EFFECT OF SCHIFF BASE LIGAND AND TRANSITION METAL COMPLEXES ON SEX HORMONES, SPERM DYNAMICS AND FERTILITY IN MALE ALBINO RATS

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Abstract: The novel organotitanium(IV) and organozirconium(IV) complexes have been synthesized by reacting Cp₂TiCl₂ and Cp₂ZrCl₂ with sulphur donor ligand (N’-[1-2-oxo-2H-chrome-3yl-ethylidene]-hydrazinecarbodithionic acid benzyl ester) and characterized on the basis of IR, UV, ¹H NMR and ¹³C NMR spectral studies, elemental analysis and molecular weight determinations. Zirconium and titanium were estimated by gravimetical method. The present research was coined at investigating the effects of metal complexes on the hormones and sperm profile in male albino rats. The effects of complexes on sex hormones, sperm density, sperm motility and fertility were observed at dose level of 30 mg/kg.b.wt./day for 45 days in male albino rats. Findings of the present investigation mention a highly significant decrease in testosterone, follicle stimulating hormone (FSH) and leutinizing hormone (LH). Decrease in sperm motility and sperm density was observed after complexes exposure, as compared to control group. Decrease in the weight of testes, epididymis, vas deferens, and seminal vesicle were also observed. In conclusion the study shows that ligand (N’-[1-2-oxo-2H-chrome-3yl-ethylidene]-hydrazinecarbodithionic acid benzyl ester) and its metal complexes alter the sex hormones and fertility in rats.

Keywords: Spectral studies, Organotitanium and organozirconium complexes, Radio immuno assay.

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INTRODUCTION

Population growth, a topic most likely insignificant to the common man, but the world’s population growth and control of that population growth is necessary for our overall survival. Over population have both major personal and societal impact and it is necessary to control it on the time. 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. 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\(^1\). The interest in the construction of Schiff-base coordination complexes by reacting transition metal ions with bidentate has been constantly growing over the past years\(^4\). Transition metals and their complexes have evolved great interest due to their biological potential. Organotitanium and organozirconion compounds of sulphur containing ligands have attracted much attention recently due to their biological importance. The sulphur containing ligands are well known for their anticarcinogenic, antibacterial, and antifungal effect. It has been reported that the activity of sulphur-containing ligands increases on complexation\(^7\). The aim of present study was to investigate the toxicity profile on male sex hormones and fertility.

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 complexes have been synthesized in our laboratory.

1. Synthesis of ligand:

1.1 Preparation of the S-benzylthiocarbazate:-
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 CS₂ 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).

1.2 Preparation of the 3-acetyl coumarin S-benzylthiocarbazate

Ligand was prepared by the condensation of 3-acetyl coumarin with S-benzylthiocarbazate in 1:1 molar ratio and refluxed on a water bath for five-six hours. Alcohol 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 (Figure. 1).
1.3 Synthesis of the complexes

For the preparation of the complexes, Cp$_2$TiCl$_2$ and Cp$_2$ZrCl$_2$ were mixed with the corresponding ligand in 1:1:1 ratio in dry THF using triethylamine (Et$_3$N) as hydrogen chloride acceptor. The solution was refluxed for a period of 8–10 hours. The precipitate of triethylamine hydrogen 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 n-hexane and then finally dried under vacuum. The purity was further checked by thin layer chromatography with silica gel-G using DMSO as a solvent. The synthetic details and elemental analyses of the resulting complexes are listed in Table I.

The ligand and complexes synthesized are soluble in DMF and DMSO. Molecular weights were determined by the Rast Camphor method. Nitrogen was estimated by the Kjeldahl’s method and sulphur was estimated by the Messenger’s method. Carbon and hydrogen analyses of the ligand and its metal complexes were carried out at CDRI, Lucknow. Infrared spectra of the ligand and its metal complexes were recorded with the help of Nicolet Megna FTIR-550 spectrophotometer on KBr pellets. $^1$H NMR spectra was recorded on a JEOL-AL-300 FTNMR spectrometer in DMSO-d$_6$ using TMS as the internal standard.

2. Spectral Studies

The molecular weights of the complexes were determined by the Rast Camphor method which indicated their monomeric nature. These complexes have low conductance values (10-15 ohm$^{-1}$ cm$^2$ mol$^{-1}$) in anhydrous DMF, which reveal their non-electrolytic nature.
2.1 Electronic Spectra

The electronic spectra of the ligand and its metal complexes were recorded in dry methanol.

The electronic spectra of the ligand exhibits two bands in the regions, 280nm and 295nm. The bands in this regions are assignable to $\pi-\pi^*$ transitions within the benzene and coumarin rings. A band around 365nm due to the >C=N chromophore shows considerable hypsochromic shift in the spectra of the metal complexes which may be attributed to the coordination of the azomethine nitrogen to the metal atom.

2.2 IR Spectra

In the IR spectra of ligands and their metal complexes the band at 3250 cm$^{-1}$ due to -NH stretching vibrations of the ligand disappears due to the complexation and a band at 1625-1590 cm$^{-1}$ is observed due to the $\nu$(C=N) vibrations. The appearance of strong and medium intensity bands in the regions 395-380 cm$^{-1}$, 520-500 cm$^{-1}$ and 375-366 cm$^{-1}$ be assigned to $\nu$(Ti$\leftrightarrow$N), $\nu$(Ti-O) and $\nu$(Ti-S) vibrations, respectively and thus confirms the formation of complexes. In 1:1 molar metal complexes band at 370-325 cm$^{-1}$ due to $\nu$(M-Cl) vibrations is also observed. In addition, the presence of cyclopentadienyl groups in these complexes is indicated by the absorption bands at ca 3000-2990 cm$^{-1}$ for $\nu$(C-H), 1430-1420 cm$^{-1}$ for $\nu$(C-C), 1020-1010 cm$^{-1}$ for $\delta$(C-H) and $\sim$ 810-800 cm$^{-1}$ for (C-H) out of plane deformation.

In ligand one strong band located at 1050 cm$^{-1}$ in the ligand was attributed to $\nu$ (C = S) moiety, which in the case of complexes shifted to lower frequency due to formation of $\nu$ (C- S) . These data on comparison with the spectrum of the ligand suggested that the azomethine nitrogen and thiol sulphur atom of the ligand are involved in coordination with the metal ion. A doublet at $\sim$ 2950 and $\sim$ 2900 cm$^{-1}$ is assigned to symmetric and asymmetric vibrations of S-CH$_2$C$_6$H$_5$ grouping. In organozirconium complexes the coordination of the azomethine nitrogen and bonding of the ketonic oxygen/thiol sulphur to the zirconium atom is supported by the appearance of three bands in the regions, 387-378, 480-473 and 372-368 cm$^{-1}$ in the complexes which may be assigned to $\nu$(Zr$\leftrightarrow$N), $\nu$(Zr-O) and $\nu$(Zr-S) vibrations, respectively.
2.3 $^1$H NMR Spectra

The $^1$H NMR spectra of the free ligand and its metal complexes were recorded in DMSO-$d_6$. The signal due to the -NH proton of the ligand $\delta$ 10.20 ppm disappears in the complexes and this confirms the deprotonation and complexation through this functional group. The spectra of the ligand shows multiplets in the region $\delta$ 6.38-7.50 ppm assignable to aromatic protons which remain almost at the same position in the free ligand as well as its respective complexes. The presence of $\eta$-C$_5$H$_5$ group in all the complexes is suggested by the signal at $\delta$ 6.10-6.19 ppm. The $^1$H NMR spectra of the ligand and its metal complexes have been recorded in Table 2.

In the $^{13}$C NMR spectra, the considerable shifts in the position of carbon atoms adjacent to the azomethine nitrogen $\delta$163.16 ppm and carbons attached to the -thiolic sulphur $\delta$ 180.30 ppm support the proposed coordination in the complexes. The $^{13}$C NMR spectra of the ligand and its metal complexes have been recorded in Table 3.

On The Basis of above Spectral studies, the structures of metal complexes considered as follows:

![Structure diagram](image)

$\text{M} = \text{Ti or Zr}$

**Figure.2 Structure of the Complexes**
3. Animal model used:

Male albino rats (Rattus norvegicus) weighing 150-200 gms were used in this study and they are housed in a separate room cages, under controlled conditions of temperature (22±3°C), humidity (50±5%) and light (12 hrs light: 12 hrs dark cycle), fed with standard pellet diet (Ashirwad Industrial Ltd., Punjab, India) and water *ad libitum*.

3.1 Ligand and metal complexes administration

For this study, the rats were divided into groups containing 5 animals each. Ligand and Metal complexes (1:2) were administered 30mg/kg.b.wt./day in the groups for 45 days to the rats. Only olive oil was given to the control group. At the end of the experimentation rats were sacrificed under light ether anesthesia for testicular and accessory sex organ weight analysis, sperm dynamics and hormonal studies.

3.2 Hormonal measurements and sperm dynamics hormonal analysis:

Radioimmunoassay of testosterone, leutinizing hormone (LH), and follicle stimulating hormone (FSH) were performed\(^\text{10}\).

3.3 Sperm motility

The epididymis was removed immediately after anesthesia and known weight of cauda epididymis was gently teased in a specific volume of physiological saline (0.9% NaCl) to release the spermatozoa from the tubules. The sperm suspension was examined within five minutes after their isolation from epididymis. The results were determined by counting both motile and immotile sperms in at least ten separate and randomly selected fields. The results were finally expressed as percent motility\(^\text{11}\).

3.4 Sperm density

Total number of sperms were counted using haemocytometer after further diluting the sperm suspension from cauda epididymis and testis. The sperm density was calculated in million per mL as per dilution\(^\text{11}\).
Statistical Analysis: The data were analyzed statistically by using ANNOVA test and the significance of differences was set at $P < 0.05$ and $P < 0.01$.

RESULTS

Analysis of male reproductive hormones and sperm dynamics is an important tool to detect male reproductive toxicity occurred by chemicals. All the results are as following:

1. Body and Organ weight determination (Table 4)

The results presented in Table 4 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.

2. Sperm Motility (Table 5)

   (i) Cauda Epididymis

   There was a significant ($P \leq 0.05$) reduction in case of ligand and highly significant reduction ($P \leq 0.01$) was observed in the motility of sperm in the rats treated with the starting materials and their complexes when treated rats were compared with the control.

   (ii) Sperm Density (Table 5)

   Testes

   Significant and highly significant reductions in testicular sperm density were observed respectively when rats were fed with the ligand, starting material and their complexes.

   (iii) Cauda Epididymis

   The density of sperm in cauda epididymis was reduced highly significantly ($P \leq 0.01$) after the treatment with the ligand, starting material and their complexes.
(iv) **Fertility test (Table 5)**

Mating exposure test showed 100% positive fertility in control rats whereas 71% negative results in group II and 53%, 92%, 56% and 90% negative results in groups III, IV, V and VI respectively.

3. **Radio-Immuno Assays (RIA) (Table 6).**

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.

**DISCUSSION**

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

The weight of testes and accessory sex organs were decreased The epididymis, seminal vesicle and Vas Deferens are all androgen dependent organs, relying on testosterone for their normal growth and function. A reduction in their weights may reflect a decreased bioavailability and production of androgens, which caused Leydig cell disintegration.

Sperm motility is considered one of the most important parameters evaluating the sperm fertilizing ability. The motility of sperm in cauda epididymis indicates less ability of sperm to interact with the oocyte plasma membrane. Decreased sperm density in the epididymis is an indicator of reduced spermatogenesis as a result of the antispermatogenic nature of any agent. Low caudal epididymal sperm density may be due to alteration in androgen metabolism. The physiological and biochemical integrity of epididymis are dependent on androgens. The negative fertility may be attributed to lack of forward progression and reduction in density of spermatozoa and altered biochemical milieu of cauda epididymis.
Researchers have demonstrated that physiologic concentrations of testosterone, LH and FSH play an important role in spermatogenesis\textsuperscript{21}, 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\textsuperscript{22}.

**CONCLUSION**

The oral administration of ligand, starting materials and their metal complexes induced reproductive toxicity in male albino rats. Toxic effects of complexes were more pronounced than ligand and starting materials and the results also revealed that the starting materials of titanium and zirconium($\text{Cp}_2\text{TiCl}_2$ and $\text{Cp}_2\text{ZrCl}_2$)are more effective than the ligand. Thus a conclusion may be drawn that ligand, starting materials and their complexes 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.

**ACKNOWLEDGEMENT**

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**Table 1. Analytical data and physical properties of the ligand and its metal complexes.**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Colour</th>
<th>Melting Point(^0\text{C})</th>
<th>Found (Calcd.)(%)</th>
<th>Mol.Wt Found (Calcd.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\text{L}^1\text{H})</td>
<td>Reddish orange 155</td>
<td>C 60.91 (61.93)</td>
<td>H 4.19 (4.38)</td>
<td>N 6.56 (7.60)</td>
</tr>
<tr>
<td>$\text{Cp}_2\text{TiCl}$</td>
<td>Brown 215(^0\text{C})</td>
<td>C 59.21 (60.16)</td>
<td>H 3.42 (4.00)</td>
<td>N 3.52 (4.84)</td>
</tr>
<tr>
<td>$\text{Cp}_2\text{Ti (L}^1\text{)}_2$</td>
<td>Brown 250(^0\text{C})</td>
<td>C 62.89 (63.28)</td>
<td>H 3.01 (4.20)</td>
<td>N 5.27 (6.15)</td>
</tr>
<tr>
<td>$\text{Cp}_2\text{ZrCl(L}^1\text{)}$</td>
<td>Brown 185(^0\text{C})</td>
<td>C 54.25 (54.94)</td>
<td>H 3.10 (4.01)</td>
<td>N 3.24 (3.84)</td>
</tr>
</tbody>
</table>

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Table 2. \(^1\)H NMR Spectral data of the ligand and its corresponding metal complexes

<table>
<thead>
<tr>
<th>Compound</th>
<th>(^1)H NMR spectral data (cm(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-NH (bs)</td>
</tr>
<tr>
<td>L(^1)H</td>
<td>10.20 (s)</td>
</tr>
<tr>
<td>Cp(_2)TiCl(L(^1))</td>
<td>-</td>
</tr>
<tr>
<td>Cp(_2)Ti(L(^1))(_2)</td>
<td>-</td>
</tr>
<tr>
<td>Cp(_2)ZrCl(L(^1))</td>
<td>-</td>
</tr>
<tr>
<td>Cp(_2)Zr(L(^1))(_2)</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 3. \(^{13}\)C NMR Spectral data of the ligand and its corresponding metal complexes

<table>
<thead>
<tr>
<th>Compound</th>
<th>(^{13})C NMR spectral data (cm(^{-1}))</th>
</tr>
</thead>
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<tr>
<td></td>
<td>-HC=N</td>
</tr>
<tr>
<td></td>
<td>C(_1)</td>
</tr>
<tr>
<td></td>
<td>C(_4)</td>
</tr>
<tr>
<td>L(^1)H</td>
<td>163.16</td>
</tr>
<tr>
<td></td>
<td>134.80</td>
</tr>
<tr>
<td>Cp(_2)TiCl(L(^1))</td>
<td>164.78</td>
</tr>
<tr>
<td></td>
<td>134.50</td>
</tr>
<tr>
<td>Cp(_2)Ti(L(^1))(_2)</td>
<td>164.12</td>
</tr>
<tr>
<td></td>
<td>133.90</td>
</tr>
</tbody>
</table>
Cp$_2$ZrCl(L$_1$)$_{166.78}$ 170.25 - 159.45 111.25 127.93 118.30 134.60 125.12 126.65 153.94 132.30
Cp$_2$Zr(L$_1$)$_2$ 166.10 170.89 - 159.40 110.62 127.70 118.20 132.35

TABLE: 4

Body and organ weight measurements of ligand (L$_1$H), starting materials and complexes treated male rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Body weight</th>
<th>Testes</th>
<th>Vas Deferens</th>
<th>Seminal Vesicle</th>
<th>Epididymis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Initial</td>
<td>Final</td>
<td>G</td>
<td>mg/100g body wt.</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>Vehicle</td>
<td>208.11±12.78</td>
<td>180.20±16.43</td>
<td>999.60±2.71</td>
<td>139.30±1.98</td>
<td>621.40±17.31</td>
</tr>
<tr>
<td></td>
<td>treated</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(Control)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>Ligand</td>
<td>206.25±19.40</td>
<td>164.24±13.54</td>
<td>882.13±1.46</td>
<td>134.28±1.14</td>
<td>577.65±11.24</td>
</tr>
<tr>
<td></td>
<td>(L$_1$H)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>Cp$_2$TiCl$_2$</td>
<td>192.21±11.87</td>
<td>160.81±13.37</td>
<td>791.64±1.65</td>
<td>130.19±0.74</td>
<td>570.14±11.3</td>
</tr>
<tr>
<td>IV</td>
<td>Cp$_2$Ti(L$_1$)$_2$</td>
<td>182.15±12.80</td>
<td>145.29±13.13</td>
<td>724.35±1.95</td>
<td>124.20±1.02</td>
<td>563.27±10.9</td>
</tr>
<tr>
<td>VI</td>
<td>Cp$_2$ZrCl$_2$</td>
<td>196.10±11.97</td>
<td>162.40±10.07</td>
<td>819.41±2.51</td>
<td>132.19±0.86</td>
<td>572.25±11.1</td>
</tr>
<tr>
<td>VII</td>
<td>Cp$_2$Zr(L$_1$)$_2$</td>
<td>187.32±14.14</td>
<td>165.54±11.76</td>
<td>792.39±2.47</td>
<td>126.45±1.73</td>
<td>567.31±11.3</td>
</tr>
</tbody>
</table>

(Mean ± SEM of 5 animals)

Group I compared with all groups

ns = Non significant

* = Significant (P< 0.05)

** = Highly significant (P< 0.01)
**TABLE 5**

Sperm motility and fertility test of ligand (L₁H), starting materials and complexes treated male rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Sperm motility (%)</th>
<th>Sperm density (million/ml)</th>
<th>Fertility (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Vehicle treated (Control)</td>
<td>73.60±4.34</td>
<td>6.03±0.50</td>
<td>43.20±0.85</td>
</tr>
<tr>
<td>II</td>
<td>Ligand (L₁H)</td>
<td>53.31±4.77</td>
<td>3.95±0.80</td>
<td>31.98±1.08</td>
</tr>
<tr>
<td>III</td>
<td>Cp₂TiCl₂</td>
<td>49.35±5.15</td>
<td>3.32±0.53</td>
<td>29.58±1.09</td>
</tr>
<tr>
<td>IV</td>
<td>Cp₂Ti(L₁)₂</td>
<td>41.87±4.28</td>
<td>2.30±0.70</td>
<td>26.87±0.86</td>
</tr>
<tr>
<td>V</td>
<td>Cp₂ZrCl₂</td>
<td>48.27±4.96</td>
<td>3.56±0.71</td>
<td>30.37±1.02</td>
</tr>
<tr>
<td>VI</td>
<td>Cp₂Zr(L₁)₂</td>
<td>43.69±5.87</td>
<td>2.41±0.62</td>
<td>27.93±1.08</td>
</tr>
</tbody>
</table>

(Mean ± SEM of 5 animals)

Group I compared with all groups

ns = Non significant

* = Significant (P < 0.05)

** = Highly significant (P < 0.01)
### TABLE 6

Serum harmonal analysis of ligand (L1H), starting materials and complexes treated male rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Testosterone (30 mg/kg b.wt./day) ng/ml</th>
<th>Luteinizing Hormone (LH) mL/l</th>
<th>Follicle Stimulating Hormone (FSH) mL/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Vehicle treated (Control)</td>
<td>3.83±0.16</td>
<td>1.83±0.15</td>
<td>0.83±0.09</td>
</tr>
<tr>
<td>II</td>
<td>Ligand (L1H)</td>
<td>1.57±0.10**</td>
<td>1.11±0.12*</td>
<td>0.58±0.06*</td>
</tr>
<tr>
<td>III</td>
<td>Cp2TiCl2</td>
<td>1.13±0.10**,a</td>
<td>0.91±0.13**,a</td>
<td>0.46±0.06**,a</td>
</tr>
<tr>
<td>IV</td>
<td>Cp2Ti(L1)2</td>
<td>0.93±0.02**,c</td>
<td>0.66±0.05**,b</td>
<td>0.35±0.04**,b</td>
</tr>
<tr>
<td>V</td>
<td>Cp2ZrCl2</td>
<td>1.21±0.17**,a</td>
<td>0.94±0.16**,a</td>
<td>0.49±0.06**,a</td>
</tr>
<tr>
<td>VI</td>
<td>Cp2Zr(L1)2</td>
<td>1.08±0.06**,b</td>
<td>0.74±0.09**,b</td>
<td>0.37±0.06**,b</td>
</tr>
</tbody>
</table>

(Mean ± SEM of 5 animals)

Group I compared with all groups

ns = Non significant

* = Significant (P< 0.05)

** = Highly significant (P< 0.01)

REFERENCES


