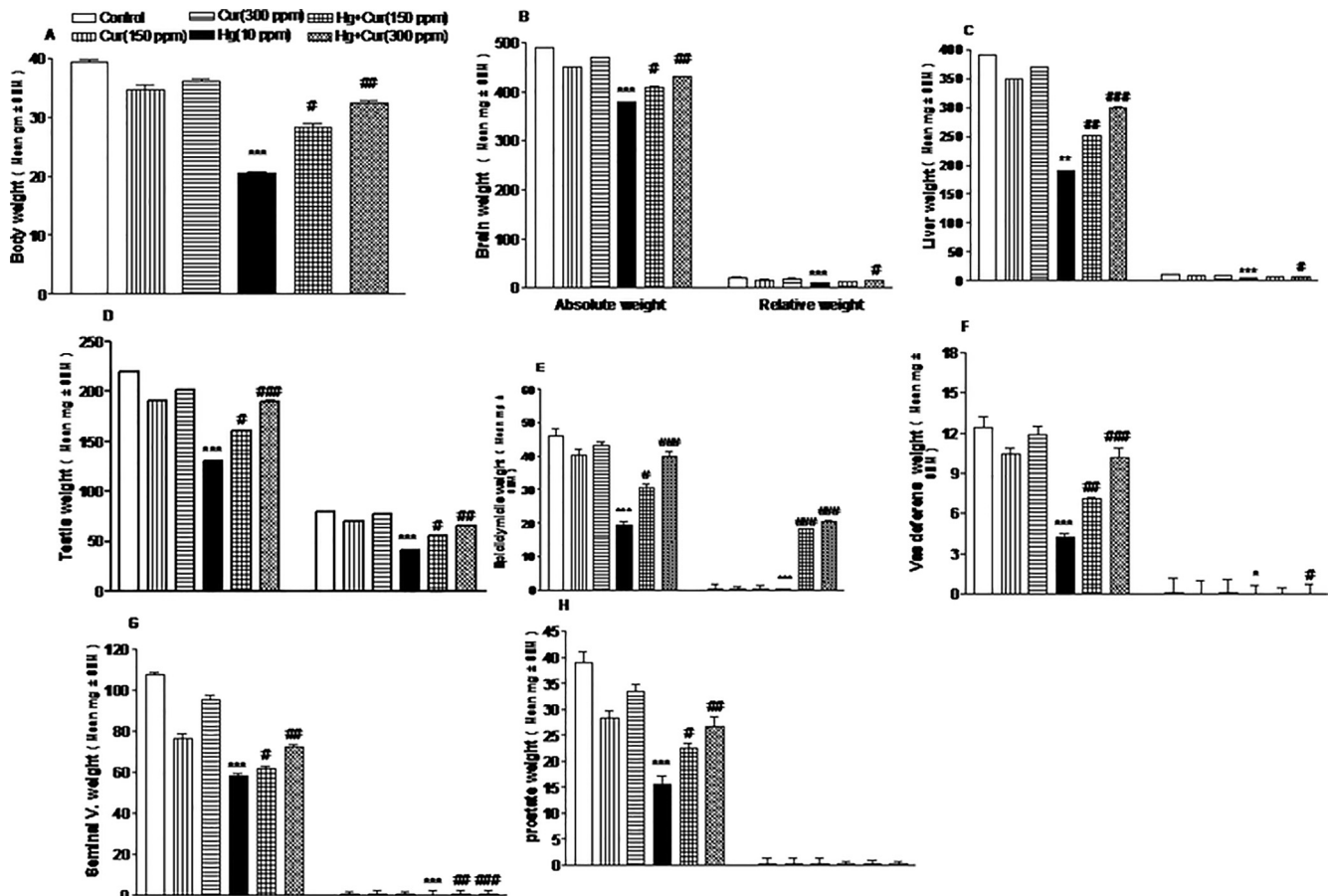
This experiment was designed to explore the impact of mercuric chloride on fertility and mating behavior of female and male offspring. The findings indicated the toxic effect of mercuric

**Table 1**

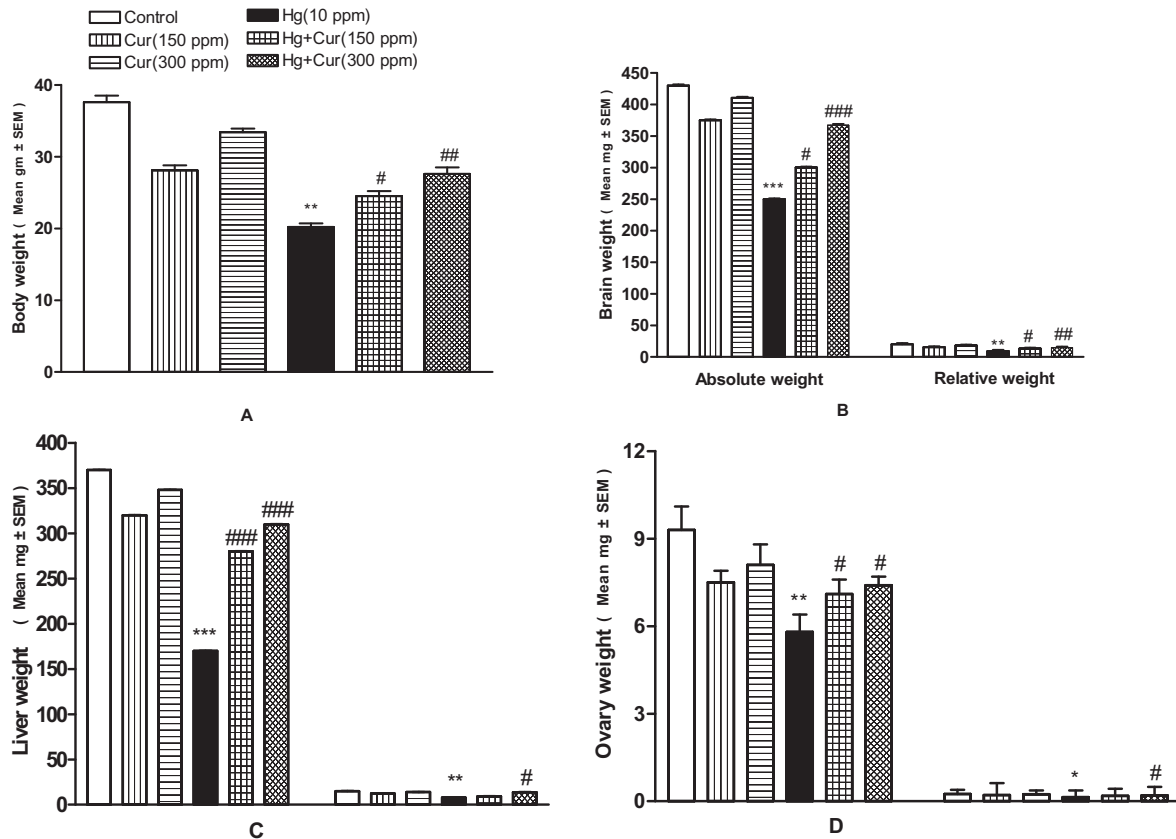
Effect of mercury on Body weight (g) and some absolute and relative weight of organs (mg) in male and female of mice offspring.

Gender	Parameters	Doses (ppm)						
		Control	Cur (1 5 0)	Cur (3 0 0)	Hg (10)	Hg + Cur(1 5 0)	Hg + Cur(3 0 0)	
Male	Body weight	39.3 ± 0.5	34.6 ± 0.8	36.1 ± 0.4	20.5*** ± 0.3	28.3# ± 0.5	32.4## ± 0.3	
	Brain A.W.	490.8 ± 0.9	450.1 ± 0.4	470.4 ± 0.5	380.1*** ± 0.2	410.5# ± 0.6	430.4## ± 0.8	
	Brain R.W.	20.7 ± 1.8	15.9 ± 1.5	18.1 ± 1.1	9.2*** ± 1.6	12.3 ± 1.2	14.2# ± 0.9	
	Liver A.W.	390.2 ± 0.12	350.3 ± 0.11	370.1 ± 0.11	190.6** ± 0.13	250.3## ± 0.16	300.3### ± 0.18	
	Liver R.W.	10.12 ± 0.14	8.21 ± 0.11	9.13 ± 0.13	5.23*** ± 0.13	6.11 ± 0.11	7.14# ± 0.15	
	Testis A.W.	220.1 ± 0.13	190.2 ± 0.17	202.12 ± 0.12	130.14*** ± 0.12	160.21# ± 0.14	190.16### ± 0.18	
	Testis R.W.	80.10 ± 0.16	70.21 ± 0.19	77.14 ± 0.17	41.18*** ± 0.16	55.17# ± 0.19	65.27## ± 0.14	
	Epididymidis A.W.	46.1 ± 2.1	40.4 ± 1.5	43.2 ± 1.1	19.3*** ± 1.2	30.4# ± 1.3	39.8## ± 1.5	
	Epididymidis R.W.	0.28 ± 1.3	0.21 ± 0.9	0.25 ± 1.1	0.13*** ± 0.11	0.18### ± 0.17	0.25### ± 0.10	
	Vas deferens A.W.	12.4 ± 0.8	10.40.5	11.9 ± 0.6	4.2*** ± 0.3	7.1# ± 0.1	10.2### ± 0.7	
	Vas deferens R.W.	0.08 ± 1.1	0.05 ± 0.9	0.06 ± 1.0	0.02 ± 0.6	0.04 ± 0.4	0.05# ± 0.7	
	Seminal V. A.W.	107.5 ± 1.4	76.1 ± 2.3	95.2 ± 2.1	57.9*** ± 1.4	61.4# ± 1.5	72.1## ± 1.2	
	Seminal V. R.W.	0.49 ± 1.0	0.41 ± 1.9	0.45 ± 1.3	0.18*** ± 2.2	0.30## ± 2.1	0.35### ± 1.9	
	Prostate A.W.	39.0 ± 2.1	28.3 ± 1.4	33.4 ± 1.3	15.4*** ± 1.8	22.4# ± 1.1	26.7## ± 1.7	
	Prostate R.W.	0.18 ± 1.2	0.14 ± 1.1	0.16 ± 1.2	0.10 ± 0.6	0.11 ± 0.7	0.13 ± 0.6	
	Female	Body weight	37.6 ± 0.9	28.1 ± 0.7	33.4 ± 0.5	20.2** ± 0.5	24.5# ± 0.7	27.6## ± 0.9
		Brain A.W.	430 ± 1.9	375 ± 1.4	410.5 ± 1.6	250*** ± 1.2	300.1# ± 1.5	367.2### ± 1.9
Brain R.W.		20.1 ± 1.8	15.4 ± 1.4	18.1 ± 1.3	9.4** ± 1.7	13.4# ± 1.4	14.9## ± 1.5	
Liver A.W.		370 ± 0.12	320 ± 0.13	348 ± 0.12	170*** ± 0.11	280### ± 0.17	310### ± 0.17	
Liver R.W.		14.9 ± 0.24	12.4 ± 0.24	13.9 ± 0.21	7.8** ± 0.23	9.1 ± 0.28	13.7# ± 0.24	
Ovaries A.W.		9.3 ± 0.8	7.5 ± 0.4	8.1 ± 0.7	5.8** ± 0.6	7.1# ± 0.5	7.4# ± 0.3	
Ovaries R.W.		0.25 ± 0.14	0.21 ± 0.41	0.24 ± 0.13	0.14* ± 0.23	0.18 ± 0.26	0.20# ± 0.29	

\*\*\* and \*\* show statistically significant at  $P < 0.05$ ,  $P < 0.01$  and  $P < 0.001$  respectively from the control and Cur groups. #, ## and ### show statistically significant at  $P < 0.05$ ,  $P < 0.01$  and  $P < 0.001$  respectively from Hg group by ANOVA and student's *t*-test.



**Fig. 1.** A–H Effect of perinatal exposure on the body, absolute and relative weight of male mice offspring. A, body weight; B, absolute and relative weight of brain; C, liver; D, testis; E, epididymidis; F, vas deferens; G, seminal vesicle and H, prostate. \*, \*\* and \*\*\* show statistically significant at  $P < 0.05$ ,  $P < 0.01$  and  $P < 0.001$  respectively from the control and Cur groups. #, ## and ### show statistically significant at  $P < 0.05$ ,  $P < 0.01$  and  $P < 0.001$  respectively from Hg group by ANOVA and student's *t*-test.



**Fig. 2.** A–D Effect of perinatal exposure on the body, absolute and relative weight of female mice offspring. A, body weight; B, absolute and relative weight of brain; C, liver and D, ovary. \*\*, \*\*\*, and \*\*\* show statistically significant at  $P < 0.05$ ,  $P < 0.01$  and  $P < 0.001$  respectively from the control and Cur groups. ### and ### show statistically significant at  $P < 0.05$ ,  $P < 0.01$  and  $P < 0.001$  respectively from Hg group by ANOVA and student's *t*-test.

**Table 2A**  
Effect of perinatal mercuric chloride exposure on sexual behavior in male mice offspring at adult age.

Group	Median number (with ranges) of acts and postures							
	Approach	Following	Number of Naso-Nasal contact	Number of Naso-Genital contact	Mount	Pelvic thrust	Threat	Bite
Control	21.00 (18.00–30.00)	33.00 (32.00–42.00)	63.00 (60.00–72.00)	33.00 (30.00–60.00)	9.00 (3.00–10 0.00)	6.00 (6.00–9.00)	9.00 (5.00–12.00)	6.00 (3.00–9.00)
Cur (150 ppm)	15.00 (10.00–25.00)	23.00 (18.00–28.00)	45.00 (28.00–43.00)	26.00 (18.00–30.00)	6.00 (4.00–6.00)	4.00 (3.00–5.00)	6.00 (3.00–8.00)	6.00 (2.00–8.00)
Cur (300 ppm)	18.00 (12.00–20.00)	30.00 (18.00–31.00)	51.00 (30.00–57.00)	30.00 (28.00–39.00)	7.00 (3.00–8.00)	5.00 (4.00–7.00)	7.00 (3.00–8.00)	5.00 (3.00–7.00)
Hg (10 ppm)	6.00 * (0.00–9.00)	10.00 *** (0.00–9.00)	28.00 ** (25.00–33.00)	11.00 ** (6.00–13.00)	1.00** (0.00–2.00)	0.00 *** (0.00–2.00)	2.00* (0.00–2.00)	22.00 *** (16.00–25.00)
Hg + Cur (150 ppm)	11.00 # (9.00–13.00)	17.00 ## (10.00–18.00)	35.00 # (29.00–39.00)	21.00 # (13.00–25.00)	4.00 # (2.00–4.00)	3.00 ### (3.00–4.00)	5.00 # (2.00–5.00)	7.00 ## (4.00–9.00)
Hg + Cur (300 ppm)	13.00 ## (9.00–15.00)	21.00 ### (15.00–33.00)	41.00 ## (35.00–41.00)	25.00 ### (15.00–25.00)	6.00 ## (3.00–7.00)	5.00 ### (3.00–6.00)	6.00 ## (3.00–7.00)	9.00 ### (3.00–10.00)

\*, \*\* and \*\*\* significantly different at ( $p < 0.05$ ,  $p < 0.01$  and  $p < 0.001$ ) respectively from the control and Cur groups. #, ## and ### significantly different at ( $p < 0.05$ ,  $p < 0.01$  and  $p < 0.001$ ) respectively from Hg group by ANOVA and Mann-Whitney *U* test.

chloride to offspring during lactation and gestation. Results revealed that reduced relative and absolute body weight and total body weight also, the relative weight of various organs including, liver, brain and reproductive organs were decreased significantly. Mercuric chloride treated mice showed unusual sexual behavior than control group. The fertility factors in female and male offspring were decreased significantly than control. Mercuric chloride change the spermatogenic germinal layers in animals. Mercuric poisoning was characterized in the presence of degenerating cells and disruption of germinal epithelium in the seminiferous tubules.

Mercuric compounds also damage DNA and maximize the action of germ cell apoptosis (Boujbiha et al., 2009). Exposure of mercury cause reduction in spermatogonia, primary and secondary spermatocytes and spermatogonia. Mercury exposure affected serum testosterone level in experimental animal. Leydig cells are predominant to produce testosterone in testis, the treatment of mercury affected Leydig cell function to affect the production of sex steroids (Shino et al., 2001; Boujbiha et al., 2009).

In the present study curcumin showed positive impact to repair cells cause by mercury toxicity. Curcumin has significant

**Table 2B**  
Effect of perinatal mercuric chloride exposure on sexual behavior in male mice offspring at adult age.

Group	Median number (with ranges) of acts and postures				
	Wall rears	Rears	Wash	Squat	Digging
Control	22.00 (17.00–30.00)	16.00 (14.00–18.00)	4.00 (3.00–6.00)	1.00 (0.00–2.00)	7.00 (2.00–10.00)
Cur (150 ppm)	18.00 (12.00–25)	13.00 (13.00–18.00)	2.00 (2.00–4.00)	3.00 (2.00–4.00)	4.00 (3.00–12.00)
Cur (300 ppm)	20.00 (15.00–30.00)	15.00 (14.00–18.00)	3.00 (2.00–5.00)	1.00 (1.00–3.00)	5.00 (2.00–6.00)
Hg (10 ppm)	4.00 *** (3.00–8.00)	2.00 *** (1.00–4.00)	1.00 ** (0.00–2.00)	9.00 *** (8.00–12.00)	2.00 ** (1.00–3.00)
Hg + Cur (150 ppm)	15.00 ### (10.00–16.00)	10.00 ### (8.00–11.00)	2.00 (1.00–2.00)	4.00 ### (2.00–5.00)	3.00 (1.00–4.00)
Hg + Cur (150 ppm)	17.00 ### (12.00–20.00)	12.00 ### (9.00–13.00)	3.00 # (3.00–4.00)	2.00 ### (0.00–3.00)	4.00 # (2.00–5.00)

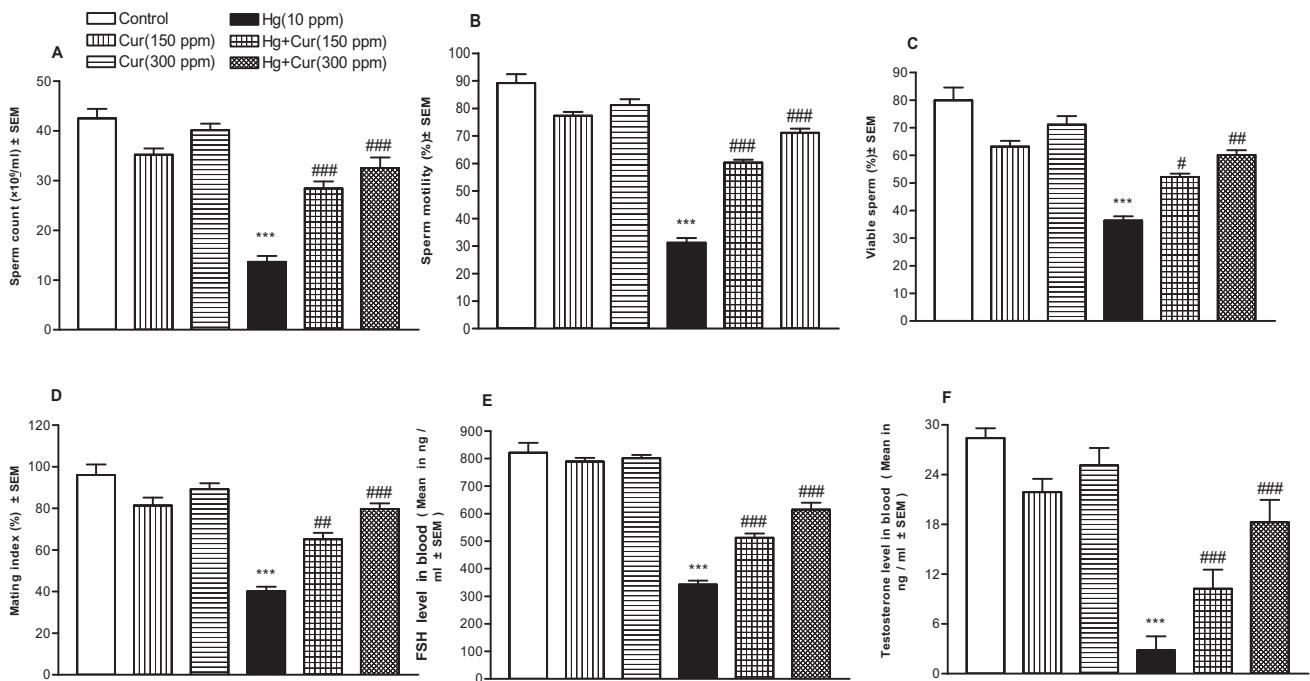
\*\* and \*\*\* show significantly different at ( $p < 0.01$  and  $p < 0.001$ ) respectively from the control and Cur groups.

# and ### show significantly different at ( $p < 0.05$ ,  $p < 0.01$  and  $p < 0.001$ ) respectively from Hg group by ANOVA and Mann-Whitney  $U$  test.

**Table 3**  
Effect of mercury on fertility parameters in male and female mice.

Gender	Parameters	Dose (ppm)					
		Control	Cur (150)	Cur (300)	Hg (10)	Hg + Cur (150)	Hg + Cur (300)
Male	Sperm count ( $\times 10^6$ /ml)	42.50 $\pm$ 1.91	35.21 $\pm$ 1.24	40.12 $\pm$ 1.31	13.64*** $\pm$ 1.18	28.41### $\pm$ 1.42	32.51### $\pm$ 2.13
	Sperm motility (%)	89.21 $\pm$ 3.2	77.3 $\pm$ 1.4	81.24 $\pm$ 2.1	31.20*** $\pm$ 1.7	60.28### $\pm$ 1.1	71.12### $\pm$ 1.5
	Viable sperm (%)	79.94 $\pm$ 4.6	63.15 $\pm$ 2.1	71.13 $\pm$ 3.1	36.41*** $\pm$ 1.5	52.19# $\pm$ 1.2	60.17## $\pm$ 1.7
	Mating index (%)	96.0 $\pm$ 5.1	81.37 $\pm$ 3.8	89.21 $\pm$ 2.9	40.21*** $\pm$ 2.1	65.17## $\pm$ 3.1	79.71### $\pm$ 2.7
	FSH	821.4 $\pm$ 35.6	789.24 $\pm$ 13.2	801.21 $\pm$ 11.9	342.3*** $\pm$ 14.3	512.67### $\pm$ 15.2	614.57### $\pm$ 24.7
	Testosterone	28.4 $\pm$ 1.2	21.9 $\pm$ 1.6	25.12 $\pm$ 2.1	2.8*** $\pm$ 1.7	10.24### $\pm$ 2.3	18.27### $\pm$ 2.7
Female	Viable embryo/litter	10.0 $\pm$ 1.7	8.0 $\pm$ 1.5	9.0 $\pm$ 1.4	3.0*** $\pm$ 1.4	5.0 $\pm$ 1.3	7.0## $\pm$ 1.5
	FSH	951.4 $\pm$ 31.8	851.3 $\pm$ 28.4	907 $\pm$ 25.7	431.1*** $\pm$ 14.3	617.2### $\pm$ 18.4	818.7### $\pm$ 21.3
	LH	68.8 $\pm$ 9.7	53.9 $\pm$ 8.1	62.1 $\pm$ 5.7	28.4*** $\pm$ 3.9	41.5## $\pm$ 4.5	50.1### $\pm$ 6.1
	Progesterone	20.16 $\pm$ 1.9	16.12 $\pm$ 1.3	18.21 $\pm$ 1.7	1.6*** $\pm$ 1.6	11.1### $\pm$ 1.8	13.4### $\pm$ 1.4

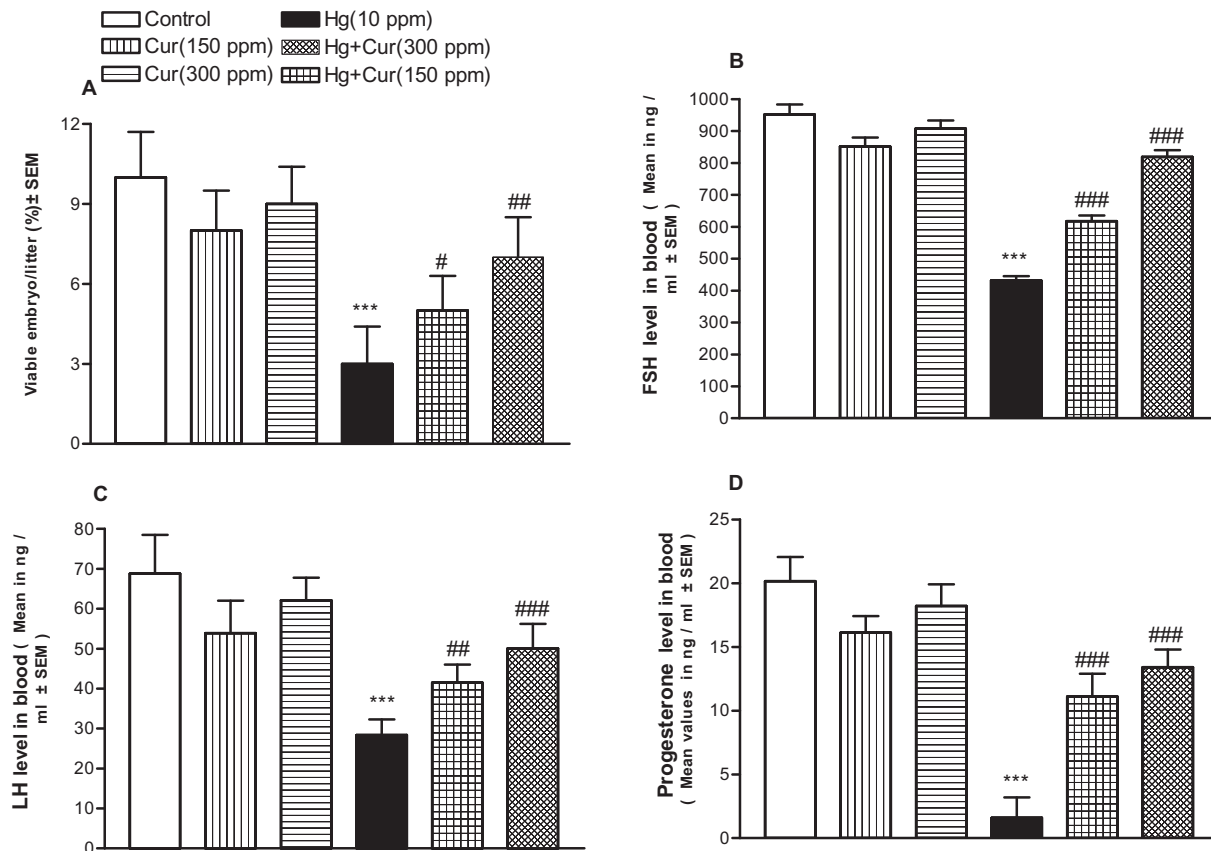
\*\* and \*\*\* show statistically significant at  $P < 0.01$  and  $P < 0.001$  respectively from the control and Cur groups. #, ## and ### show statistically significant at  $P < 0.05$ ,  $P < 0.01$  and  $P < 0.001$  respectively from Hg group by ANOVA and student's  $t$ -test.



**Fig. 3.** A–F. Effect of perinatal HgCl<sub>2</sub> exposure on male mice offspring fertility. A, Sperm count; B, Sperm motility; C, viable sperm; D, mating index; E, FSH; F, testosterone. \*\*\* shows statistically significant at  $P < 0.001$  from the control and Cur groups. #, ## and ### shows statistically significant at  $P < 0.001$  respectively from the Hg group by ANOVA and student's  $t$ -test.

ameliorating effect on the mercury induced infertility, sexual behavior and reproductive disorders. The experimental animal treated with only curcumin did not show any negative impact to the animal. This clearly shows the beneficial and non toxic effect of curcumin. Administration of curcumin after the exposure of

mercuric chloride restored some extent of biochemical and behavioral changes. [Stajin et al. \(1997\)](#) reported antioxidant properties of curcumin and also showed anti-inflammatory activity ([Motterlini et al., 2000](#)). Curcumin also has chemoprotective properties and has been reported by [Ray \(2005\)](#). The biochemical changes cause



**Fig. 4.** A–D Effect of perinatal HgCl<sub>2</sub> exposure on female mice offspring fertility. A, Viable embryo/litter; B, FSH; C, LH; D, progesterone. \*\*\* shows statistically significant at  $P < 0.001$  from the control and Cur groups. #, ## and ### shows statistically significant at  $P < 0.05$ ,  $P < 0.01$  and respectively from Hg group by ANOVA and student's *t*-test.

by mercury and ameliorating potential of curcumin in the present investigation may be due to the fact that curcumin has various health benefits, however much work is needed to analyze the ameliorating properties.

## 5. Conclusion

In this study, perinatal exposure of mercuric chloride on behavior and fertility of female and male mice was studied. The present findings showed significant disruption in fertility of female and male and changes in sexual behavior due to mercuric chloride toxicity. Exposure of mercury poses serious threat to fetus and neonates and easily passes through milk or placenta. Based our finding, curcumin is a key to protect the animals from mercury poisoning.

## Declaration of Competing Interest

The author declared no conflict of interest in this manuscript.

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