

Effects of Manganese(II) Complexes of 2-[(1-Thienyl)Ethylidene]-Hydrazinecarbothioamide on Reproductive Function of Male Rats

N. FAHMI, ANIL BANSAL, S.C. JOSHI† AND R.V. SINGH*

Department of chemistry, University of Rajasthan,
Jaipur-302 004, India

The animals treated with ligand 2-[(1-thienyl) ethylidene] hydrazinecarbothioamide (TSCZH) and its manganese(II) complexes $[MnCl(TSCZ)H_2O]$ and $[Mn(TSCZ)_2]$ resulted in a significant reduction in the weight of testes, and other sex accessories. They also bring about reduction in sperm motility and sperm density in testes and cauda epididymis. The sialic acid, protein and fructose contents of various reproductive organs were significantly lowered. The results of these findings have been presented and discussed.

INTRODUCTION

The synthesis, characterization and antimicrobial activity of the ligand 2[(1-thienyl)-ethylidene]hydrazinecarbothioamide (TSCZH) and its manganese(II) complexes have already been reported¹. They were found to exhibit good antifungal activity against *Macrophomina phaseolina*, *Aspergillus niger* and *Fusarium oxysporum* and antibacterial activity against *Pseudomonas cepacicola*, *Escherichia coli*, *Klebsiella aerugenus* and *Staphylococcus aureus*. Manganese chloride causes loss of testicular germ cells in rats and rabbits² and decreased libido and impotency were noted in men occupationally exposed to manganese³, but the data is inconclusive. Hence, the aim of the present study was to assess the effect of manganese(II) complexes on fertility and to contribute to a better understanding of the reproductive function of male albino rats. In the present study we investigated a variety of male reproductive end points following acute exposure to mono and bis-manganese complexes of a biologically active ligand (TSCZH).

EXPERIMENTAL

Thirty-two adult male albino rats of inbred colony were housed in an air-conditioned animal room at $24 \pm 2^\circ\text{C}$ with 14 h light. Water and food was given *ad libitum*. They were divided into four groups containing eight animals each. The first group animals received dose of ligand (TSCZH) 50 mg/kg body

†Reproductive Physiology Section, Department of Zoology, University of Rajasthan, Jaipur-302 004, India

TABLE-1
CHANGES IN THE BODY WEIGHT AND ORGANS WEIGHTS OF REPRODUCTIVE ORGANS AFTER TREATMENT WITH LIGAND AND ITS MANGANESE COMPLEXES

Treatment	Body weight (g)		mg/100 g body weight			
	Initial	Final	Testes	Epididymis	Seminal vesicle	Ventral prostate
Control	190.0 ± 12.0	220.0 ± 9.50	1050.0 ± 70.5	400.0 ± 28.5	340.0 ± 27.8	250.75 ± 30.50
(TSCZH)	180.0 ± 15.0	215.0 ± 10.5 ^c	805.0 ± 50.0 ^a	345.0 ± 20.5 ^a	300.0 ± 10.0 ^a	200.0 ± 10.7 ^a
[MnCl(TSCZ)H ₂ O]	195.0 ± 10.0	225.0 ± 15.0 ^c	780.0 ± 15.0 ^b	290.0 ± 20.3 ^b	280.0 ± 15.0 ^b	185.0 ± 15.3 ^a
[Mn(TSCZ) ₂]	185.0 ± 15.0	210.0 ± 17.0 ^c	715.0 ± 30.0 ^b	281.75 ± 10.5	250.0 ± 17.3 ^b	155.0 ± 10.9 ^a

a = P ≤ 0.05 Values means ± SE of six determinations.

b = P ≤ 0.001

c = NS

TABLE-2
ALTERED SPERM DYNAMICS AND FERTILITY TEST AFTER TREATMENT WITH LIGAND AND ITS MANGANESE COMPLEXES

Treatment	Sperm density (million/mL)		Sperm motility		Fertility test (%)
	Testis	Epididymis	Cauda epididymis	Fertility test (%)	
Control	1.75 ± 0.09	45.52 ± 1.5	72.0 ± 5.21	95 (+ve)	
(TSCZH)	1.93 ± 0.10 ^b	35.0 ± 0.5 ^a	51.0 ± 3.7 ^b	70 (-ve)	
[MnCl(TSCZ)H ₂ O]	0.85 ± 0.19 ^b	30.0 ± 0.4 ^b	47.0 ± 3.8 ^b	80 (-ve)	
[Mn(TSCZ) ₂]	0.69 ± 0.15 ^b	25.0 ± 0.3 ^b	40.0 ± 3.8 ^b	90 (-ve)	

a = P ≤ 0.05 Values means ± SE of six determinations

b = P ≤ 0.001

TABLE-3
EFFECTS OF LIGAND AND ITS MANGANESE COMPLEXES ON VARIOUS BIOCHEMICAL
PARAMETERS OF REPRODUCTIVE ORGANS

Treatment	Sialic acid (mg/g)			Total protein (mg/g)			Total cholesterol (mg/g)			Fructose (mg/g)
	Testis	Epididymis	Seminal vesicle	Ventral prostate	Testis	Epididymis	Seminal vesicle	Ventral prostate	Testis	
Control	7.30 ± 0.9	6.30 ± 1.2	6.80 ± 1.3	6.90 ± 0.5	225.0 ± 17.0	205.0 ± 19.3	250.0 ± 10.8	230.0 ± 20.5	7.30 ± 0.52	450.0 ± 30.0
(TSCZH)	5.80 ± 0.7 ^a	4.90 ± 1.3 ^b	5.0 ± 0.8 ^a	5.1 ± 0.3 ^a	150.0 ± 13.0 ^a	170.0 ± 15.0 ^a	190.0 ± 11.3 ^a	185.0 ± 15.35 ^a	8.10 ± 0.20 ^a	360.0 ± 40.0 ^a
[MnCl(TSCZ)-H ₂ O]	4.50 ± 0.7 ^b	4.30 ± 0.8 ^b	4.20 ± 0.9 ^b	4.28 ± 0.5 ^b	1.4 ± 5.0 ^b	138.0 ± 10.3 ^b	150.0 ± 11.7 ^b	158.0 ± 20.0 ^b	8.30 ± 0.59 ^b	330.0 ± 20.0 ^b
[Mn(TSCZ) ₂]	3.90 ± 0.9 ^b	3.70 ± 0.1 ^b	3.80 ± 0.8 ^b	3.10 ± 0.7 ^b	130.0 ± 10.70 ^b	125.0 ± 15.4 ^b	139.0 ± 12.5 ^b	135.0 ± 17.7 ^b	8.9 ± 0.52 ^b	310.0 ± 35.0 ^b

a = P ≤ 0.05 Values means ± SE of six determinations

b = P ≤ 0.001

weight suspended in 0.2 mL olive oil, orally, for a period of 60 days. The animals of group third and fourth received the same dose of compounds $[\text{MnCl}(\text{TSCZ})\text{H}_2\text{O}]$ respectively for a similar period. The animals were screened for fertility test and autopsied for determination for detailed biochemical studies. Reproductive organs were excised, blotted free of blood, weighed and were frozen for biochemical estimations. The sperm motility and density of cauda epididymal spermatozoa was assessed by the method of Prasad *et al.*⁴ The total protein⁵, sialic acid⁶, total cholesterol⁷ and fructose⁸ were determined.

RESULTS AND DISCUSSION

Body and Organ Weight: No significant change in the body weight was observed after treatment with ligand and its complexes. The weights of testes, epididymis, seminal vesicles and ventral prostate were decreased significantly in ligand ($P < 0.05$) and complex $[\text{MnCl}(\text{TSCZ})\text{H}_2\text{O}]$ and $[\text{Mn}(\text{TSCZ})_2]$ treated animals ($P < 0.001$) (Table-1).

Sperm Motility and Sperm Density: A significant ($P < 0.001$) decline in sperm motility in cauda epididymis was observed in animals treated with ligand and its complexes. A significant decrease in sperm density in cauda epididymis and testes was also observed (Table-2).

Biochemical Analysis (Table-3)

Total Cholesterol: The total cholesterol content of testes increased in all experimental groups when compared with controls (ligand, $P < 0.05$; compounds $[\text{MnCl}(\text{TSCZ})\text{H}_2\text{O}]$ and $[\text{Mn}(\text{TSCZ})_2]$, $P < 0.001$).

Sialic Acid: The sialic acid content of testes, epididymis, seminal vesicles and ventral prostate was reduced significantly in (TSCZH) ($P < 0.05$) and $[\text{MnCl}(\text{TSCZ})\text{H}_2\text{O}]$ and $[\text{Mn}(\text{TSCZ})_2]$ treated animals ($P < 0.001$).

Total Protein: Ligand treatment resulted in a significant reduction in total protein content of testes, epididymis, seminal vesicles and ventral prostate ($P < 0.05$). A highly significant ($P < 0.001$) decrease in protein content was observed in both the compounds treated animals.

Fructose: A significant reduction in the fructose content of seminal vesicles was observed in ligand ($P < 0.05$) and its both complexes ($P < 0.001$) treated animals.

The present study evaluated the effects of the ligand (TSCZH) and its manganese complexes $[\text{MnCl}(\text{TSCZ})\text{H}_2\text{O}]$ and $[\text{Mn}(\text{TSCZ})_2]$ on male fertility and biochemical parameters of the reproductive organs. The administration of these compounds resulted in a significant reduction in the weight of testes, epididymis, seminal vesicles and ventral prostate. The reduction in their weight indirectly suggests suppression of testosterone synthesis or binding to receptors⁹. There was a reduction in both density and motility of the epididymal spermatozoa after treatment with the ligand and its compounds. Ligand and compounds hamper the fertility because a sufficient number of normal fast progressive spermatozoa are necessary for fertilization of ovum¹⁰. The reduction in sialic acid content of testes and other sex accessories also supports the

antiandrogenic nature of these compounds. Sialic acid is involved in maintaining the structural integrity of acrosomal and plasma membrane of sperm and plays a role in sperm maturation and transport in the epididymis¹¹. Further reduction in the fructose (a free sugar secreted by seminal vesicle) concentration of seminal vesicles supports the antiandrogenic nature of these compounds¹². Increased concentration of cholesterol in testes suggests the androgenesis and impairment of spermatogenesis. In our study, the reduction in protein contents of testes, epididymis, seminal vesicle and ventral prostate may be due to suppression of testosterone biosynthesis.

Manganese has been found to cause testicular injury and to inhibit spermatogenesis in experimental animals^{13, 14}. It is interesting that the compound [Mn(TSCZ)₂] has more pronounced effects of fertility and various biochemical parameters of reproductive organs. Treatment with different organochlorine compounds causes a reduction in steroidogenic enzymes¹⁵. The greater activity of [Mn(TSCZ)₂] is presumably due to the presence of two ligand moieties in this manganese complex¹⁶. From the above data we conclude that the ligand and its manganese complexes have a sperm inhibiting effect with change in the biochemical milieu of the reproduction accessories.

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