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ORGANOSILICON COMPLEXES AS GOOD FERTILITY INHIBITOR: EFFECT OF (N′ - [1-2-OXO-2H-CHROME-3YL-ETHYLIDENE]-HYDRAZINECARBODITHIONIC ACID BENZYL ESTER) AND ITS ORGANOSILICON COMPLEX ON SEX HORMONES AND TESTES HISTOPATHOLOGY IN MALE ALBINO RATS.

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1. ABSTRACT

The present study was designed to synthesize and characterize the ligand (N′ -[1-2-oxo-2H-chrome-3yl-ethylidene]-hydrazinecarbodithionic acid benzyl ester) and its organosilicon complex and also to evaluate their antifertility potentials. The tested doses were given orally to the male albino rats for 45 days at the dose level 30 mg/kg. b.wt./day. Findings of the present investigation mention a decrease in the weight of testes, epididymis, vas deferens, and seminal vesicle and highly significant decrease in testosterone, follicle stimulating hormone (FSH) and leutinizing hormone (LH). Histopathological changes in testes were also studied which show many degenerative changes i.e. spermatogenesis, disruption in normal epithelial organization, decreased number of elongated sperm, depletion in germinal layer and dilatation of interstitial space. In conclusion the study shows that ligand (N ′ -[1-2-oxo-2H-chrome-3yl-ethylidene]-hydrazinecarbodithionic acid benzyl ester) and its silicon complex alter the sex hormones and fertility in rats.

2. KEYWORDS: Organosilicon Complex, Radioimmuno Assay, Spectral Studies, Hormones, Fertility Inhibitor.

3. INTRODUCTION

One of the important concerns of present age is the problem of over population. If the population increase is not controlled or checked, it will lead to several problems.[1] Various

methods of contraception were used for fertility control. There are a variety of methods available and are in use but most of them are for female contraception.[2] In contrast, except for the barrier method and vasectomy, there are no methods available for male contraception. Thus, there is a need to develop multiple male contraceptive methods. As we know the entire available contraceptive in the market are not safe, mostly they are steroid in nature and they have more or little hazardous side effects. It includes adverse effects on sexual function and fertility in adult males and females, as well as developmental toxicity in the offspring.[3] The use of histopathological evaluations of testes shows prominent role in male reproductive risk assessment. Histopathological evaluations are especially useful in providing information about the antifertility effects of chemicals on male reproduction.[4]

Attention has now been focused on safe chemicals for possible contraceptive effect. The chemical control of fertility in the male has received attention since quite some time and a large number of synthetic compounds have been tested for their antispermatogenic and antiadrogenic effects.[5,7] Organosilicon compounds of sulfur containing ligands have attracted much attention due to their biological importance.[8,9]

The aim of present study was to evaluate the antifertility effects as well as histopathological alteration in male albino rats after administration of ligand and its organosilicon complex.

4. MATERIALS AND METHODS

All reagents were obtained commercially and were purified by standard procedures. All solvents were of reagent grade. The reactions were carried out under strictly anhydrous conditions. In the present investigation, ligand and its complex has been synthesized in our laboratory**.**

Synthesis of ligand

Preparation of the S-benzyldithiocarbazate

A cold solution of KOH (11.4 g) in 90% ethanol (70 mL) was added to hydrazine hydrate (10 g) 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°C. 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 g) 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°C).

Preparation of the 3-acetyl coumarin S -benzyldithiocarbazate

Ligand was prepared by the condensation of 3-acetyl coumarin with S-benzyldithiocarbazate in 1:1 molar ratio and refluxed on a water bath for five-six hours. Ethanol was used as the solvent. The solution then concentrated under reduced pressure. On cooling overnight, crystals separated out which were further purified by washing with ethanol and finally recrystallized with acetone (Fig. 1 & 2).

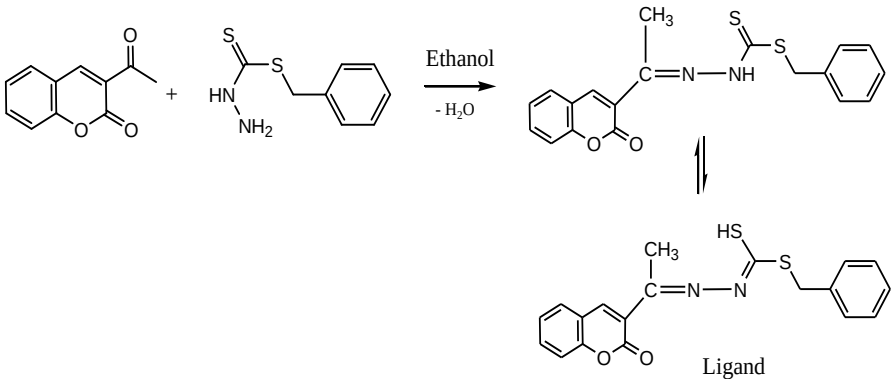


Fig.1: Synthetic scheme of the ligand (L^1H).

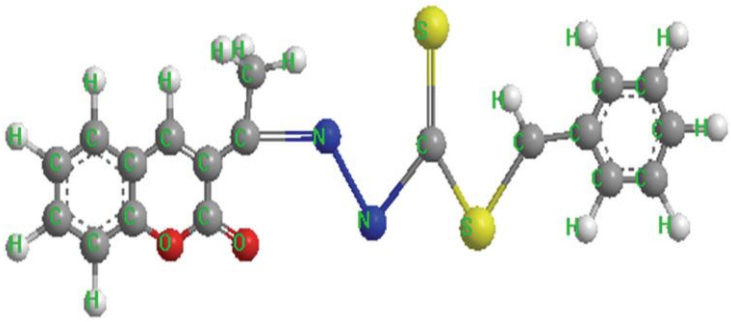


Fig. 2. 3-D structure of the ligand (L^1H).

Synthesis of the complex

For the preparation of the complex, methanolic solution of Ph_2SiCl_2 was mixed with the corresponding sodium salt of the ligand in 1:2 ratios using methanol as a solvent. The solution was refluxed for a period of 5–7 hours. The white precipitate of sodium chloride formed during the course of the reaction was removed by filtration and the filtrate was dried under reduced pressure. The resulting product was repeatedly washed with a mixture of methanol and *n*-hexane (1:1) and then finally dried under vacuum. The purity was further checked by thin layer chromatography with silica gel-G using DMSO as a solvent.

Analytical methods and physical measurements

Nitrogen and sulfur were estimated by the Kjeldahl's and Messenger's methods[10], respectively. Silicon was determined gravimetrically as SiO_2. The conductance was measured with a conductivity bridge type 304 Systronics model and the molecular weights were determined by the Rast Camphor method. Infrared (IR) spectra were recorded on a Perkin-Elmer 577 Grating Spectrophotometer in the range 4000-200 cm^{-1}, as Nujol mulls using CsI Cell. ^1H NMR spectra were recorded in DMSO-d6 using TMS as the internal standard.

Analyses of the above ligand L^1H and its complex are as follows.

(a) N'-[1-(2-oxo-2H-chrome-3-ylethylidene]hydrazine- carbthionic acid benzyl ester (L^1H)

($C_{19}H_{16}N_2O_2S_2$) Colour, reddish orange, Mol. Wt. 366.85 (368.47), M. P. 155^oC, C 60.91 (61.93), H 4.19 (4.38), N 6.56 (7.60), S 16.54 (17.40).

(b) Organosilicon complex with L^1H ($N^{\frown}S$ donor ligand)

$Ph_2Si(N^{\frown}S)_2$, Colour, black, Mol.Wt. 914.56 (917.24), M.P.115^oC, C 63.91 (65.47), H 3.99 (4.40), N 5.56 (6.11), S 12.63 (13.98), Si 2.69(3.06).

ANIMAL MODEL USED

Matured male albino rats (150-200g) were used for this experimental study. The animals were kept in clean polypropylene cages covered with chrome plates grills and maintained under controlled environmental conditions (12-h light: 12-h dark). They were provided with rat pellet and water *ad libitum* throughout the period of the experiment. 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.[11]

Calculation of Median Lethal Dose (LD50)

In the present study, ligand and its organosilicon complex were given orally with the help of hypodermic syringe having pearl point needle. Five-five animals were tested for ligand and complex. Control rats were given equivalent amount of vehicle. Poisoning symptoms and mortality were observed daily for three days following the treatment. Results of the toxicity were analyzed statistically for the determination of LD50 values of the compound.[12]

Treatment Protocol

Animals were divided into three groups having 5 animals each. Group I animals were kept as

control and were administered olive oil only. Animals of Group II received ligand emulsified in olive oil and Group III were administered organosilicon complex emulsified in olive oil at a dosage of 30 mg/kg b. wt./day for 45 days.

PARAMETERS STUDIED

Body and Organ weight measurements

At the end of the experimentation, the rats were weighed and sacrificed under light ether anesthesia. The initial and final body weights of the animals were recorded. The male reproductive organs were removed, weighed and processed for detailed biochemical and histopathological studies.

Radioimmunoassay

Testosterone, Follicle Stimulating Hormone (FSH) and Leutinizing Hormone (LH) concentrations of control and treated groups were measured by radioimmunoassay (RIA) to observe the changes in the gonadal hormone. The autopsy was performed under light ether anaesthesia and the blood from the heart was collected in pre heparinized tubes. Serum was than obtained by centrifugation at 3000 rpm and stored at -20°C for the determination of testosterone, FSH and LH concentrations.[13]

Histopathological Studies

The main reproductive organs testis was fixed in Bouin's fixative and cut into pieces and processed through ethanol-xylene series. The tissues were than embedded in paraffin and bee wax (3:1 ratio; M.P. 55-62°C). Sections were cut at 5 μm thickness and stained with Harris haematoxylin and eosin (H and E).

Statistical Analysis

The data were analyzed statistically by using ANNOVA test and the significance of differences was set at $P < 0.05$ and $P < 0.01$.

5. RESULTS

Electronic Spectra

The electronic spectra of the ligand N′-[1-2-oxo-2H-chrome-3yl-ethylidene]-hydrazine carbodithionic acid benzyl ester and its complex show bands at 260 and 324 nm, assigned to π - π * electronic transitions within the benzene ring. Another band observed at 365 nm in the spectrum of the ligand is due to the n- π * transitions of the azomethine (> C = N) group

which undergoes a blue shift in the complex due to the polarization within the >C=N chromophore caused by the silicon–ligand electron interaction during the chelation.

IR Spectra

The IR spectrum of the ligand displays two sharp bands around 3450 – 3300 cm^{-1} and 3550 – 3450cm^{-1}, assignable to ν sym and ν asym vibrations of the NH_2 group, respectively. These bands remain unchanged in the silicon (IV) complex. Furthermore, strong band at 3250 cm^{-1} due to ν (NH) vibration is observed. This band disappears in the complex. A sharp and strong band at 1625 cm^{-1} is due to the azomethine group of the ligand which shows a lower shift of the order 20 cm^{-1} in the complex indicating the coordination of the azomethine nitrogen to the silicon atom. One strong band located at 1050 cm^{-1} in the ligand was attributed to ν (C = S) moiety, which disappears in the case of complex. These data on comparison with the spectrum of the ligand suggested that the azomethine nitrogen and thiolic sulfur atom of the ligand are involved in coordination with the silicon ion.

^{1}H NMR Spectra

The ^{1}H NMR spectral data of the ligand and its corresponding organosilicon (IV) complex were recorded in DMSO-d 6 with TMS as an internal standard. The ^{1}H NMR spectrum of the ligand exhibits -CH_2 -proton signals at δ4.15 – 4.16 ppm and aromatic proton signals at δ6.38 – 7.50 ppm and these remain at the same position in the spectrum of the silicon complex. The proton of the -NH group of the ligand gives a signal at δ10.20 ppm, which is absent in the spectrum of silicon complex indicating the chelation of the ligand moiety to silicon with the sulfur atom. The proton signals of the methyl groups appear at δ1.15 – 1.19 ppm in the organosilicon (IV) complex.

^{13}C NMR Spectra

^{13}C NMR spectra were recorded in dry methanol using TMS as the internal standard and these spectra also support the authenticity of the proposed structures. The considerable shifts in the positions of carbons of the silicon complex attached to N and S, respectively, clearly indicate that the nitrogen and sulfur of the ligand group participate in the complexation reaction. The signals due to the carbon atoms attached to the thionic and azomethine groups in the ligand appear at δ176.20 ppm and 164.25 ppm, respectively. However, in the spectrum of the corresponding silicon (IV) complex, these appear at δ 168 ppm (thionic group) and at δ 160 ppm (azomethine group), respectively. The considerable shifts in carbons attached to S and N indicate the involvement of sulfur and nitrogen atoms in coordination.

On the basis of spectral studies as well as the analytical data octahedral geometry has been established for the organosilicon(IV) complex (Fig.3.).

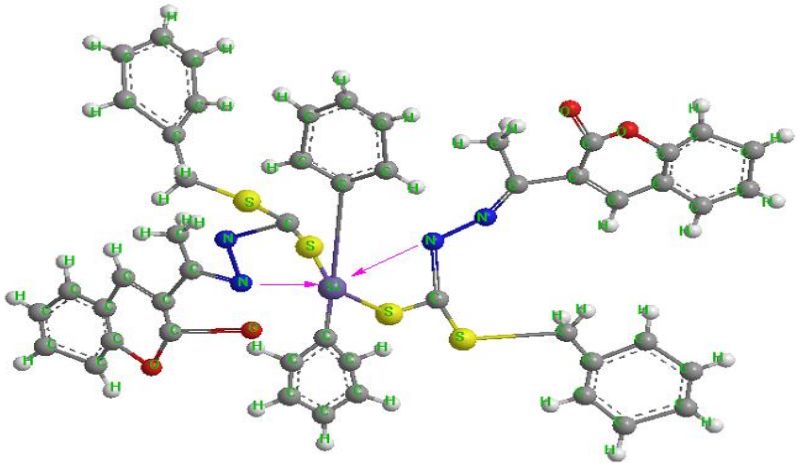


Fig. 3: 3-D Molecular structure of $Ph_2Si(N^{\frown}S)_2$.

After oral administration of chemicals at the dose level of 30 mg/k.b.wt./day for 45 days exhibit adverse effects on male albino rats. The results have been explained here.

Body and organ weight determination

Table 1: Body and organ weight measurements of (L^1H) and its complex of treated male rats.

Group	Treatment (30mg/kg b.wt./day)	Body weight		Testes	Vas Deferens	Seminal Vesicle	Epididymis
		Initial	Final				
I	Vehicle treated (Control)	208.11± 12.78	180.20± 16.43	999.60± 2.71	139.30±1 .98	621.40± 17.31	366.80± 10.41
II	Ligand (L^1H)	206.25± 19.40^{ns}	164.24± 13.54^{ns}	882.13± 1.46*	134.28±1 .14^{ns}	577.65± 11.24^{ns}	341.74± 12.32^{ns}
III	$Ph_2Si(L^1)_2$	172.32± 10.41^{ns}	170.52± 11.55^{ns}	718.21± 1.80*	120.64±0 .96^{ns}	556.60± 12.53^{ns}	313.32± 12.33^{ns}

(Mean ± SEM of 5 animals) Group I compared with Group II and ns = Non significant Group II compared with III Group.

* = Significant (P< 0.05).

** = Highly significant (P< 0.01**).**

The results presented in **Table 1** clearly revealed that the weights of testes of the treated rats decreased significantly in comparison to the control group, even though non- significant reductions were observed in the weight of Vas Deferens, Seminal Vesicle and Epididymis

and a normal decrease in the body weight was found in both the treated as well as control groups.

Radio-Immuno Assays (RIA) (Table. 2)

Leutinizing Hormone (LH) and Follicle Stimulating Hormone (FSH) decreased significantly in group II and highly significantly in groups III to VI whereas Serum testosterone level decreased highly significantly in all groups.

Table 2: Serum hormonal analysis of (L^1H) and its complex treated male rats.

Group	Treatment (30 mg/kg b.wt./day)	Testosterone ng/ml	Luteinizing Hormone (LH) mIu/ml.	Follicle Stimulating Hormone (FSH) mIu/ml.
I	Vehicle treated (Control)	3.83±0.16	1.83±0.15	0.83±0.09
II	Ligand (L^1H)	1.57±0.10**	1.11±0.12*	0.58±0.06*
III	$Ph_2Si(L^1)_2$	0.88±0.04**	0.62±0.05**	0.21±0.04**

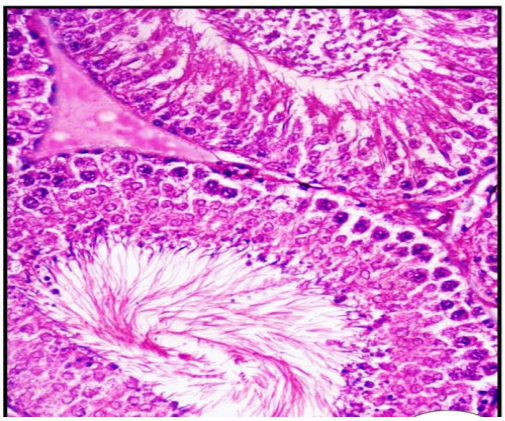
(Mean ± SEM of 5 animals) Group I compared with Group II and ns = Non significant Group II compared with III Group.

* = Significant (P< 0.05).

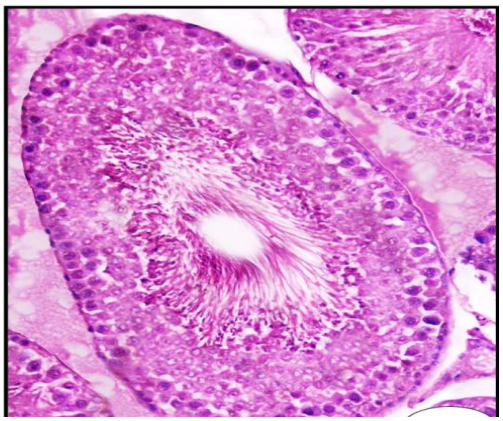
** = Highly significant (P< 0.01**).**

Histopathological Analysis

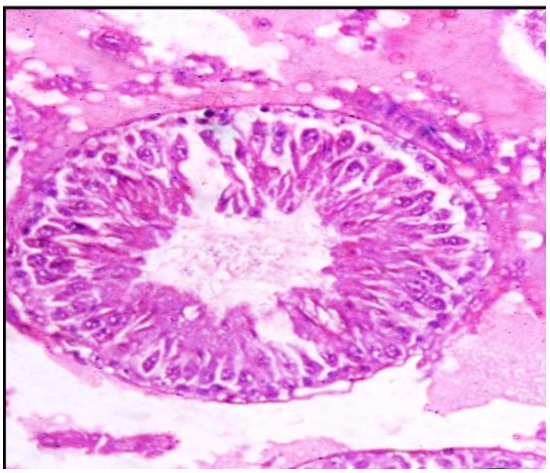
The histoarchitecture of the testes in the rats receiving ligand and its complex showed degenerative changes. The characteristics tiered arrangement was also disturbed. The number of spermatogonia and spermatocyte remain unaffected. The Leydig cells showed atrophic changes. Intertubular space is increased.



(a). Control.



(b). Ligand (L1H).



(c). Organosilicon Complex.

Fig.4 (a) Microphotograph of testes of control rats showing normal morphology of seminiferous tubules with all successive stages of spermatogenesis. Lumen filled with spermatozoa.

(b). Microphotograph of testes showing irregular germinal epithelium and lumen with less sperm is visible.

(c). Seminiferous tubules exhibiting inhibition of spermatogenesis predicting lack of sperm and presence of germ cells in the tubular lumen. culd be seen.

6. DISCUSSION

The present study revealed that administration of ligand (N′ -[1-2-oxo-2H-chrome-3yl-ethylidene]-hydrazinecarbodithionic acid benzyl ester) and silicon complex at dose level of 30 mg/kg/b.wt./day for 45 days to male rats resulted in antifertility.

Non -significant decrease in intial and final body weight was observed whereas the weight of testes were decreased significantly indicating that the level of androgen was not enough to maintain the weight of sex organ. Any small change in the androgen content may alter the pattern of cellular proliferation and affect the weights of reproductive organs.

The testis is the main organ of male reproductive system and main organ for production of spermatozoa.[14] Normal spermatogenesis can be hindered by chemicals through direct interaction with target cells within the testis or indirectly by interfering with hormonal stimulation or alteration in blood supply. Researchers have demonstrated that physiologic concentrations of testosterone, LH and FSH play an important role in spermatogenesis[15], so a significant decrease of these hormones in our study could decrease the number and function of somatic and germinal cells of testis. The reduction in the serum testosterone, FSH and LH

clearly demonstrate the inhibitory effects on the secretion of pituitary gonadotrophins and in turn on the testosterone biosynthesis in the testis of rats.[16]

The production of the sperm cells (spermatozoa) and testosterone in the testis are mainly regulated by the follicle stimulating hormone (FSH) and luteinizing hormone (LH), which are released from the anterior pituitary and necessary for the development and regulations of testicular functions.

Testosterone and FSH are required for maintaining normal spermatogenesis. FSH and LH are necessary for normal sperm production, development and maturation in testes.

The histological picture of the testis of control rats displayed normal process of spermatogenesis. The seminiferous epithelium showed characteristic arrangement of all successive germ cell types along with mature sperms. The presence of large number of normal Leydig cells was also conspicuous in the interstitium. The histoarchitecture of the testis in the rats receiving chemicals showed degenerative changes.

Histopathological data indicated that after oral administration of ligand and its complex on male rats shows inhibition of spermatogenesis in certain areas. Therefore, it can be concluded that ligand and its complex produces significant toxic changes in functions of test. The degenerative changes observed in the testes of ligand and complex treated rats include tubular shrinkage which occurs due to the cell death or sloughing of epithelial cell.[17]

The antiandrogenic effects of the compound are reflected in the arrest of the spermatogenesis as seen by significant reduction in number of spermatocytes, spermatids, sperms and cell debris in the lumen of seminiferous tubules, which may be due to the non availability of the androgens.[18]

Decreased androgen level affected the numbers and volume of mature leydig cells also. Degenerative changes in leydig cells indicate effect on functional ability of these cells to synthesized testosterone which is required in the maintenance of spermatogenesis.[19]

7. CONCLUSION

The oral administration of ligand and its silicon complex induced reproductive toxicity in male albino rats. Toxic effects of complex was more pronounced than ligand and the results also revealed that the scomplex is more effective than the ligand. Thus a conclusion may be

drawn that ligand and its complex may be a potential source for the development of an antifertility drug for males because of their antispermatogenic nature and some antifertility effects on reproductive organs.

8. ACKNOWLEDGEMENT

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