



## Original article

## A study on the potential reprotoxic effects of thimerosal in male albino rats



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## ABSTRACT

Thimerosal is ethyl mercury based compound which is being used as a preservative in vaccines since decades. Pharmaceutical products and vaccines that contain thimerosal are among the potential source of mercury exposure. Current research was intended to ascertain the reprotoxic effects of thimerosal on rat testes. Twenty-four adult male albino rats were sorted into four groups (n = 6). The first group was a control group. Rats of experimental Group 2, 3 and 4 were treated with various dosages of thimerosal (0.5, 10, 50 mg/kg) respectively. Rats were decapitated after thirty days of trial and different parameters were analyzed. Thimerosal exposure resulted in a significant decrease in antioxidant enzyme activities including catalase (CAT), peroxidase (POD), superoxide dismutase (SOD), glutathione reductase (GSR) and increased levels of thiobarbituric acid reactive substances (TBARS). Different doses of thimerosal significantly decreased (p < 0.05) the concentration of plasma testosterone, luteinizing hormone (LH) and follicle stimulating hormone (FSH). Additionally, Daily sperm production (DSP) and efficiency of daily sperm production were significantly reduced followed by thimerosal exposure. Moreover, thimerosal significantly (p < 0.05) decreased the primary spermatocytes, secondary spermatocytes, number of spermatogonia along with spermatids. Thimerosal induced adverse histopathological and morphological changes in testicular tissues such as decreased Leydig cells, diameter of seminiferous tubules, tunica albuginea height and epithelial height. On the other hand, the increase in tubular lumen and interstitial spaces was observed due to thimerosal. These outcomes indicated that thimerosal has potential reprotoxic effects in male albino rats.

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## 1. Introduction

The bioaccumulation ability of the heavy metals is a matter of great concern (Sharaf et al., 2020). Mercury, a heavy metal which

exists in the environment in three states; inorganic (mercury sulphide, mercury chloride), organic (ethyl mercury, methyl mercury) and elemental or metallic (Clarkson and Magos, 2006). There is a broad range of toxicological consequences of mercury on humans affecting immune system, CNS, cardiovascular system and kidneys (Clarkson et al., 2003). Pharmaceutical products, thimerosal containing vaccines, fish polluted with mercury and cinnabar which is used in Chinese medicine are the major sources of mercury exposure in humans (Rizzetti et al., 2013; Geier et al., 2015).

Mercury is one of the most damaging sources of the reproductive system in animals and humans (Boujbiha et al., 2009). By disturbing the thyroid, pituitary, pancreas and adrenal glands, mercury can affect the endocrine systems of humans and animals

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even at very low concentration (Rice et al., 2014). Mercury affects the function of the endocrine system by decreasing its hormone-receptor binding ability (Iavicoli et al., 2009). Previous studies have reported that thimerosal critically affected the metabolism of thyroid hormones in rats by reducing the activity of deiodinase D1 and D2 in tissues by binding directly to the selenium of catalytic site (Pantaleao et al., 2017). It has been experimentally proved that mercury causes damage to male gonads and disrupts steroidogenesis and spermatogenesis (Zhu et al., 2000). Administration of mercury affects the sertoli cells, which plays an important role in spermatogenesis (Monsees et al., 2000).

Thimerosal is used in vaccines, which breaks down into ethyl mercury (Et-Hg) and thiosalicylic acid and readily accumulates in the tissues (Magos, 2003). Et-Hg, which is released from thimerosal is more lethal as compared to the parent compound (Clarkson et al., 2003). Due to lack of knowledge, the risk assessments for Et-Hg was made on the basis of toxicity caused by Me-Hg. Nonetheless, recent data have displayed that Me-Hg is not a proper reference for risk assessment for mercury released from thimerosal as there is a large difference between the kinetics of metabolism of both methyl and ethyl mercury (Magos, 2003; Burbacher et al., 2005).

The harmful impacts of thimerosal are abnormal pain sensitivity (Olczak et al., 2009), neuro-degradation of hippocampus (Olczak et al., 2010) and modification in dopaminergic pathways with successive behavioral disorganization (Olczak et al., 2011). Likewise, it is reported that neonatal administration of thimerosal may cause poor regulation of neurodevelopment, endocrine system and synaptic activity, which could be incidentally linked with mice autistic behavior (Li et al., 2014). Despite the harmful effects, thimerosal is still being used in antiseptics and vaccines (Sykes et al., 2014). Current research was planned to estimate the damaging effects of thimerosal on the testicular tissues of adult male rats.

## 2. Materials and methods

### 2.1. Experimental design

Adult male albino rats (170–200 g), *Rattus norvegicus* were used in the research trial. Rats were placed in the animal house at 24–27± °C for 30 days. 12 h. The dark / light cycle was maintained. Rats were fed with proper food and tap water. Four groups of rats (n = 6) were ascertained. Group 1: This group was taken as control and given with normal food and tap water. Group 2: 5 mg/kg dose of thimerosal was given to this group via oral gavage. Group 3- (n = 6): This group was treated with 10mg/kg dose of thimerosal. Group 4-(n = 6): 50 mg/kg dose of thimerosal was given.

### 2.2. Biochemical analysis

Activity of CAT and POD was assessed by the procedure of Chance and Maehly (1955). SOD activity was measured through the process developed by Kakkar et al. (1984). The activity of GSR was estimated by the procedure developed by Carlberg and Mannervik (1975). In order to determine the lipid peroxidation (TBARS), procedure of Wright et al. (1981) was followed.

### 2.3. Hormonal analysis

Plasma testosterone concentration in the testicular homogenates was measured by using ELISA (enzyme-linked immunosorbent assay) kits. FSH and LH concentrations were evaluated through Immuno-Assay Test Kits (Gen-Way Biotech. Inc.).

### 2.4. Daily sperm production (DSP)

DSP was measured through the process of Robb et al. (1978). Number of spermatids that remained resilient through the homogenization process were divided by 6.3 for calculation of DSP.

$$DSP = Y/6.3$$

Where, Y = spermatids present in homogenate  
6.3 = Entire days through spermatids remained in the seminiferous tubules epithelial part.

### 2.5. Tissue histology

After dissection testicular tissues were fixed in 10% formalin and dipped in alcohol of different grades, cleaned with cedarwood oil then fixed in paraffin. Sections of 5 µm thickness were slashed out, placed on a glass slide, stained through eosin and hematoxylin and then examined under Nikon microscope (187842, Japan). Image J2x software was used to study different parameters of histology.

### 2.6. Statistical analysis

The data was tabulated as means (±SEM). The experiment was factorial design by One-way ANOVA followed by Dunnett's test was applied to compare the treated groups with the with the control. The data were analyzed by using Minitab software. The significance was checked at a level of p < 0.05.

## 3. Results

### 3.1. Effect of thimerosal on antioxidant enzymes and TBARS

Results presented that there was significant (p < 0.05) reduction in activities of CAT, SOD, POD and GSR in thimerosal administered groups in comparison to control in dose dependent manners (5, 10 and 50 mg/kg). Instead, TBARS level was substantially (p < 0.05) increased as a result of thimerosal administration dose dependently in comparison to control. Changes in antioxidant enzyme activity and TBARS level are presented in Table 1.

### 3.2. Effect of thimerosal on concentration of plasma testosterone, LH and FSH

Thimerosal treatment displayed remarkable (p < 0.05) decline in the concentrations of testosterone, LH and FSH compared to control and this decrease in concentration was in dose dependent manners (Table 2).

### 3.3. Effect of thimerosal on DSP and efficiency of DSP

Thimerosal treatment at various doses remarkably (p < 0.05) decreased the DSP and efficiency of DSP in thimerosal administered groups when matched with a control group as shown in Table 2.

### 3.4. Effect of thimerosal on tissue histopathology

A remarkable (p < 0.05) increase in the interstitial spaces and diameter of tubular lumen were detected in thimerosal administered groups when matched to the control group. The height of the tunica albuginea, and diameter of seminiferous tubule was decreased considerably (p < 0.05) followed by treatment of thimer-

**Table 1**  
Effect of thimerosal on activity of CAT, SOD, POD, GSR and TBARS level in the testis of treated groups.

Groups	CAT (U/mg protein)	Activity of POD (nmole)	SOD (U/mg protein)	GSR (nM NADPH oxidized/ min/mg tissues)	TBARS (nM TBARS/min/mg tissues)
Control	6.13 ± 0.08 <sup>a</sup>	3.40 ± 0.03 <sup>a</sup>	4.25 ± 0.05 <sup>a</sup>	2.46 ± 0.07 <sup>a</sup>	14.07 ± 0.07 <sup>a</sup>
Thimerosal (5 mg/kg)	5.66 ± 0.06 <sup>b</sup>	3.13 ± 0.04 <sup>a</sup>	3.96 ± 0.04 <sup>b</sup>	1.96 ± 0.07 <sup>b</sup>	16.10 ± 0.07 <sup>b</sup>
Thimerosal (10 mg/kg)	5.45 ± 0.08 <sup>b</sup>	3.01 ± 0.05 <sup>b</sup>	3.87 ± 0.06 <sup>b</sup>	1.83 ± 0.11 <sup>b</sup>	17.78 ± 0.11 <sup>c</sup>
Thimerosal (50 mg/kg)	4.29 ± 0.09 <sup>c</sup>	2.68 ± 0.05 <sup>c</sup>	3.04 ± 0.05 <sup>c</sup>	1.22 ± 0.09 <sup>c</sup>	19.05 ± 0.09 <sup>d</sup>

Means that do not shared similar letters are significantly different.

**Table 2**  
Concentrations of plasma testosterone, LH, FSH, DSP and efficiency of DSP in thimerosal administered groups.

Groups	Plasma testosterone conc.(ng/ml)	LH conc. (mIU/ml)	FSH conc. (mIU/ml)	DSP × 10 <sup>6</sup> / testis	Efficiency of DSP × 10 <sup>6</sup> /testis
Control	7.10 ± 0.06 <sup>a</sup>	2.88 ± 0.05 <sup>a</sup>	3.43 ± 0.06 <sup>a</sup>	21.31 ± 0.59 <sup>a</sup>	13.29 ± 0.35 <sup>a</sup>
Thimerosal (5 mg/kg)	6.44 ± 0.06 <sup>b</sup>	2.47 ± 0.04 <sup>b</sup>	3.14 ± 0.01 <sup>b</sup>	17.98 ± 0.44 <sup>b</sup>	12.08 ± 0.14 <sup>b</sup>
Thimerosal (10 mg/kg)	6.13 ± 0.06 <sup>b</sup>	2.35 ± 0.03 <sup>b</sup>	3.02 ± 0.04 <sup>b</sup>	16.73 ± 0.26 <sup>c</sup>	11.75 ± 0.21 <sup>c</sup>
Thimerosal (50 mg/kg)	5.30 ± 0.08 <sup>c</sup>	2.08 ± 0.04 <sup>c</sup>	2.93 ± 0.04 <sup>c</sup>	15.74 ± 0.20 <sup>d</sup>	10.83 ± 0.20 <sup>d</sup>

Means that do not shared similar letters are significantly different.

**Table 3**  
Cell-types number in the testicular tissues of thimerosal administered groups.

Groups	Spermatogonia	Primary spermatocyte	Secondary spermatocyte	Spermatids	Leydig cells
Control	43.2 ± 0.90 <sup>a</sup>	38.3 ± 0.52 <sup>a</sup>	31.2 ± 0.68 <sup>a</sup>	48.1 ± 0.65 <sup>a</sup>	4.5 ± 0.20 <sup>a</sup>
Thimerosal (5 mg/kg)	38.2 ± 0.96 <sup>b</sup>	33.8 ± 0.79 <sup>b</sup>	26.9 ± 0.89 <sup>b</sup>	42.9 ± 1.31 <sup>b</sup>	3.7 ± 0.11 <sup>b</sup>
Thimerosal (10 mg/kg)	38.8 ± 0.91 <sup>b</sup>	28.0 ± 1.41 <sup>c</sup>	21.1 ± 0.70 <sup>c</sup>	37.0 ± 0.85 <sup>c</sup>	2.7 ± 0.11 <sup>c</sup>
Thimerosal (50 mg/kg)	22.9 ± 1.39 <sup>c</sup>	20.0 ± 0.67 <sup>d</sup>	18.3 ± 0.82 <sup>d</sup>	32.6 ± 1.12 <sup>d</sup>	1.9 ± 0.06 <sup>d</sup>

Means that do not share similar letters are significantly different.

**Table 4**  
Effect of thimerosal on morphometry of testes of treated groups.

Groups	Interstitial spaces (μm)	Height of tunica albuginea (μm)	Seminiferous tubule epithelial height (μm)	Diameter of seminiferous tubule (μm)	Tubular lumen (μm)
Control	9.63 ± 0.35 <sup>a</sup>	25.5 ± 0.55 <sup>a</sup>	72.6 ± 1.16 <sup>a</sup>	222.6 ± 0.13 <sup>a</sup>	14.8 ± 1.15 <sup>a</sup>
Thimerosal (5 mg/kg)	11.4 ± 0.29 <sup>b</sup>	22.1 ± 0.92 <sup>b</sup>	67.5 ± 1.63 <sup>b</sup>	214.5 ± 0.35 <sup>b</sup>	10.5 ± 0.54 <sup>b</sup>
Thimerosal (10 mg/kg)	15.5 ± 0.55 <sup>c</sup>	18.4 ± 0.86 <sup>c</sup>	58.5 ± 0.57 <sup>c</sup>	209.9 ± 0.15 <sup>c</sup>	7.6 ± 0.54 <sup>c</sup>
Thimerosal (50 mg/kg)	18.7 ± 0.81 <sup>d</sup>	12.9 ± 0.75 <sup>d</sup>	55.7 ± 0.55 <sup>d</sup>	201.4 ± 0.28 <sup>d</sup>	5.3 ± 0.41 <sup>d</sup>

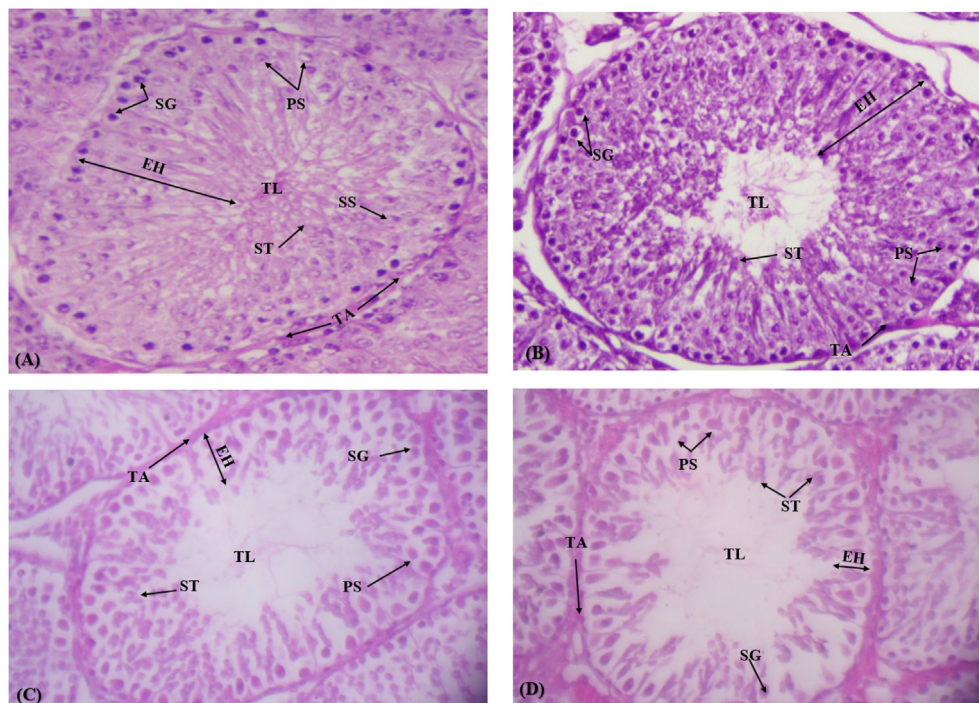
Means that do not shared similar letters are significantly different.

osal matched with a control (Table 3). Thimerosal treatment generated a substantial ( $p < 0.05$ ) decline in the primary and secondary spermatocytes numbers, spermatogonia, spermatids and Leydig cells when matched with a control group (Table 4). All these alterations due to exposure of thimerosal were found in dose dependent ways (Fig. 1).

#### 4. Discussion

Mercury derivative, thimerosal is made up of thiosalicylic acid and ethyl mercury, which has been extensively used in ocular, dermatological preparations and as a preservative in vaccines. In the hospitals, vaccines containing the thimerosal are major passage of exposure to mercury (Bigham and Copes, 2005). Both ethyl and methyl mercury can exist in the environment as deciles. These dialkyls are unstable and hard to manage for practical use, including studies of toxicology. Moreover, Et-Hg (Thimerosal) and Me-Hg get immediately absorbed via the skin and air passages and are very toxic even at very low levels (Carocci et al., 2014).

Thimerosal generates ROS (Kim et al., 2002) and the toxicity caused by thimerosal in HeLa S cells was due to ROS generation. Viability of HeLa S cells was remarkably reduced by thimerosal, and it is also linked with the reduction of intracellular levels of glutathione (Lee et al., 2006). ROS generates oxidative stress in the cells and ultimately decreased the activity of antioxidant enzymes. Animals exposed to different forms of Hg suffer induced oxidative stress and ROS mediated cell death (Park and Park, 2007). CAT is an essential antioxidant to make the cells resistant against dangerous effects of ROS and hydrogen peroxide (Coban et al., 2007). There is a coordination in the functions of anti-oxidant enzymes, including CAT, POD and SOD, to prevent the cell from oxidative stress (Palermo et al., 2015). In our study, it has been determined that thimerosal reduced the antioxidants (CAT, SOD, POD and GSR) activity. Activities of antioxidant enzymes decreased and oxidative stress increased in the testes of rats after exposure of HgCl<sub>2</sub> (El-Desoky et al., 2013). Decreased activity of antioxidant enzymes leads to increase in lipid peroxidation indicated by elevated levels of TBARS. Thimerosal administration results in an increased TBARS level in doctored rats, that is in line with the



**Fig. 1.** Microphotographs of the rats testicular tissues (Hematoxylin-Eosin-40X): (A) Control group exhibiting regular morphologic structure of testes. (B) Thimerosal (5 mg/kg) showing slight in TL, decrease in EH and slightly disturbed morphometry of tubule. (C) Thimerosal (10 mg/kg) showing increased size of lumen, damage SG, decreased EH, less SG, PS and SS in numbers (D) Thimerosal (50 mg/kg) showing severely damaged tubule including increased TL, disturbed TA, decreased EH and less number of SG, PS and SS. Primary spermatocytes = PS; Secondary spermatocytes = SS, Tubular lumen = TL, Tunica albuginea = TA, Spermatogonia = SG.

increase in TBARS in the head and body of flies treated with thimerosal (Bianchini et al., 2019). Natural antioxidants containing plant extracts have shown useful curative abilities against chemically persuaded damages (Bakr et al., 2019).

Thimerosal resulted in a decrease in concentration of LH and FSH. The pituitary gland secretes the LH hormone which further starts the testosterone production. However, testosterone production is required to sustain sperm production (Mantovani, 2002). Furthermore, testosterone and FSH accelerate the sperms release and growth of spermatids (Chauhan et al., 2007). In this analysis, the thimerosal mediated decreased concentration of testosterone, LH and FSH probably due to the effect on the hypothalamic pituitary gonadal axis. Exposure of mercury in adult albino rats causes reduction in the levels of follicle stimulating hormone and luteinizing hormone (Ramalingam et al., 2003). Thimerosal caused a decrease in the concentration of plasma testosterone. There is a dramatic decrease in plasma testosterone in animals exposed to mercury (Moussa et al., 2011). Thimerosal mediated the reduction in testosterone concentration, perhaps as a consequence of the drop in approachability of gonadotropin to Leydig cells in the testes. As spermatogenesis relies on the reproductive hormones (FSH and testosterone) (Schulz and Miura, 2002) the decreased concentration of testosterone led to a reduction in the number of sperm production. Thus, minimized levels of these hormones due to thimerosal intoxication lead to DSP reduction in rats.

The present study reports that thimerosal exposure resulted in a decreased number of germ cell population at various stages within experimental groups. The outcomes of our study are in line with Altunkaynak et al., 2015 who reported a decrease in primary, secondary spermatocytes and the number of spermatids after Hg exposure, which is an integral part of thimerosal. Penna et al. (2009) has suggested that mercury damage to spermatogenesis. Number of Leydig cells were significantly reduced after treatment

with thimerosal. Vachhrajani and Chowdhury (1990) reported that, rats treated with Me-Hg for three months displayed degenerated Leydig cells and a reduction in their numbers. As our results demonstrated, height of the tunica albuginea, lumen diameter, diameter of seminiferous tubules decreased and interstitial space increased in thimerosal treated groups which is attributed to reduced testosterone and increased oxidative stress.

## 5. Conclusions

In conclusion, our findings show that exposure to thimerosal results in increased oxidative stress and decreased activities of antioxidant enzymes, which ultimately lead to impairment in reproductive hormones and eventually decreased daily sperm production in testicular tissues of treated rats. Our findings provided information about the safe use of low concentrations of thimerosal in vaccines. Thus, the use of thimerosal as animal and human vaccine preservative should be of great concern, specifically till the efficient risk evaluation. More studies are required to investigate the molecular basis of these changes both in vivo and in vitro, which will help to identify that how thimerosal influences the physiology of various tissues within the body.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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