Toxicological Studies of Orally Administered Triphenyl Tin Complex [$(C_6H_5)_3Sn$(Sal. Benz. H.)] on the Reproductive Organs of Male Albino Rats

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Summary

The present investigation is an attempt to determine the possible toxicity potentials of orally fed triphenyl tin complex [$(C_6H_5)_3Sn$(Sal. Benz. H.)] in the reproductive system of male rats (*Rattus norvegicus*) at the dose level of 10mg/kg body weight and 20 mg/kg body weight for 60 days. Treated rats showed decline in the weight of reproductive organs in a dose-response fashion. The treatment also diminished the sperm motility and density highly significantly; the fertility percent was reduced to 95% at 20 mg dose level. Serum testosterone levels were also diminished significantly. Significant reduction was also found in the biochemical parameters such as cholesterol, glycogen, protein and sialic acid content of the reproductive tissues in a dose dependent manner. Investigations through hematology and serology showed no signs of clinical toxicity. Histological studies of the sections of testis of treated rats showed absence of spermatozoa in the lumen of seminiferous tubules along with highly reduced seminiferous tubular diameter and increased intertubular space in the treatment groups when compared to the control counterparts. These results indicate that the complex is antispermatogenic in nature and oral administration in male rats caused sterility i. e. reproductive toxicity. A comparison indicates that 20 mg/kg dose level was more effective pertaining to its antifertility than the corresponding dose level of 10 mg/kg dose level.

Key words: Triphenyl tin complex, testis, Histopathology, *Rattus norvegicus*, reproductive toxicity, testosterone.

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Introduction

The male reproductive system and its related endocrine system can be very sensitive to the exposure of chemical and physical agents which may cause adverse effects on gamete production and cycle normality [1, 2].

Organotin compounds are a broad group of chemicals widely used in agriculture and industry. Trisubstituted organotin compounds are the most important class of organotin chemicals, are biologically active, and widely used as biocides. The two most important groups of triorganotin compounds are triphenyltin and tributyltin derivatives. Triphenyltins (TPT'S) are used as agricultural fungicides, rodent repellents, molluscicides and antifoulants in ship paints and underwater coatings [3-6]. TPT'S have been reported to be chemosterilants [7].

The aim of present study was to evaluate the toxic effects of the triphenyltin complex on the reproductive system of male albino rats.

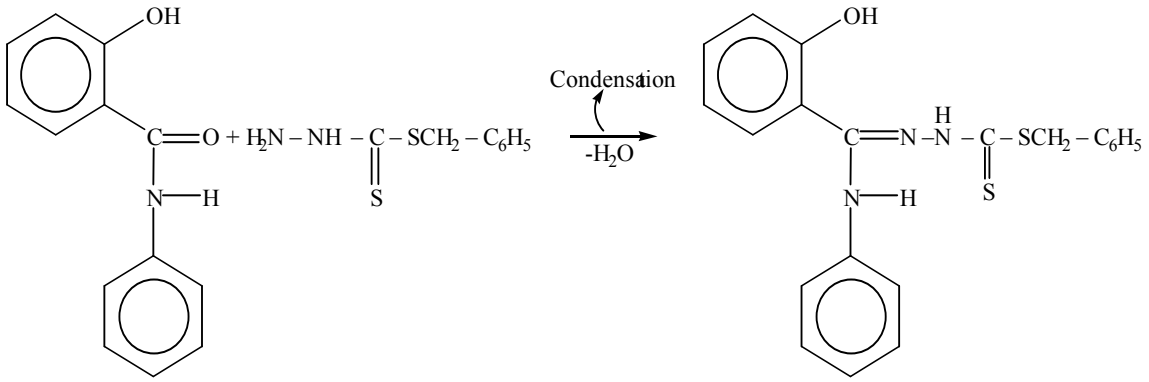
Materials and Methods

Preparation of Test Complex:

All reagents were obtained commercially (Sarabhai and Glaxo Make, India) by standard procedures. All solvents were of reagent grade. In the present investigation, ligand (Salicylanilide-S-benzyldithiocarbazate) and it's triphenyltin complex have been synthesized in our laboratory.

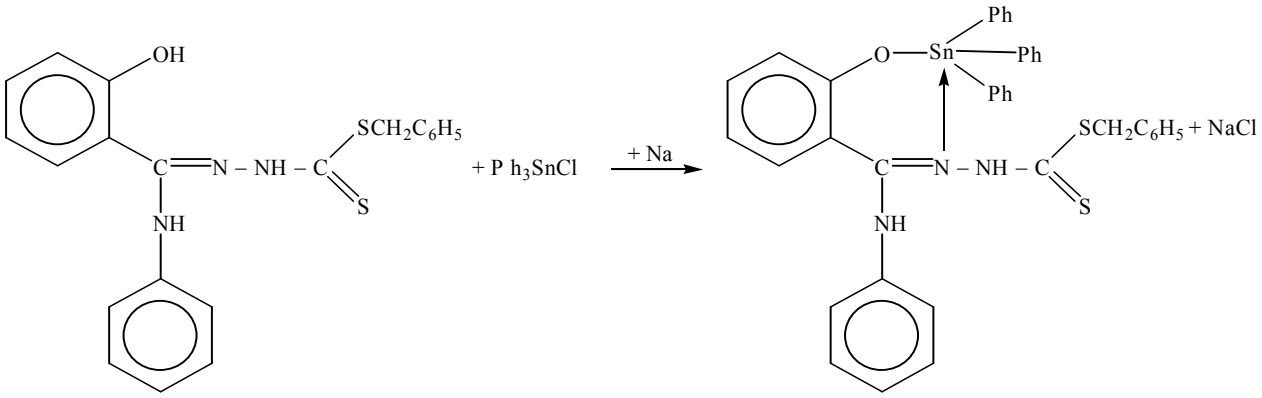
Synthesis of Ligand:

For the preparation of the ligand (S-benzyldithiocarbazate) to a cold solution of KOH (11.4 gm) in 90% ethanol (70 ml) added hydrazine hydrate (10 gm) with constant stirring. A solution of CS_2 was added drop wise with continuous stirring over a period of one hour and temperature of the reaction mixture was kept below 10^oC. During the addition, the oily layer so formed was separated and dissolved in cold 40% ethanol (60 ml). The solution was cooled in a freezing mixture and benzyl- chloride (25 gm) was added drop wise while stirring for two hours. The white solid was separated by filtration, washed with water and dried in air. The crude product was recrystallized from benzene (M.P., 125^o C).



Synthesis of Complex

For the preparation of the metal complex $(C_6H_5)_3$ SnCl and the ligand in amounts in 1:1 molar ratio was refluxed in dry methanol for about 14 hours and during which a white solid (NaCl) was separated by filtration. The excess of the solvent from filtrate was removed under reduced pressure and the complex was then subsequently dried for 3-4 hours in vacuum. The products were repeatedly washed with ether and n-hexane and again dried for 3-4 hours in vacuum.



The complex thus formed was characterized by elemental analysis and molecular weight determinations.

Animal Model Used:

Thirty colony bred adult male albino rats (*Rattus norvegicus*) of Sprague-Dawley strain, 4-5 month old and weighing between 175-200 gm with proved fertility were marked properly and housed two or three animals in polypropylene cages under controlled environmental conditions (12-h light: 12-h dark). They were fed pelleted standard rat feed (Aashirwad Foods, Chandigarh, India) supplemented with soaked gram and wheat and allowed free access to water. Animal procedures were approved by the Institutional Ethical Committee and conducted in compliance with the Guidelines for Care and Use of Animals for Scientific Research [8].

Calculation of Median Lethal Dose (LD_{50})

In the present study 40 mg and 60 mg dose levels of Phenyl tin complex were given orally with the help of hypodermic syringe having pearl point needle. Ten animals

were tested for each dose level. Control rats were given equivalent amount of vehicle poising symptoms and motility was observed daily for ten days, following the treatment. Results of the toxicity were analyzed statistically for the determination of LD_{50} value of the compound [9].

Treatment Protocol:

The experiments consisted of the following three groups of 10 rats per group. All the animals were treated orally with the vehicle, 0.5 ml Olive Oil, given daily for 60 days. Due care was taken to assure that the animals received the complete dose.

Group I : Control received the vehicle only i.e. 0.5 ml Olive Oil.

Group II : Animals received [$(C_6H_5)_3Sn$(Sal. Benz. H.)] emulsified in 0.5 ml Olive Oil at a dosage of 10 mg/kg b. wt./day for 60 days.

Group III : Animals received [$(C_6H_5)_3Sn$(Sal. Benz. H.)] emulsified in 0.5 ml Olive Oil at a dosage of 20 mg/kg b. wt./day for 60 days.

Fertility Test:

Fertility of each treated male and their control counterparts was estimated by mating with two virgin untreated females at day 50-60 of the treatment. They were left together for 10 days during which two estrus cycles should have elapsed [10].

Sacrification Schedule and Body & Organ weight measurements:

Twenty-four hours after their last dose, the rats were weighed and sacrificed under light ether anesthesia. The initial and final body weights of the animals were recorded. The testes, epididymis, seminal vesicle and ventral prostate were dissected out along with some vital organs like liver, heart, kidney and adrenal, freed from adherent tissues and blood, and weighed to the nearest milligram.

Sperm Dynamics:

Cauda epididymal sperm motility and count and testicular sperm count (maturing spermatozoa with head and tail) were made using a haemocytometer [11, 12].

Assay for circulatory level of Testosterone

Blood samples were collected for estimations of serum testosterone by radio immunoassay (RIA). Serum samples were separated by standard procedures and stored at -20°C for the analysis. Serum levels of testosterone were assayed in duplicate using specific RIA method [13]. As the present study deals with only reproductive organs and not the endocrine system so only the levels of testosterone hormone were measured.

Tissue Biochemistry:

Assays were conducted to determine levels of Cholesterol [14] in testes and liver, Glycogen [15] in testes and liver, Protein [16] in testes, epididymis, seminal vesicle and prostate gland and Sialic acid [17] in testes, epididymis, seminal vesicle and prostate gland.

Clinical Toxicity studies:

To evaluate the effects of $[(C_6H_5)_3Sn(Sal.\ Benz.\ H.)]$ on the general body metabolism and normal functioning of the animals the following clinical toxicity studies were performed:

Haematological profiles:

The counts of TEC (Total Erythrocyte Count) and TLC (Total Leucocyte Count) , haemoglobin, haematocrit and standard haematological indices (mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration) and blood urea were determined from the blood collected directly from the heart at the time of sacrification according to standard methods.

Serological profiles:

The serum was separated from the blood samples by standard procedures and kept at -20°C until assayed for ACP (Acid Phosphatase), ALP (Alkaline Phosphatase), SGOT (Serum Glutamic Oxaloacetic Transaminase), SGPT (Serum Glutamic Pyruvic Transaminase), protein, cholesterol, phospholipids and triglycerides.

Histological Studies of the Testis:

The effects of test substance on the reproductive system of male rats could be assessed by the histological examination of the reproductive tissues mainly testis as testis is the site of sperm production. For histology the testicular tissues of all the groups were processed for histopathological evaluation. For histology the tissues were fixed in Bouin's fluid, dehydrated and embedded in paraffin for sections at 5μm. These sections were stained with Harris hematoxylene and eosin.

Statistical Calculations:

All the values of body and organs weights, sperm dynamics, blood toxicity profiles and tissue biochemistry were expressed in terms of mean ± SEM. The treated groups were compared to their respective controls using Student's t-test [18].

Results

Effects of [$(C_6H_5)_3Sn$(Sal. Benz. H.)] administration on body and organ weight measurements:

The results presented in Table 1 clearly revealed that the weights of reproductive organs of the treated rats decreased highly significantly ($p \leq 0.001$) in comparison to the control group, even though no change in the vital organs weight (liver, heart, kidney and adrenal) was observed and a normal increase in the body weight was found in both the treated as well as control groups.

Table 1: Body and organ weight measurements of Control and Phenyl tin Complex [$(C_6H_5)_3Sn$(Sal. Benz. H.)] treated male albino rats.

Treatment	Body weight (gm)		Reproductive organs weight (mg/100 gm b. wt.)				Vital organs weight (mg/100 gm b.wt.)			
	Initial	Final	Testes	Epididymis	Seminal vesicle	Ventral prostate	Liver	Heart	Kidney	Adrenal
Group I Control	175.0 ± 6.20	187.0 ± 5.2	1329.49 ± 19.8	399.70 ± 20	297.5 ± 12.37	580.5 ± 20.56	3438.29 ±176.20	297.38 ±9.45	784.0 ± 31.4	24.6 ± 1.2
Group II 10 mg/kg b.wt./day for 60 days	189.2 ± 5.9	198.1 ± 7.0	1028.8 ± 32. 0**	262.31 ± 7.9**	208.0 ±11.9**	508.8 ± 9.3**	3395.26 ±125.00 ns	284.95 ±6.38 ns	802.0 ± 41 ns	27.0 ± 2.6 ns
Group III 20 mg/kg b.wt./day for 60 days	190.0 ± 8.1	199.4 ± 5.4	830.21 ±40.25**	227.51 ±16.2**	189.09 ±9.9**	468.21 ± 8.4**	3376.90 ±169.76 ns	276.22 ±7.20 ns	789.0 ± 29.7ns	26.23 ± 1.48 ns

(Mean ±SEM of 10 Animals) Group II and III compared with Group I ns = non-significant ** = highly significant (p≤0.001)

Effects of [$(C_6H_5)_3Sn$(Sal. Benz. H.)] administration on the sperm dynamics and circulatory level of serum testosterone:

Number of implantation sites and number of viable fetuses were significantly reduced (p≤0.001) in the treatment groups in a dose dependent manner. A highly significant decrease (p≤0.001) in sperm motility and sperm density of cauda epididymis was observed in both the treatment groups. 95% sterility was observed at 20 mg/day dose level (Table 2). The level of serum testosterone also dwindled remarkably (p≤0.001) in both the treatment groups.

Table 2: Sperm dynamics, Fertility test and Cicrculatory level of testosterone of Control and Phenyl tin Complex [$(C_6H_5)_3Sn$(Sal. Benz. H.)] treated male albino rats.

Treatment	Number of mated Males	Number of mated Females	Number of Pregnant Females	Number of Implantation Sites/rat	Number of Viable Fetuses /rat	Sperm motility (Cauda epididymis) (%)	Sperm density (million/ml)		Fertility Test (%)	Serum Testosterone (ng/ml)
							Testes	Cauda epididymis		
Group I Control	10	20	20/20	10.32 ±2.36	8.32 ±1.46	70.16 ± 0.22	4.95 ± 0.01	39.32 ± 0.59	100% (+ve)	4.33 ±0.76
Group II 10 mg/kg b.wt./day for 60 days	10	20	5/20	3.00 ±0.10**	2.08 ±0.75**	37.91 ± 1.3**	1.91 ± 0.2**	18.97 ± 1.07**	75% (-ve)	1.82 ±0.03**
Group III 20 mg/kg b.wt./day for 60 days	10	20	0/20	Nil	Nil	19.68 ± 4.9**	0.99 ±0.06**	9.75 ± 1.1**	100% (-ve)	0.83 ±0.02**

(Mean ±SEM of 10 Animals) Group II and III compared with Group I
ns = non-significant
** = highly significant ($p \leq 0.001$)

(Abbreviations Used: +ve = Positive; -ve= Negative)

Effects of [$(C_6H_5)_3Sn$(Sal. Benz. H.)] administration on the tissue biochemistry:

Testicular cholesterol and glycogen showed highly significant ($p \leq 0.001$) depletion, while in liver the cholesterol and glycogen levels were significantly reduced ($p \leq 0.01$). Protein and Sialic acid contents of testes, cauda epididymis, seminal vesicle and prostate gland showed a significant decrease ($p \leq 0.01$) in dose dependent fashion (Table 3).

Table 3: Tissue biochemistry of Control and Phenyl tin Complex [$(C_6H_5)_3Sn$(Sal. Benz. H.)] treated male albino rats.

Treatment	Cholesterol (mg/gm)		Glycogen (mg/gm)		Protein (mg/gm)				Sialic Acid (mg/gm)			
	Testes	Liver	Testes	Liver	Testes	Epidid-ymis	Seminal Vesicle	Prostate Gland	Testes	Epidid-ymis	Seminal Vesicle	Prostate Gland
Group I Control	9.81 ±0.50	16.59 ±0.4	2.69 ±0.22	4.66 ±0.57	209.2 ±14.3	220.0 ±15.12	161.1 ±8.19	198.4 ±13.18	5.16 ±0.05	5.7 ± 0.18	5.3 ± 0.01	4.96 ± 0.06
Group II 10mg/kg b.wt./day for 60 days	4.9 ±1.2*	16.9 ±0.18^{ns}	1.59 ±0.05**	3.85 ±0.02^{ns}	118.09 ±9.17**	128.1 ±7.9**	107.6 ±11.17*	110.8 ±5.2**	2.80 ±0.03**	4.54 ± 0.07**	4.02 ±0.03**	4.36 ±0.02**
Group III 20mg/kg b.wt./day for 60 days	3.6 ±0.17**	16.34 ±0.2^{ns}	1.32 ±0.07**	3.63 ±0.2^{ns}	105.0 ±10.0**	113.7 ±9.4**	97.02 ± 9.8**	100.0 ±9.9**	2.72 ±0.02**	4.26 ± 0.09**	3.04 ± 0.2**	4.17 ±0.06**

(Mean ±SEM of 10 Animals) Group II and III compared with Group I ns = non-significant ** = highly significant ($p \leq 0.001$)

Effects of [$(C_6H_5)_3Sn$(Sal. Benz. H.)] administration on the Clinical toxicity:

Effects of [$(C_6H_5)_3Sn$(Sal. Benz. H.)] administration on the Haematological profiles:

Hematological parameters TEC (Total Erythrocyte Count) and TLC (Total Leucocyte Count), haemoglobin, haematocrit, standard hematological indices and blood urea varied within the control range. No drug-related effects on any of these parameters were observed in any group when compared with vehicle treated control group (Table 4).

Table 4: Hematological parameters of Control and Phenyl tin Complex [$(C_6H_5)_3Sn$(Sal. Benz. H.)] treated male albino rats.

Treatment	Total Erythrocyte Count (TEC) million/mm^3	Total Leukocyte Count (TLC) per cu. mm	Hemoglobin (Hb) gm%	Hematocrit (PCV) %	Mean Corpuscular Volume (MCV) per cu. µm	Mean Corpuscular Hemoglobin (MCH) Pg.	Mean Corpuscular Hemoglobin Concentration (MCHC) %	Blood Urea mg/dl
Group I Control	5.23 ± 0.25	8086.3 ±303.32	14.1 ± 0.13	47.6 ± 1.76	82.0 ± 5.5	29.2 ± 2.10	30.29 ± 2.3	39.0 ±6.1
Group II 10mg/kg b. wt./day for 60 days	5.06 ± 0.65 ns	8280.0 ±406.20 ns	13.6 ±0.95 ns	46.5 ±4.7 ns	84.5 ±3.5 ns	25.2 ± 2.1 ns	31.66 ± 1.52 ns	42.2 ±4.0 ns
Group III 20mg/kg b.wt./day for 60 days	4.90 ± 0.16 ns	9210.0 ±393.80 ns	13.2 ± 0.77 ns	43.5 ± 2.5 ns	81.0 ± 4.2 ns	27.3 ± 0.3 ns	32.9 ± 1.5 ns	41.1 ±3.8 ns

(Mean ±SEM of 10 Animals) Group II and III compared with Group I ns = non-significant
Abbreviations Used:
Per. Cu. Mm.= per cubic millimeter ; Per. Cu. µm = per cubic micrometer ; Pg.= Pico gram

Effects of [$(C_6H_5)_3Sn$(Sal. Benz. H.)] administration on the Serological profiles:

The values of serological parameters viz. ACP (Acid Phosphatase), ALP (Alkaline Phosphatase), SGOT (Serum Glutamic Oxaloacetic Transaminase), SGPT (Serum Glutamic Pyruvic Transaminase), protein, cholesterol, phospholipids and triglycerides remained unchanged in both the treated groups (Table 5).

Table 5: Serological parameters of Control and Phenyl tin Complex [$(C_6H_5)_3Sn$(Sal. Benz. H.)] treated male albino rats.

Treatment	Acid Phosphatase (ACP) mm/gm/hr	Alkaline Phosphatase (ALP) mm/gm/hr	Serum Glutamic Oxaloacetic Transaminase (SGOT) I.U./L	Serum Glutamic Pyruvic Transaminase (SGPT) I.U./L	Protein mg/dl	Phospholipids mg/dl	Triglycerides mg/dl	Cholesterol mg/dl
Group I Control	3.36 ±0.24	1.75 ±0.08	74.00 ±2.38	71.95 ±2.00	13436.20 ±111.63	128.37 ±10.07	132.48 ±14.35	128.26 ±13.45
Group II 10mg/kg b. wt./day for 60 days	3.24 ±0.36 ns	1.67 ±0.04 ns	72.95 ±2.25 ns	70.63 ±3.05 ns	13395.63 ±104.29 ns	126.29 ±9.87 ns	130.09 ±13.06 ns	127.45 ±11.98 ns
Group III 20mg/kg b.wt./day for 60 days	3.19 ±0.27 ns	1.59 ±0.07 ns	71.63 ±1.60 ns	69.29 ±2.48 ns	13376.09 ±108.00 ns	125.69 ±10.03 ns	129.38 ±12.65 ns	126.22 ±10.32 ns

(Mean ±SEM of 10 Animals) Group II and III compared with Group I ns = non-significant
Abbreviations Used:
mm/gm/hr = millimeter per gram per hour ; I.U./L =Units per liter ; Mg/dl = milligram per deci liter

Effects of [$(C_6H_5)_3Sn$(Sal. Benz. H.)] administration on the testicular histopathology:

Vehicle treated control rats showed round or oval seminiferous tubules with normal Sertoli cells and germ cells at various stages covering the complete spermatogenesis, and an interstitium containing distinct Leydig cells (Figure 1). By contrast, marked alterations were observed in the histoarchitecture of the testes of treated rats.

In Group II treated rats non-uniform degenerative changes were observed in same sections of testes. Secondary spermatocyte and spermatid are absent. Seminiferous tubules were devoid of spermatozoa (Figure 2).

In Group III severe effects were observed seminiferous tubules showed depletion of germ cells. The epithelium consisted of only a layer of spermatogonia. Lumen was filled with debris of dead and broken sperm tails (Figure 3).

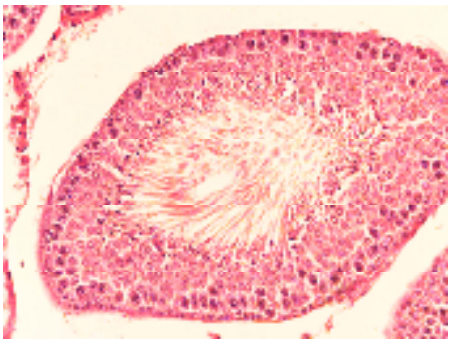


Figure. 1. Showing testicular histopathology of testis of control rats showing normal stages of spermatogenesis. Seminiferous tubules with all stages of spermatogenesis. Normal intertubular spaces with connective tissue. The lumen is filled with healthy spermatozoa.

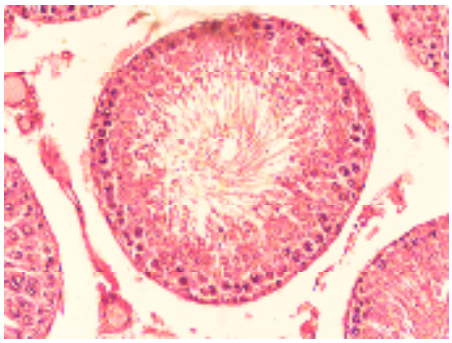


Figure. 2. Showing testicular histopathology of testis of male rats fed with 10 mg/kg b. wt. of $[(C_6H_5)_3Sn(Sal. Benz. H.)]$ for 60 days. Damage was not uniform, lumen of the tubule contains broken tail spermatozoa and the Leydig cells are normal in structure.

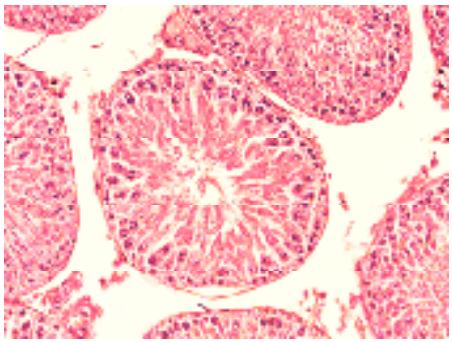


Figure. 3. Showing testicular histopathology of testis of male rats fed with 20 mg/kg b. wt. of $[(C_6H_5)_3Sn(Sal. Benz. H.)]$ for 60 days. Degeneration of spermatogenic elements could be seen as rounded and elongated spermatids are absent. Lumen contains cellular debris. An increased interstitial space is seen.

Discussion

In the current study, the administration of [$(C_6H_5)_3Sn$(Sal. Benz. H.)] decreased the weight of testes and accessory sex organs. The epididymis, seminal vesicle and ventral prostate are all androgen dependent organs, relying on testosterone for their normal growth and function [19]. A reduction in their weights may reflect a decreased bioavailability and production of androgens, which caused Leydig cell disintegration [20]. The nucleus of Leydig cells was shrunken and pycnosis was very evident on the histopathological observation of the testes.

The sperm density and motility have direct relationship to the fertility [21]. In the present study, sperm motility and density of testis and cauda epididymis after [$(C_6H_5)_3Sn$(Sal. Benz. H.)] treatment were significantly declined. Rao and Shah [22] have reported declined sperm motility, resulting in to decreased fertility. Inadequate concentration and sluggishly motile or immotile sperm could not penetrate the cervical mucus and thus failed to fertilize the ova [23]. The significant decline in serum testosterone might be due to adverse effect of the treatment on hormonal milieu of the testes. The reduced weights of seminal vesicle and ventral prostate support the suppressed concentration of testosterone in circulation [24].

The glycogen content in the cell represents the energy storage. The Sertoli cells contain glycogen and provide nourishment to the seminiferous tubular cells, and glycogen content is also found to be directly proportional to the steroid hormone levels [25]. A decrease in the glycogen content of the testis reduces the energy source for spermatogenic activity. Changamma and Redanna[26] and Sisodia et al [27] suggested that the decrease in glycogen content could also be due to increased glycogenolysis.

$[(C_6H_5)_3Sn(Sal.\ Benz.\ H.)]$ administration caused a highly significant decrease in protein and sialic acid. Sialic acid is secreted by the epididymal epithelium and is coated on spermatozoa as they pass through the epididymis. They are concerned with changing the membrane surface of maturing spermatozoa, coating of spermatozoa with certain antigens, and in the development of their fertilizing capacity [28-30] Low level of sialic acid confirms the decreased fertilizing capacity.

$[(C_6H_5)_3Sn(Sal.\ Benz.\ H.)]$ administration to the male rats did not alter the general body metabolism of the test animals as reveled by the data obtained after examination of the clinical toxicity studies i.e. haematological and serological profiles as the values of all the parameters studied were in normal range.

It is concluded that $[(C_6H_5)_3Sn(Sal.\ Benz.\ H.)]$ is a reproductive toxicant, but is effective in the terms of male contraception and that the effects could be mediated through the testis without untoward side effects on the general metabolism at 20 mg dose level and that the compound may be helpful for developing an oral contraceptive for males. However, the substance is identified as a reproductive toxicant in rats, further studies are in progress to determine the complete and reversible fertility with this complex.

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