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Assessment of the Effects Following Subchronic Dosing with Sodium Tungstate on Male Reproductive System in Wistar Rats

Geeta Pandey*, Prof Gyan Chand Jain, Nidhi Mathur

Lab no. 15, Reproductive Physiology Lab, Department of Zoology, University of Rajasthan, Jaipur, India

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*Corresponding Author

Tel : +91-9784390737

Email:geetika_pandey12@yahoo.co.in

Abstract

Sodium tungstate is one of the inorganic compound with insulinomimmetic properties. The present study was conducted to investigate the effects of sodium tungstate on male reproductive system. The experimental rats were randomly divided into two groups (n=8/ group). The first group was used as a control, the second group was used to study the effect of sodium tungstate. Sodium tungstate was orally administrated to male Wistar rats at a dose level of 50mg/kg.bd wt. /day for 60 days. The daily administration produced no significant changes in the body weight gain. No significant changes were observed in the weight of testis, epididymis, seminal vesicles, ventral prostate, liver, kidney, and adrenal gland. Exogenous supplementation with Sodium tungstate produce non significant depletion in protein, glycogen and sialic acid content in testis and epididymis and fructose level in seminal vesicle. Cholesterol level showed no significant change in testis and epididymis. However, Spermatozoon motility and epididymal sperm concentration showed no significant changes. Plasma testosterone, FSH and LH level showed non significant decline in the sodium tungstate treated animals when compared to the control animals.The histology of testis and cauda epididymis showed no severe histological alterations when compared to control group. The results conclude that sodium tungstate (50 mg/kg bd.wt./day) did not cause any severe histological and biochemical alteration in male reproductive system.

Introduction

Tungsten (chemical symbol W, atomic number 74, atomic weight 183.85) is a greyish-white lustrous metal which is solid at room temperature [1]. Tungsten is an element belonging to group VIb of the periodic system.Tungsten is one of the rare metals, comprising only about 1.5 ppm of the earths crust. It occurs naturally as tungstate [2]. Tungstate is the most soluble form of W, it is the most frequently encountered form in ecological and biological systems [3].

For the general population, exposure to tungsten is possible from breathing air, drinking water or ingestion of products containing tungsten or its compounds. For examples, beverages such as wine, mineral water, beer, brewed tea and instant coffee were found to significantly contribute to the total dietary intake of tungsten. The production and use of tungsten compounds (example, as catalyst and dyes) may result in the release of tungsten to the environment through waste streams. [4,5]. Anthropogenic activities, such as agriculture and mining, can increase the concentration of tungsten in soils through direct application or atmospheric deposition into soil [6,7,8]. Tungsten uptake by agricultural crops is of concern because of the potential for tungsten getting into the food supply. Tungsten concentrations have been demonstrated to be highest (approximately 1.5 mg/kg) in phosphate fertilizers [6].

Sodium tungstate is used for fire- and waterproofing fabrics, in the preparation of complex compounds (e.g., phosphotungstate and silicotungstate), as a reagent for biological products, and as a precipitant for alkaloids. It is also used as a catalyst in the oxidation of maleic acid, as pigment in ceramics and colour resistant mordants for textiles, in cathode-ray tubes and x-ray screens[9]. In military applications, heavy metal tungsten alloys (HMTA) have recently gained interest as a replacement for lead and uranium. Plants provide a potential source of antidiabetic drugs. In India, most of the people, especially in rural areas use traditional medicine of plants to treat many diseases including diabetes[10].The medicinal property of metals are also being described. Some metals are essential for life, others have unknown biological functions, either favorable or toxic and some others have the potential to caused toxicity [11]. The biological function of selenate, molybdate, tungstate and vandate is related to reduction of blood glucose concentration and increase of glucose uptake. The antidiabetic and antiobesity properties of tungstate have been widely reported in several animal models of diabetes [12]. Oral administration of sodium tungstate is an effective treatment for diabetes in animal models. Sodium tungstate has shown a remarkable normoglycemic effect in several animal models of diabetes and low toxicity in diabetic and healthy animals [13]. Morever, these effects are maintained for long term and undesirable effects such as hypoglycemic episodes or tungstate intolerance do not appears during the treatment [12]. Marc Claret *et al*. [14] evaluated that tungstate, by

increasing thermogenesis and lipid oxidation in adipose tissue, prevents body weight gain of obese rats on a high-fat diet.

McCain *et al*. [15] concluded that administration of sodium tungstate at 200 mg/kg to male and female Sprague-Dawley rats via oral gavage for 90 consecutive days resulted in pronounced renal changes, specifically renal tubular necrosis. In a study by Guandalini *et al*. [16] following oral exposed laboratory mice to different oral doses of sodium tungstate (0, 62.5, 125, and 200 mg/kg/d) for 28 days. Sodium tungstate, tungsten mainly accumulates in the bone and spleen, but retention is also observed in the colon, kidney, liver, and brain (from highest to lowest concentration). Kalinich *et al*. [17], embedded tungsten alloy pellets in rat (91.1% tungsten, 6% nickel, 2.9% cobalt) not only resulted in aggressive localized tumors (high-grade pleomorphic rhabdomyosarcomas) that rapidly metastasized to the lungs, but also caused significant hemopoietic changes well before the carcinogenic effect was observed. Tungstate also activates adenylate cyclase in the brain, heart, lungs, kidneys, and liver of the rat [18].

The NTP is currently conducting systematic studies on the toxicity of tungstate administered orally (via drinking water) to mice and rats. Although a direct association between W and leukemia has not been made, the uncertainty in W toxicity reinforces the need to systematically characterize its toxicity.In order to find out the toxic effects on male reproductive toxicity of sodium tungstate, following study was conducted.

Materials and Methods

Chemicals:

Sodium tungstate was purchased from the Merck India Ltd., Mumbai, India.

Animals treated:

In this investigation, Colony bred adult healthy male and female Wistar rats weighing170–200 g were used in the present investigation. The rats were housed in standard rat cages and maintained under standard conditions (12-h light/dark cycle; 25±3°C temperature), and provided a standard laboratory chow (Aashirwad Food Industries, Chandigarh, India) and water *ad libitum*. Test substance and/or vehicle were administered to all male rats by oral intubation. The animals were maintained as per guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) regulations. The study was approved by the institutional ethical committee of the Department of Zoology, University of Rajasthan.

Dose and duration of treatment

The rats were divided into two groups of eight rats each. The daily dose of the chemical was dissolved in 0.5 ml of distilled water and orally administered to each experimental animal every morning for 60 days.

Group A: Control rats received 0.5 ml/day of the vehicle, that is, distilled water.

Group B: Rats treated with Sodium tungstate ($Na_2WO_4 \times 2H_2O$) 50 mg/kg bd.wt./day.

Rat in each cage received water from a separate glass container. Containers were checked each day and protected from light. Animals were observed twice daily.

Autopsy schedule

The animals were weighed and autopsied under light ether anesthesia 24 h after last dose of the treatment.

Body and organ weight

Body weight was recorded before the beginning of treatment, at weekly intervals and at the end of treatment. Food consumption was monitored daily. The testes, epididymides, vas deferens, seminal vesicles and ventral prostate, kidney, adrenal and liver were removed, cleared of the adhering connective tissue and weighed.

Tissue biochemistry

Testes, epididymis and accessory sex organs were frozen at – 20°C for the biochemical estimations. Testes and epididymis were assayed for protein [19], sialic acid [20], glycogen [21] and cholesterol [22]. Fructose in seminal vesicle was also estimated [23].

Radioimmunoassay of testosterone, FSH and LH

Blood samples were also collected for estimations of serum testosterone by radioimmunoassay (RIA) [24]. Follicle-stimulating hormone and Luteinizing Hormone [25].

Epididymal sperm concentration and motility

Spermatozoa from cauda epididymis were counted by Prasad *et. al.* [26] method Briefly, One hundred milligram of the epididymis was finely minced with anatomical scissors in 1 mL of physiological saline, placed in a rocker for 10 min then allowed to sit at room temperaturefor 2 min. Total sperm number was determined by using a hemocytometer. Approximately 10 mL of the diluted sperm suspension was transferred to each counting chamber of the hemocytometer and was allowed to stand for 5 min. The cells settled during this time were counted with the help of light microscope (Magnification, 200x). Cauda epididymal sperm counts expressed as million/mm3 of suspension . For sperm motility, one drop of evenly mixed sample was applied to a glass slide under a cover glass. The percent motility was determined by counting both motile and immotile spermatozoa per unit area [27].

Fertility test

Male rats were introduced to parous females, 170-200 gm (male:female ratio, 1:2) at 17:00 h after 55 days of treatment. The successful mating was confirmed in the forthcoming mornings from 56 to 61 days by vaginal plug and spermatozoa in the vaginal smear. The inseminated females were separated and allowed to deliver at term. The number of pups delivered and their characteristics were noted [27].

Histological examination

Testes were fixed in Bouin's fluid. Paraffin section were cut (5µm) and stained with hematoxylin and eosin. Mean seminiferous tubular diameter was determined by measuring 100 round sections of seminiferous tubule with the help of ocular micrometer.

Diameter of Leydig cells nuclei were measured at×800.

Statistical analysis

Data are expressed as mean ± S.E.M. one-way analysis of variance (StatPlus, 2007) was used for statistical comparison.

Results

During the experimental procedure, no deaths or any other clinical signs of toxicity were observed.

Effect of sodium tungsate on Body and organ weight

The growth and general appearance of the sodium tungstate treated animals was normal throughout the experiment. Sodium tungstate did not cause any significant adverse effect on the body weight of treated rats.The weight of testes, epididymides, vas deferens, seminal vesicle and ventral prostate was also showed no significant reduction as compared to control. The weight of other vital organs remained significantly unchanged as compared to the control rats.

Table 1: Effect of Sodium tungstate treatment on the body and organ weight of male albino rats

	Body weight (g)		***Reproductive organs weight (mg/100 g body weight)***					Vital organ weight (mg/100 bd.wt)		
Treatment	Initial	Final	Testes	Epididymides	Ventral prostate	Seminal vesicle	Vas deferens	kidney	Adrenal	Liver
Group A, control (vehicle-treated)	173.75 ±5.66	201.87 ±4.33	1174.50 ±43.84	497.51 ±62.56	283.08 ±16.63	401.31 ±14.95	104.58 ±4.75	590.97 ±21.36	22.43 ± 1.02	3229.53 ± 72.25
Group B, 50 mg/kg body weight / day for 60 days	172.5 ±2.84	221.62 ±3.54	1089.08^{ns} ±19.4 (p<0.125)	474.03^{ns} ±10.32 (p<0.2119)	267.57^{ns} ±10.81 (p<0.5036)	395.59^{ns} ±8.36 (p<0.759)	98.34^{ns} ±2.61 (p<0.271)	550.17^{ns} ±11.61 (p<0.137)	19.92^{ns} ± 1.12 (p<0.189)	3183.75^{ns} ± 71.88 (p<0.669)

Data are expressed as Mean ± SEM of eight animals. ANOVA analysis of variance; Groups B was compared with Group A, [a] Highly significant (p≤0.0001) ; [b] Significant (P≤.001); [ns] non significant

Tissue biochemistry

Protein, glycogen and Sialic acid content of testes and epididymides was reduced non significantly after sodium tungstate treatment as compared with controls. Fructose level in seminal vesicle also non significantly declined. However, the cholesterol content of testes and epididymides was non significantly increased after the administration of sodium tungstate(Table-2).

Table 2: Effect of Sodium tungstate treatment on tissue biochemistry of male albino rats

Treatment	***Cholesterol (mg/g)***		***Sialic acid (mg/g)***		***Glycogen (mg/g)***		***Total protein (mg/g)***		***Fructose***
	Testis	Epididymis	Testis	Epididymis	Testis	Epididymis	Testis	Epididymis	Seminal vesicle
Group A, control(vehicle-treated)	4.98 ± 0.30	4.72 ± 0.17	4.63 ± 0.16	4.51 ± 0.16	3.43 ± 0.14	3.96 ± 0.13	194.37 ± 6.81	198.12 ± 8.59	3.12 ± 0.21
Group B, 50 mg/kg body weight /day for 60 days	5.06^{ns} ± 0.21 (p<0.801)	5.03^{ns} ± 0.04 (p<0.110)	4.45^{ns} ± 0.15 (p<0.446)	4.35^{ns} ± 0.20 (p<0.5461)	3.13^{ns} ± 0.15 (p<0.178)	3.39^{ns} ± 0.17 (p<0.127)	192.63^{ns} ± 5.75 (p<0.855)	197.37^{ns} ± 5.07 (p<0.941)	3.20^{ns} ± 0.12 (p<0.737)

Data are expressed as Mean ± SEM of eight animals. ANOVA analysis of variance; Groups B was compared with Group A, [a] Highly significant (p≤0.0001) ; [b] Significant (P≤.001); [ns] non significant

Hormonal assays

Non Significant decline was noticed in serum testosterone levels, FSH and LH level in treated group when compared with group A (Table 3).

Table 3: Effect of Sodium tungstate treatment on serum testosterone, serum FSH, serum LH, cauda epididymis sperm analysis, fertility test and morphometry of male albino rats

Treatment	*Serum testosterone (ng/mL)*	*Serum FSH (ng/mL)*	*Serum LH (ng/ml)*	*Sperm count(million/mm³)*	*Motility (%) Cauda epididymis*	*Fertility test (%)*	*Seminiferous tubule diameter (µm)*	*Leydig cell nuclei diameter (µm)*
Group A, control (vehicle-treated)	4.06 ±0.11	112.87 ±1.39	4.04 ±0.057	44.14 ±0.628	63.33 ±1.27	93.75% (15/16)	258.12 ±4.79	6.07 ±0.089
Group B, 50 mg/kg body weight / day for 60 days	3.95^{ns} ±0.10 (p<0.4745)	108.25^{ns} ±1.164 (p<0.381)	3.93^{ns} ±0.063 (p<0.212)	42.95^{ns} ±0.66 (p<0.2094)	60.31^{ns} ±1.69 (p<0.172)	87.5% (14/16)	248.12^{ns} ±4.29 (p<0.1343)	5.93^{ns} ±0.13 (p<0.383)

Data are expressed as Mean ± SEM of eight animals. ANOVA analysis of variance; Groups B was compared with Group A, [a] Highly significant (p≤0.0001); [b] Significant (P≤.001); [ns] non significant

Effect on sperm motility and sperm count

Sodium tungstate significantly reduced sperm count (Table 3) and sperm motility (Table 3) in cauda epididymis.

Fertility test

The fertility of the male rats was assessed by the incidence of pregnancy in females. There was no significant change in the fertility of male rats (Table 3).

Histopathological observation

Control testes showed well-organized seminiferous tubules. All stages of transformation of the seminiferous epithelium from spermatogonia to mature spermatozoa could be seen in the tubules. Testicular tissues from control animals were considered normal, with no cellular exfoliation, abnormal cellular necrosis, or apparent change in spermatogenesis (Fig. 1). Treated groups were essentially normal, containing only incidental microfocal lesions of seminiferous or epididymal tubule. Spermatogenesis does not appear to be affected. However, testicular sections of treated rats showed no change in the diameter of seminiferous tubular and number of Leydig cells as compared to that of control rats though this was not quantified (Fig. 2). There were no other significant observed histopathological changes found in Cauda epididymis of treated rats.The epithelial cell height of cauda epididymis in treated rats showed no decreament as compared to control rats. The lumen is filled with spermatozoa (Fig 3,4).

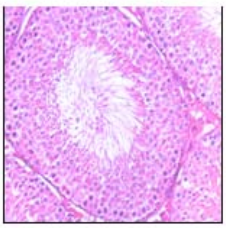

Fig.1. Photomicrograph of testis of a rat of group A (vehicle treated control) showing normal features with successive stages of transformation of seminiferous epithelium to spermatozoa. H & E×200.

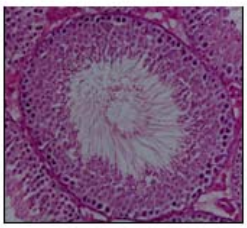

Fig.2. Photomicrograph of testis of a rat of group B (50 mg/kg body weight, sodium tungstate) after 60 days of treatment showing no significant change in seminiferous tubular diameter and number of spermatozoa. H &

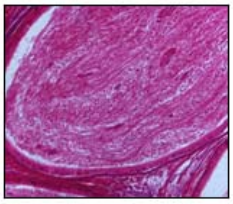

Fig.3. Photomicrograph of cauda epididymis of a rat of group (vehicle treated control) after 60 days showing normal architecture of cauda epididymis. H & E×200.

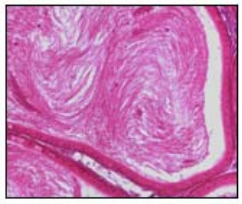

Fig.4. Photomicrograph of cauda epididymis of a rat of group B (50 mg/kg body weight, sodium tungstate) after 60 days of treatment showing no severe alterations in epithelial cell height or number of spermatozoa in lumen. H & E×200.

Discussion

Tungsten is a widely used transition metal for which very limited information on environmental and toxicological effects is available. Of particular interest is the lack of information linking tungsten speciation and environmental effects. [28]. Sodium tungstate has been found to be an effective hypoglycemic and antiobese agent in several animal models. Although very little

has been reported about the toxicity of the different chemical forms of tungsten, including tungstate.

In rats, the oral and iv LD50 (median lethal dose) values were 1928.4 and 61.0 mg/kg, respectively [29]. These LD50 values indicate that this compound has a rather low toxicity. Findings of present study are also in favour of previous studies. Our results showed no severe toxic effects of sodium tungstate on male reproductive system at the dose level of 50 mg/kg bd. Wt/day. There is no significant change in the body and organ weight which showed that the compound don't effect the metabolic activities at all. Our results are in accordance with the previous studies. Pharmokinetics studies indicate that tungsten is rapidly and thoroughly absorbed orally and rapidly eliminated in urine (50% in 24 h) but less quickly in feces (50% in 4 days)[2,30,31,32] .Although these data suggest that there is little bioaccumulation of tungsten. [33]. The protein content in testis and epididymis reduced non significantly.The normal protein content represent normal protein metabolism. A non significant decrease was noted in glycogen amount in testis and epididymis.The glycogen content in the cell represents the energy storage. The Sertoli cells and spermatogonia contain glycogen and provide nourishments to the seminiferous tubular cells and the glycogen content is found to be directly proportional to the steroid hormone levels [34].The non significant increased cholesterol content of testis may reflect a slight reduced conversion of cholesterol to androgens, which is dependent on the availability of LH/ ICSH. The results of present studies reflect non significant increment in cholesterol and non-significant decrease in testosterone, FSH, LH. Non significant decline level of sialic acid in testes, epididymides may be correlated with loss of androgen [35]. The testicular sperm counts and daily sperm production are important indicators for investigators to detect the adverse effects of various factors on spermatogenesis [36]. Our results suggest that sodium tungstate showed no significant change in cauda epididymal sperm counts and sperm motility.Normal histoarchitecture of testis and epididymis can be explained by normal level of testosterone,FSH and LH.

Conclusions

In conclusion, although the results of the present study did not indicate that sodium tungstate exposure resulted in direct male reproductive toxicity in the rat, but fertility problems might occur. So, additional studies, including genotoxic assessment should be undertaken to assess whether sodium tungstate can harm the fertility of the exposed male. Further studies should be conducted to determine the level at which it is no toxic and the extent to which humans might be exposed to the substance. To date, data on the beneficial/adverse effects of tungstate administration in diabetic patients are not available. However, as for any metal, tissue accumulation and potential toxicity derived from the chronic use of tungstate cannot be dismissed. Studies to evaluate the potential adverse effects derived from the prolonged use of tungstate in diabetes treatment are also clearly required.

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