Reproductive Toxicity of Vanadyl Sulphate in Male Rats

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The reproductive functions were evaluated by epididymal sperm counts, motility, fertility rate, reproductive organ weights, biochemistry and histological examination of testes in vanadyl sulphate treated adult male Wistar rats. Oral administration of vanadyl sulphate (100 mg/kg b.wt./day) for 60 days caused a decrease ($p < 0.05$) in the weights of testes and accessory reproductive organs. Cauda epididymal sperm analysis exhibited a significant decline in the number ($p < 0.01$) and motility ($p < 0.001$). Atrophy of seminiferous tubules was observed in histopathological examination. The diameter of seminiferous tubules and Leydig cells nuclei were reduced. Biochemical analysis of marker parameters indicated alteration in biochemical milieu of the genital organs. The mating tests with untreated females revealed a decrease in pregnancy rate and mean number of the pups delivered. As such present investigation indicate an adverse effect of vanadyl sulphate on male reproductive functions.

Key words —— vanadyl sulphate, testes, reproductive toxicity, fertility, rats

INTRODUCTION

Vanadium occurs in the natural (water, rocks and soil) environment at low concentrations. Vanadium exists in oxidation states from −1 to +5. It is a natural component of fuel oils (crude petroleum deposits). It's occupational exposure is in oil-fired electricity generating plants, where high levels (upto 50%) may appear in fuel gas; also in steel, mining and petrochemical industries.[1)] Recent research suggests that vanadium may help regulate the Na^+ pump and it has been characterized as a constituent of several enzyme systems and complexes within living beings. Vanadium is probably an essential trace element, although vanadium — deficiency disease has not been detected in humans.[2)]

Vanadium compounds exert a variety of biological responses; Vanadyl ion and its complexes are effective not only in treating or relieving of diabetes mellitus but also in preventing the onset of this disease. Vanadyl sulphate is thought to mimic the physiological effects of insulin by an unclear mechanism. Through this insulin — mimetic effect vanadium is thought to promote glycogen synthesis, maintain blood glucose level and stimulate muscle anabolism.[3,4)] Some of the vanadium complexes have been also found to possess the anti tumor activity.[5)] Vanadium compounds have been used as pharmacological tools to investigate signaling pathways.[6,7)] The toxicity of vanadium compounds is low and usually increases with an increase in valancy. Vanadyl compounds are reported to cause DNA lesions by hydrogen peroxide (H_2O_2) formed by dismutation of superoxide anion radicals[8)] and in long term can become genotoxic.[9)] However, limited information is available about the reproductive toxicity of Vanadyl sulphate.

The absorption of dietary vanadium is poor, it is estimated that less than 2% of dietary vanadium is absorbed. Most of the absorbed vanadium is converted to cationic vanadyl in the blood.[10)] Large doses (100 mg/kg b.wt./day and above) of Vanadyl sulphate are necessary to lower plasma glucose in the animal studies.[11)] Therefore, the present investigation was undertaken to assess the reproductive toxicity of Vanadyl sulphate (100 mg/kg b.wt./day) in a sub-chronic exposure in the male Wistar rats.

MATERIALS AND METHODS

Test Chemical —— Vanadyl sulphate ($VOSO_4$) (Procured from Central Drug House, Delhi, India).

Animals —— Colony bred adult healthy male albino rats of Wistar strain, weighing 170–200 g were used in the study. The rats were housed in polypropylene cages under standard husbandry conditions (12 hr light/dark cycle: 25 ± 3°C). Rats were provided water and pellet diet *ad libitum*. The institutional ethical committee for animal care approved the study.

Experimental Design —— Male rats of proven fertility were divided into two groups of 7 rats each.

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Group I: Rats served as control and received the vehicle (0.5 ml distilled water/day/rat).

Group II: Rats were administered Vanadyl sulphate (100 mg/kg b.wt./day) dissolved in distilled water.

All the rats received treatment for 60 days duration.

On day 61, animals were sacrificed under mild ether anesthesia. The testes and accessory sex organs were dissected out, cleared and weighed. The body weight of each animal was recorded initially and then at the time of autopsy.

Fertility Test —— The mating tests were performed during last five days of the treatment. The male rats were cohabited with pro-estrus females in the ratio of 1:2 respectively. The presence of vaginal plug and sperm in the vaginal smear in the next morning were considered the index for positive mating. The mated females were separated and allowed to deliver after full term.

Sperm Analysis —— Sperm density and motility of cauda epididymal spermatozoa were evaluated by the method of Prasad *et al*. (1972).[12)]

Tissue Biochemistry —— Testis and accessory sex organs were frozen at −20°C for the estimation of protein,[13)] sialic acid,[14)] glycogen,[15)] cholesterol[16)] and fructose.[17)]

Histological Analysis —— Testes were fixed in Bouin's fluid. Paraffin sections were cut (5 μm) and stained with hematoxylin and eosin. Mean tubular diameter was determined by measuring 100 round sections of semeniferous tubules with the help of ocular micrometer. Diameters of Leydig cells nuclei were measured at × 800.

Statistical Analysis —— Data were expressed as mean ± SEM and the significance of difference was analyzed by the Student's *t*-test.

RESULT AND DISCUSSION

The present investigation revealed that the body weight of the control rats showed a significant ($p < 0.05$) gain in comparison to initial weight, while the treated rats exhibited no body weight gain; instead a non-significant decline was recorded as compared to their initial body weight, which reflect general toxicity. The relative weights of testes and accessory sex organs decreased slightly ($p < 0.05$) (Table 1) in treated rats, which stems from reduced availability of androgens.[18)] The reduction in the weight of testes in vanadyl sulphate treated rats might be due to the decreased number of germ cells.[19)]

A significant decline in the cauda epididymal sperm density ($p < 0.01$) and motility ($p < 0.001$) (Table 2) may be attributed to impairment of sperm maturation and secretory functions of epididymal cells, which might be due to insufficiency of androgens.[20)] Alternatively, the decline in sperm motil-

Table 1. Body and Organ Weights of Rats Treated with Vanadyl Sulphate

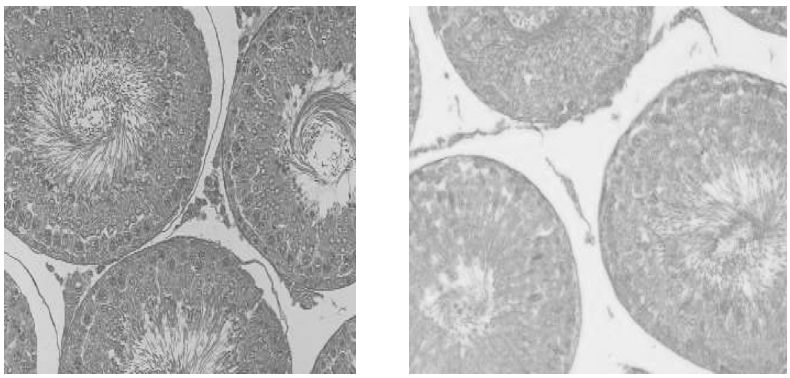
Group and Treatment	Body Weight (g)		Organ Weight (mg/100g b.wt.)			
	Initial	Final	Testes	Epididymides	Seminal vesicle	Ventral prostrate
I Control	180.0	190.2*	1345	580.33	561.00	302.20
(Vehicle treated)	(± 2.4)	(± 2.7)	(± 43.74)	(± 15.79)	(± 15.47)	(± 16.76)
II Vanadyl sulphate	185.1	179.8	1174**	505.24**	492.35**	246.82**
(100 mg/kg.wt./day)	(± 2.3)	(± 4.25)	(± 50.60)	(± 20.30)	(± 18.20)	(± 12.64)

Levels of significance values are (mean ± SEM) of 7 rats. *$p < 0.05$ compared with initial body weight. **$p < 0.05$ compared with control rats.

Table 2. Cauda Epididymal Sperm Analysis, Fertility Performance and Morphometery of Rats Treated with Vanadyl Sulphate

Group and Treatment	Sperm count (million/mm^3)	Motility (%)	Fertility test (%)	Litter size	Seminiferous tubules diameter (μm)	Leydig cell nucleidiameter (μm)
I Control	46.80	74.40	10/10	7.7	270.00	6.68
(Vehicle treated)	(± 2.50)	(± 2.70)	(100%)	(± 0.70)	(± 8.75)	(± 0.30)
II Vanadyl sulphate	35.20**	42.0***	5/10	5.0**	232.40*	5.40*
(100 mg/kg.wt./day)	(± 1.85)	(± 4.47)	(50%)	(± 0.45)	(± 7.75)	(± 0.27)

Fertility test = No. of females delivered/No. of females inseminated. Levels of significance values are (mean ± SEM) of 7 rats. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared with control rats.



(a) (b)

Fig. 1. C. S. of testis (a) Control rat showing active spermatogensis. (b) Vanadyl sulphate treated rat showing degenrative changes.

Table 3. Effect of Vanadyl Sulphate Treatment on Tissue Biochemical Parameters in Rats

Group and Treatment	Protein (mg/g)		Sialic Acid (mg/g)			Glycogen (mg/g)		Cholesterol (mg/g)	Fructose (mg/g)
	Testis	Cauda epididymis	Testis	Cauda epididymis	Seminal vesicle	Testis	Epididymis	Testis	Seminal vesicle
I Control	178.80	205.62	4.46	5.11	4.92	3.12	3.08	5.66	5.28
(Vehicle treated)	(± 8.16)	(± 4.85)	(± 0.22)	(± 0.16)	(± 0.22)	(± 0.19)	(± 0.18)	(± 0.22)	(± 0.28)
II Vanadyl sulphate	149.86*	172.25**	3.82*	4.22*	4.26	3.59	3.48	7.19**	4.39*
(100 mg/kg.wt./day)	(± 6.67)	(± 6.64)	(± 0.17)	(± 0.21)	(± 0.26)	(± 0.16)	(± 0.21)	(± 0.32)	(± 0.23)

Levels of significance values are (mean ± SEM) of 7 rats. $^{*}p < 0.05$, $^{**}p < 0.01$ compared with control rats.

ity might be due to oxidative stress or impairment of energy generating metabolic enzymes.[21–23] A sharp decline in fertility (50% negative) and litter size ($p < 0.01$) in vanadyl sulphate treated rats might be result of decreased sperm count, motility and alteration in biochemical milieu of genital organs. Similar results were obtained in other studies with vanadium compounds, which have reported an arrest of spermatogenic activity and decline in fertility.[24–27]

Histological observation of testes showed a significant reduction in diameters of seminiferous tubules and Leydig cells nuclei when compared with that of control rats (Table 2), which indicates testosterone biosynthesis hampering either due to direct action on Leydig cells or through suppression of hypothalamus — pituitary-gonadal axis. This is supported by the study of Dehghan *et al*. (2002)[27] who reported a significant decline in blood testosterone in rats, when treated with low dose (32 mg/kg body.wt/d) of vanadyl sulphate for 30 days.

Histopathology of testes showed exfoliation and degeneration of spermatogenic cell and decline in post meiotic germ cells and spermatozoa in the lumen of seminiferous tubules indicating impairment of spermatogenesis (Fig. 1) which might be due to, an increase in the number of germ cells apoptosis, as a consequence of adverse effects on Sertoli cells and germ cell interaction.[28] In an another study Musali-Galante *et al*. (2005)[29] reported that inhalation of vanadium pentaoxide in male mice lead to damage in spermatogenesis by decreasing the percentage of gamma tubulin in testicular cells which adversely affects the microtubules involved in cell division.

The biochemical observations (Table 3) revealed, that protein and sialic acid contents of testis and cauda epididymis were significantly reduced ($p < 0.05$ and $p < 0.01$ respectively) in vanadyl sulphate treated rats indicating adverse effect on biochemical milieu of these organs. The principal cells of epididymis synthesize protein and other secretory materials, which have important role in maturation of spermatozoa.[30] Decrease in the level of sialic acid also shows the necrotic condition of testis.[31] Glycogen contents of testis and epididymis did not depict any significant change, while the cholesterol level in the testis was enhanced significantly indicating impairment of steriodogenesis by virtue of suppression of pituitary gonadotropins or direct action on Leydig cells.[32] The fructose contents of seminal vesicle reduced significantly ($p < 0.05$), this may be another cause of reduction in sperm motility, as

the fructose is important source of energy in seminal plasma. The reduction in fructose content is also indicating under supply of androgens.[17] We conclude that vanadyl sulphate adversely affects the reproductive functions and fertility of male rats, probably by virtue of suppression of androgen biosynthesis.

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