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Synthesis, Spectroscopic Studies and Antispermatogenic Activity of Tri- and Pentavalent Organoantimony Derivatives of Schiff Bases

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ABSTRACT

Diphenyl antimony(III) and triphenylantimony(V) derivatives of Schiff bases having general formulae $Ph_2Sb[OC(R)CHC(R')NC_6H_5]$ and $Ph_3Sb[OC(R)CHC(R')NC_6H_5]_2$ (where R=R′=CH_3,C_6H_5; R = CH_3, R′=C_6H_5), respectively have been prepared. Trivalent organoantimony derivatives have been synthesized by the reaction of $RC(O)CHC(R')$-$NH(C_6H_5)$ with Ph_3Sb in 1: 1 molar ratio whereas, pentavalent organoantimony derivatives have been synthesized by the reaction of Ph_3SbBr_2 with $Na[OC(R)CHC(R')N(C_6H_5)]$ in 1:2 molar ratio, respectively in benzene solution. The structures of these complexes have been assigned on the basis of chemical analysis, molecular weight measurements and spectral (IR ^1H & ^{13}C NMR) studies. Schiff base (R = CH_3, R′ = C_6H_5) and its corresponding organoantimony(III) and antimony(V) compounds were used for evaluation of reproductive physiological activity in male rats and for studying antifertility activity. Compounds were administrated orally for 30 days to male albino rats and after termination of treatment, animal tissue and serum were taken for histopathological, biochemical and hematological parameters.

KEYWORDS

Organoantimony derivatives, Schiff Bases, Antispermatogenic activity.

INTRODUCTION

Bio-organometallic chemistry is an area which encompasses organometallic chemistry into biology, medicine and bioanalytics. Schiff bases represent one of the most widely utilized classes of ligands in metal coordination chemistry. The application of organometallic compounds in biology and medicine is nothing new, indeed nature has been using organometallic systems to sustain life for a rather long. Contraceptive pills available in market were mostly for females but males do not have sufficient attention in family planning program. However, in present scenario research is diverting for male contraceptive development. In the previous research many chemical compounds are evaluated for fertility suppression activity in male [1-4]. The development of additional male methods of fertility control can provide tremendous social and public health benefits. There are relatively few realistic approaches currently being pursued which include (i) the suppression of sperm production (ii) disruption of sperm maturation and/or function and (iii) interruption of sperm transport contraceptive vaccines and inhibitors of spermatogenesis and sperm motility, provide

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potential for non-hormonal male contraceptives. Many chemical synthetic compounds were used in research for developing a male contraceptive drug but till now no significant success is achieved.

In this communication, the synthesis and characterization of diphenylantimony(III) and triphenyl antimony(V) derivatives of Schiff bases and their antispermatogenic and antifertility activities in male rats is reported.

EXPERIMENTAL

Solvents used in the work were dried by standard method [5] before use. Ph_3Sb (Sigma-Aldrich) has been used as supplied. Ph_3SbBr_2 [6] and Schiff bases [7] have been synthesized by the literature methods. Antimony was estimated by iodometric method [8]. The elemental analysis (C, H, N) was obtained by using a Coleman CHN analyzer. Molecular weights have been determined cryoscopically in freezing benzene solution using a Beckmann's thermometer. Infrared spectra of these compounds have been recorded as Nujol mull using KBr cells from 4000-400 cm^{-1} on FTIR spectrophotometer. ^1H & ^{13}C NMR spectra have been recorded in $CDCI_3$ solution on a JEOL-FT AI 300 MHz spectrometer using TMS as an internal reference. All these compounds have been synthesized by a similar method, for convenience the synthesis of one representative compound of the series is described.

Synthesis of $Ph_2Sb[OC(R)CHC(R')NC_6H_5]$: A weighed amount of Schiff base $CH_3C(O)CHC(C_6H_5)NC_6H_5$ (0.81g, 3.41 mM) was added to benzene solution of Ph_3Sb (1.20 g, 3.40 mM). The reaction mixture was refluxed for about 4 h. After the completion of reaction, the excess solvent was removed under reduced pressure to yield colored viscous product. The compound was recrystallized from benzene/*n*-hexane mixture (1:1).

Synthesis of $Ph_3Sb[OC(R)CHC(R')NC_6H_5]_2$: A benzene solution of Schiff base $CH_3C(O)CHC(C_6H_5)NH(C_6H_5)$ (1.23 g, 5.18 mM) was added to sodium methoxide solution prepared *in situ* by the interaction of sodium (0.12 g, 5.21mM) with CH_3OH and the reaction mixture was refluxed for ~ 4 h. The benzene solution of Ph_3SbBr_2 (1.33 g, 2.59 mM) was added to the above reaction mixture after cooling. The reaction mixture was refluxed for ~4 h and the precipitated NaBr was filtered off. The removal of volatile component from the filtrate under reduced pressure yielded a light yellow viscous. It was recrystallized from benzene/*n*-hexane mixture (1:1).

Ethics: Healthy albino wistar stain male rats (*R. norvegicus*) weighing about 150-180 g. All the animal investigations were permitted by the Institutional Animal Ethics Committee of Department of Zoology, University of Rajasthan, Jaipur, India. The experiments were carried in agreement with the Committee for the purpose of control and supervision of experiments on animals (CPCSEA) guidelines for laboratory animal facility. Informed consents were obtained from the healthy patients for blood samples as per medical ethics.

Anti-spermatogenic activity (B)

Experimental design: Animal were divided in four groups. **Group I:** Seemed as control, treated with 0.5 mL vehicle (olive oil). **Group II:** Animals in this group were treated with ligand (A) 25 mg/kg bw/day. **Group III:** Animals in this group were treated with diphenylantimony(III) compound (B) 10 mg/kg bw/day. **Group IV:** Animals in this group were treated with triphenylantimony(V) compound (C) 10 mg/kg bw/day. Treatment was carried out for 30 days in all four groups.

Biological Parameters: Body and organ weights measurement, fertility test, sperm density, sperm motility, sperm morphology and histopathological analysis were preformed as described earlier [9].

Antioxidant parameter: Lipid peroxidation (LPO) [10], reduced glutathione (GSH) [11] and superoxide dismutase (SOD) [12] were also conducted as described elsewhere.

Biochemical test: Cholesterol [13], glycogen [14], protein [15], epididymis, seminal vesicle, epididymis and liver were aslo estimated as per described in the literature.

Hematology: Total RBC count, total WBC count [16], haemoglobin% [17] and hematocrit value [18] was conducted in blood.

Hormonal analysis: Testosterone [19], FSH and LH [20] tests were also conducted.

Statistical analysis: Data are expressed as mean ±SD and analyzed for statistical significance by using student's *t*-test. Results were considered and compared at the three levels of significance *i.e.* $p \leq 0.05$, $p \leq 0.01$ and $p \leq 0.001$ levels [21].

RESULTS AND DISCUSSION

Reaction of Schiff base with Ph_3Sb and Ph_3SbBr_2 in 1:1 and 1:2 molar ratio using reflux benzene give corresponding diphenylantimony(III) and triphenylantimony(V) derivatives.

$$Ph_3Sb + OC(R)CHC(R')NH(C_6H_5) \longrightarrow Pb_2Sb[OC(R)CH\text{-}C(R')NC_6H_5 + C_6H_6$$

$$2NaOMe + 2OC(R)CHC(R')NHC_6H_5 \longrightarrow 2Na[OC(R)CHC(R')N(C_6H_5)] + MeOH$$

$$2Na[OC(R)CHC(R')N(C_6H_5)] + Ph_3SbBr_2 \longrightarrow Ph_3Sb[OC(R)CHC(R^1)N(C_6H_5)]_2 + 2NaBr\downarrow$$

where R = R′ = CH_3, C_6H_5; R = CH_3, R′ = C_6H_5.

All these compounds are coloured viscous/solid, soluble in common organic solvents and monomeric in nature. Tentative structure of these compounds have been proposed on the basis of spectral studies.

IR analysis: A comparative study of IR spectra of ligand and its corresponding organoantimony(III) and antimony(V) derivatives show disappearance of NH band observed at 3100-2900 cm^{-1} in the spectra of free ligands indicating the deprotonation of NH group. This is evidenced by the appearance of a new band at 475-450 cm^{-1} assignable to Sb-N [22] in the spectra of the corresponding metal complexes. The intense absorption bands observed at 1600-1586 and 1537-1500 cm^{-1} may be attributed to C=N and C=O vibrations. These bands undergo considerable shift towards the lower frequency by 8-10 cm^{-1} compared to the free ligand values, indicating the involvement of these groups in bonding which is further supported by the appearance of a new band at 555-550 cm^{-1} for Sb-O vibrations [23].

^1H NMR analysis: The characteristic signals in the ^1H NMR spectra of all these derivatives are summarized in Table-1. Formation of Sb-N bond, which was indicated by IR spectra

TABLE-1
^{1}H NMR DATA (ppm) OF TRI AND PENTA VALENT ORGANOANTIMONY DERIVATIVES OF SCHIFF BASES

S. No.	Compounds	CH_3	CH	C_6H_5
	a b	a, 2.01 (s)		
1	$Ph_2Sb[OC(CH_3)\ CH{:}C(CH_3)NC_6H_5]$	b, 1.96 (s)	5.00 (s)	6.92-7.34 (m)
2	$Ph_2Sb[OC(CH_3)\ CH{:}C(C_6H_5)NC_6H_5]$	1.93 (s)	4.81 (s)	6.23-7.45 (m)
3	$Ph_2Sb[OC(C_6H_5)\ CH{:}C(C_6H_5)NC_6H_5]$	–	5.00 (s)	7.20-7.96 (m)
	a b	a, 2.32 (s)		
4	$Ph_3Sb[OC(CH_3)\ CH{:}C(CH_3)NC_6H_5]_2$	b, 2.00 (s)	5.12 (s)	7.10-7.85 (m)
5	$Ph_3Sb[OC(CH_3)\ CH{:}C(C_6H_5)NC_6H_5]_2$	2.00 (s)	5.30 (s)	7.20-7.90 (m)
6	$Ph_3Sb[OC(C_6H_5)\ CH{:}C(C_6H_5)NC_6H_5]_2$	–	5.20 (s)	7.18-7.90 (m)

is also supported by ^{1}H NMR spectra. Absence of NH signal (9.1-10.2 ppm) from the spectra of these derivatives show the complexation of antimony with Schiff base through Sb-N bond. The methyl and methine protons have been observed as singlet at 4.81-5.13 and 1.93-2.00 ppm in the spectra of these derivatives, respectively. A small down field shift in the position of these signals may be due to the quasiaromatic character of the chelate ring, which leads to the delocalization. The phenyl protons are observed as multiplet at δ 6.23-7.96 ppm in these derivatives.

^{13}C NMR analysis: ^{13}C NMR chemical shifts of these derivatives are summarized in Table-2. ^{13}C NMR spectra exhibit carbon signals for C=O and C=N at 193.4-196.2 and 164.3-166.0 ppm, respectively. These carbon signals show small down field shift (2-4 ppm) compared to free ligands. This indicates the involvement of these groups in bonding. A down field shift in the position of methyl and methine carbon as compared to their position in the ligand further support the delocalization of electron in chelate ring. Signals for phenyl carbons are observed in the region 126.3-142.3 ppm as multiplet.

Anti-spermatogenic activity: The protocol of the experiments is outlined in Table-3. Body weights of treated rats were taken weekly to ensure their well being. Fertility test showed that female cohabitated with controlled rats have 100% fertility but in treated rats 33.33%, 22.22% and 0% fertility was observed in group II, III and IV, respectively after treatment (Table-4). Sperm density and motility decrease highly significantly in treated groups in comparison to control (Table-5). Male reproductive potency was suppressed due to reduced sperm dynamics. Inadequate sperm concentrations with sluggish or immotile sperm tend not to penetrate the cervical mucosa. Sperm morphological alterations in treated groups were observed *i.e*. ruptured head, Tail without head, Head without tail and banded tail (Fig. 1).

Antioxidant levels *i.e*. GSH and SOD decreased in highly significant manner in testes whereas; liver and kidney showed no significant changes in in all treated group in comparison to control. Elevation of LPO level in testes was observed in all treated group, however in group IV shows highly significant elevation than group I (Table-6). Cholesterol level in testes, epididymis and seminal vesicle in treated groups were showed highly significant increase. Protein and glycogen level in all reproductive organs was showed reduced highly significantly manner when compare to control whereas, Ascorbic acid in adrenal and fructose in SV were decrease significantly in treated groups (Table-7).

TABLE-2
^{13}C NMR CHEMICAL SHIFTS OF DERIVATIVES

S. No.	Compounds	CH	CH_3	CO	CN	C_6H_5
1	a b	93.2	a,23.2	193.4	164.3	128.2-139.8 (m)
	$Ph_2Sb[OC(CH_3)\ CH{:}C(CH_3)NC_6H_5]$		b,21.2			
2	$Ph_2Sb[OC(CH_3)\ CH{:}C(C_6H_5)NC_6H_5]$	94.0	22.3	194.0	165.0	127.3-141.2 (m)
3	$Ph_2Sb[OC(C_6H_5)\ CH{:}C(C_6H_5)NC_6H_5]$	95.0	–	195.0	164.3	126.3-140.8 (m)
4	a b	96.1	a,24.3	196.1	165.0	128.3-140.8 (m)
	$Ph_3Sb[OC(CH_3)\ CH{:}C(CH_3)NC_6H_5]_2$		b,22.3			
5	$Ph_3Sb[OC(CH_3)\ CH{:}C(C_6H_5)NC_6H_5]_2$	95.2	23.3	195.2	164.8	127.8-142.3 (m)
6	$Ph_3Sb[OC(C_6H_5)\ CH{:}C(C_6H_5)NC_6H_5]_2$	96.2	–	196.2	166.0	126.8-140.3 (m)

TABLE-3
BODY AND ORGAN WEIGHT MEASUREMENT OF COMPOUND

Group	Body weight (g)	Organ weight (mg/100 g)								
		Testes	SV	VD	Prost	EPI	Liver	Heart	Kidney	AD
I	252.53	1123.19	458.25	182.11	252.01	382.81	3809.96	328.71	282.98	20.21
	±14.13	±13.58	±19.22	±9.29	±18.38	±15.11	±32.73	±16.32	±5.15	±2.13
II	225.11^{ns}	752.18**	298.46**	109.00**	163.58**	235.38**	3672.63^{ns}	280.769^{ns}	259.29^{ns}	17.22^{ns}
	±6.52	±9.18	±6.21	±15.77	±3.88	±6.18	±109.62	±19.19	±6.12	±2.33
III	238.22^{ns}	673.67**	315.39**	128.32*	180.12*	258.40**	3682.92^{ns}	292.91^{ns}	242.18^{ns}	16.92^{ns}
	±13.19	±10.66	±12.31	±4.82	±1.67	±2.37	±97.67	±13.28	±9.29	±2.82
IV	227.50^{ns}	640.56***	264.39***	112.39***	153.61***	239.00***	3557.11^{ns}	357.03^{ns}	235.65^{ns}	19.83^{ns}
	±1.71	±5.16	±6.97	±2.55	±2.98	±1.40	±54.12	±12.05	±9.25	±0.32

Level of significant: ns = Non-significant, *P ≤ 0.05, **P ≤ 0.01 and ***P ≤ 0.001.

TABLE-4
FERTILITY INDEX OF COMPOUND TREATED RATS

	No. of males	No. of females	Pregnant	Fertility (%)	Litters/ pregnant female
I	10	30	30	100	9
II	10	30	7	23.33	3
III	10	30	10	33.33	3
IV	10	30	0	0	0

Level of significant: ns = Non-significant, *P ≤ 0.05, **P ≤ 0.01 and ***P ≤ 0.001.

Testosterone, FSH and LH in serum also decreased in all treated group when compared to control level. In hematology parameters, RBC, WBC, PCV and haemoglobin show no significant changes as compared to control (Tables 5 and 8). Histopathological observations showed a significant decrease in cellular architecture of treated rats when compared to control. In controlled rats, very well developed and round seminiferous

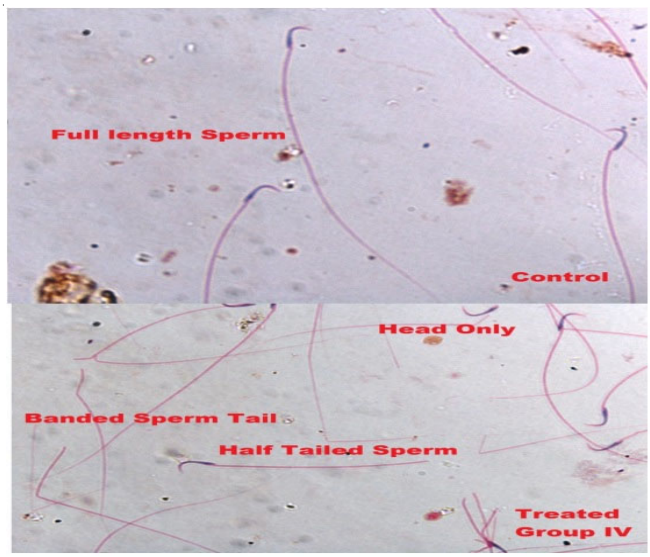

Fig. 1. Sperm morphological alterations

TABLE-5
PROTEIN, SPERM DYNAMICS, HORMONE LEVELS MEASUREMENT OF COMPOUND

Parameter group	Protein (mg/g)			Sperm density (L/mL)	Sperm motility (%)	Testosterone (ng/dl)	FSH	LH
	Testes	Epi	SV					
I	434.22	359.54	411.91	54.33	70.54	3.21	4.70	4.10
	±8.24	±11.03	±10.07	±4.14	±10.04	±0.09	0.02	±0.06
II	252.51**	215.61**	297.18**	23.72**	31.13***	2.60**	3.8*	3.20*
	±10.73	±6.18	±6.58	±1.13	±1.17	±0.07	0.06	0.06
III	277.71**	258.37**	315.16*	27.31**	34.61***	2.40**	2.92**	2.64**
	±9.13	±9.71	±13.17	±2.38	±1.03	±0.03	0.05	0.02
IV	230.15***	180.25***	385.26***	12.36***	22.56***	1.81***	2.33***	2.11***
	2.56	5.32	2.56	8.23	1.54	±0.04	0.03	0.02

Level of Significant: ns = Non-significant, *P ≤ 0.05, **P ≤ 0.01 and ***P ≤ 0.001.

TABLE-6
ANTIOXIDANT PARAMETER OF TREATED RATS

Parameter group	SOD (µmol/mg protein)			LPO (N mol MDA/mg protein)			GSH		
	Liver	Testes	Kidney	Liver	Testes	Kidney	Liver	Testes	Kidney
I	3.92	1.39	1.19	5.21	1.89	3.73	3.5	2.25	2.38
	±1.37	±0.04	±0.53	±0.41	±0.15	±1.74	±0.07	±0.06	±0.05
II	2.97 ns	0.71**	1.01 ns	3.25 ns	2.98**	2.74 ns	3.45 ns	1.21	2.28 ns
	±0.91	±0.04	±0.32	±1.13	±0.08	±0.18	±0.01	±0.06***	±0.01
III	2.88 ns	0.82*	0.97 ns	4.10 ns	2.54*	2.51 ns	3.12	1.18**	2.22 ns
	±0.72	±0.12	±0.27	±0.58	±0.03	±0.37	0.09	0.05	0.03
IV	3.50 ns	0.52**	1.22 ns	5.02 ns	3.85**	3.24 ns	3.5 ns	1.01***	2.35 ns
	0.12	0.06	0.12	0.24	0.09	0.21	0.06	0.02	0.05

Level of significant: ns = Non-significant, *P ≤ 0.05, **P ≤ 0.01and ***P ≤ 0.001.

TABLE-7
TISSUE BIOCHEMISTRY OF COMPOUND TREATED RATS

Parameter group	Cholesterol (mg/g)			Glycogen (mg/g)			Ascorbic acid (mg/g)	Fructose (mg/g)
	Testes	Epi	SV	T	Epi	SV	Adrenal	Seminal vesicle
I	14.67	15.46	18.93	2.92	3.93	3.29	2.86	6.52
	±4.43	±0.96	±0.86	±0.48	±0.67	±0.13	±0.17	±0.31
II	51.91**	32.56**	35.75**	1.28**	0.78**	0.72**	2.27*	4.92**
	±4.18	±6.28	±4.19	±0.32	±0.04	±0.38	±0.05	±0.44
III	47.63**	54.13**	48.56*	1.37 **	0.97**	0.86**	2.21**	3.85**
	±2.22	±4.17	±7.01	±0.08	±0.07	±0.19	±0.05	±0.13
IV	57.25**	68.67**	64.92**	1.09**	0.52***	0.56***	2.02**	3.30***
	5.45	1.95	1.68	0.05	0.02	0.01	±1.92	±2.62

Level of Significant:- ns-non significant, *P ≤ 0.05, **P ≤ 0.01and ***P ≤ 0.001.

TABLE-8
HEMATOLOGY OF COMPOUNDS TREATED RATS

Parameter group	Hb (g/dl)	TLC (Thousand/mm^3)	PCV (%)	TRBC (Million/cu mm)
I	14.44±1.08	11.8±0.69	30.55±4.01	5.5±0.52
II	13.0 ns±2.71	11.20 ns±0.31	37.50 ns±3.42	5.43 ns±1.04
III	13.30 ns±1.87	10.60 ns±0.13	45.45 ns±6.71	5.34 ns±0.73
IV	14.00 ns±0.19	12.40 ns±0.26	38.46 ns±0.45	5.51 ns±0.35

tubule was present but in treated animals it showed negative effect. Controlled rats' seminiferous tubules showed all spermatogenic stages, where as both treated group show alteration in these stages. Seminiferous tubular diameter reduced and spermatogenic stages were degraded in all treated groups (Figs. 2-4).

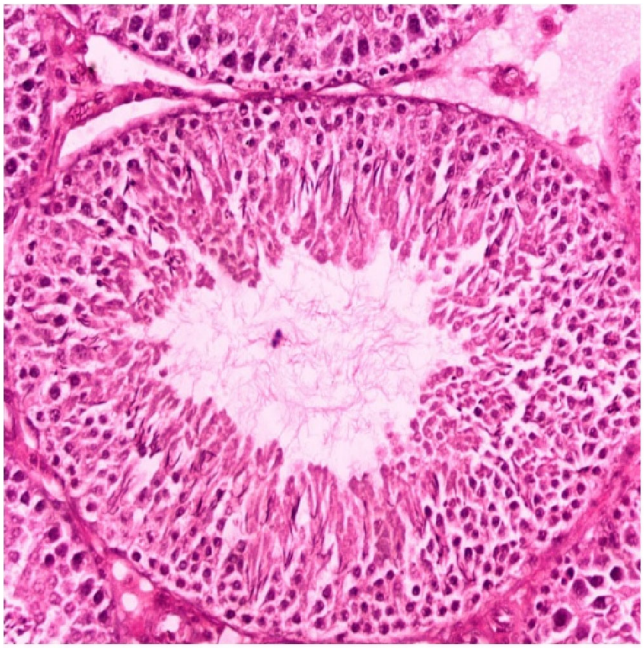

Fig. 2. Histopathology of seminiferous tubule in controlled group

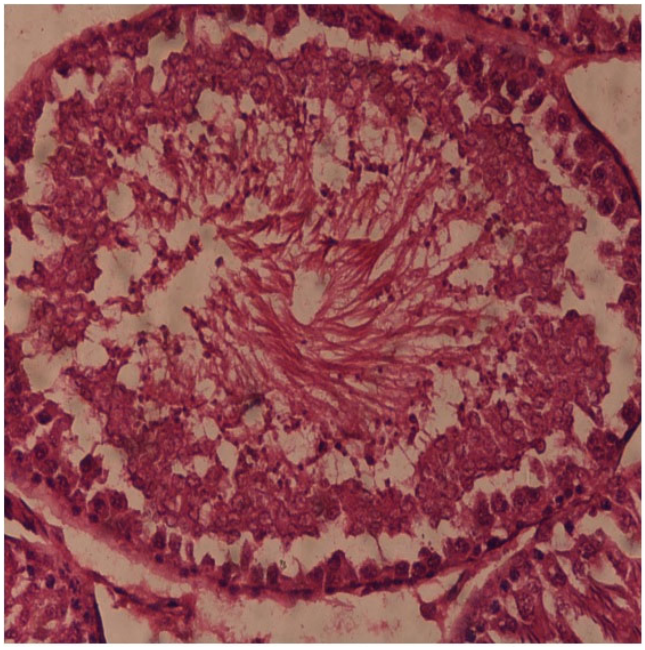

Fig. 3. Histopathology of seminiferous tubule in treated group

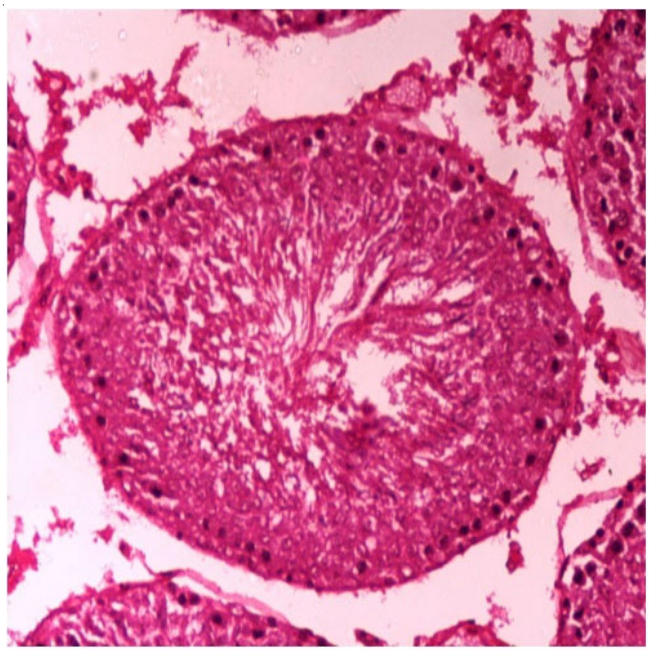

Fig. 4. Histopathology of seminiferous tubule in treated group

On the basis of spectral evidences, it is observed that the Schiff base ligand behave as monofunctional bidentate moiety [24-26]. In view of the presence of one monofunctional bidentate ligand and two phenyl rings in the compound $Ph_2Sb[OC(CH_3)$-$CHC(C_6H_5)NC_6H_5]$, following structure may be proposed in which antimony atom acquires trigonal bipyramidal geometry. (Fig. 5). Whereas, in compound $Ph_3Sb[OC(CH_3)CHC(C_6H_5)$-$NC_6H_5]_2$ due to the presence of two chelate ring and three phenyl groups, following structure in which antimony acquire pentagonal bipyramidal geometry is proposed (Fig. 6).

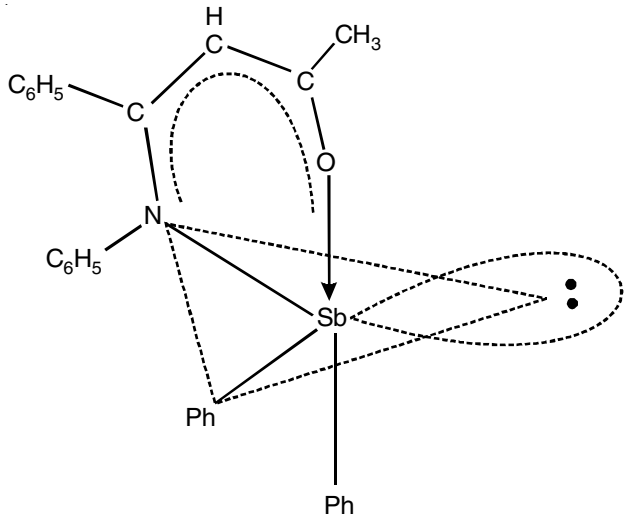

Fig. 5. Organoantimony(III) derivatives of Schiff base

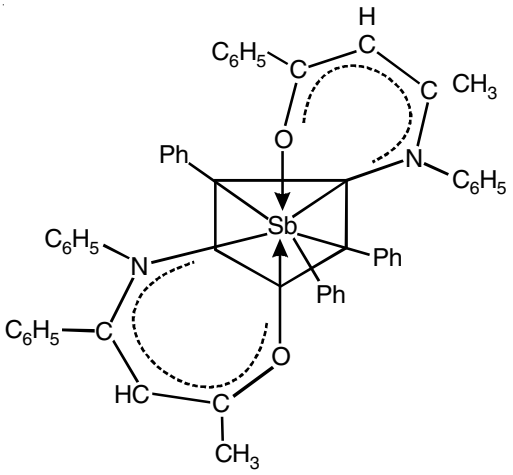

Fig. 6. Organoantimony(V) derivatives of Schiff base

This study revealed adverse effects of compounds on reproductive organ weight, epididymal sperm dynamics and cholesterol, glycogen, protein as well as hormones levels in rats. Results indicated that these compounds reduced testicular weight in rats indicating some adverse effect on reproduction. Decrease in sperm counts and motility in epididymis with increased reactive oxygen species support the evidence antispermatogenic nature [27]. Plasma membrane has a high level of polyunsaturated fatty acids which are easily susceptible to lipid per oxidation caused by oxidative stress. Altered antioxidant level in reproductive organ affect reproductive physiology of male rats [28].

Blood parameters are important to find toxic effect of any compound or products that are injected in the body [29]. This study reveals that hematological analysis shows no significant change in all group of experiment define not cause any adverse effects on health of rats [30]. As a male reproductive system, testosterone plays an important role in maintaining morphology and physiology. Significant reduction of testosterone concen-tration effects reproduction function of male rats and reprodu-ction organ′s cellular structure and produce infertility [31]. Treatment directly affects the testicular hormonal system without changing the levels of other endocrine hormones which is also supported by the decreased number of mature Leydig cells, the main source of testosterone [32]. Decrease in protein and glycogen level may be due to the decrease in formation of new cells in the reproductive organ [33]. Increase in cholesterol level in reproductive organ results from reproductive organ cell degradation, because cell membrane with high content of chole-sterol and cholesterol is a precursor of androgens in treated animals therefore testosterone level decreases so it may increase cholesterol level [34]. Altered histology of testes of treated rats also supports the antifertility investigation. Reduction in cellular protein, glycogen ascorbic, fructose and testicular hormone effect seminiferous tubule diameter and cellular architecture that brings potent antifertility and antispermatogenic activity of the synthesized compound [35].

Conclusion

Spectroscopic studies and analytical data of synthesized compounds concluded the above structural formula. Reproductive physiological analysis of compound on male albino rats shows reduction in sperm density, motility, testicular glycogen, protein contents and hormone level. It indicates adverse effect on reproductive physiology. Sperm morphological and testes histopathological observations also support antispermatogenic and antifertility activity of the synthesized compounds. In this study, triphenylantimony(V) complex is more efficient to reduce fertility in comparison to diphenylantimony(III) compound. So, it may be concluded that both compounds have potent antifertility and antifertility activity however, triphenylantimony(V) complex showed a highly significant effect.

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