Revelation of the Antifertility and Antispermatogenic Activity of newly synthesized thioisatin based heterocyclic compounds

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Abstract

A variety of novel thioisatin-based heterocyclic compounds were synthesized, characterized, and evaluated for their potential as contraceptive agents by assessing their impact on the reproductive physiology of male albino rats. The results revealed a significant reduction in reproductive organ weight, sperm density, sperm motility, testicular glycogen, testicular protein content, and serum hormone levels, ultimately leading to decreased fertility. Furthermore, changes in antioxidant levels and sperm morphology were observed, accompanied by histopathological evidence of reduced seminiferous tubule diameter and degeneration of interstitial cells.

Keywords: Benzothiazolidinone; Fertility test; Sperm motility and count; Hematology

Introduction

In the current scenario, most drugs available on the market are chemically synthesized. Numerous synthetic compounds have been screened for their efficacy in curing diseases and maintaining good health. Fertility regulation remains a major concern, and many chemically synthesized compounds have been investigated in preliminary studies for contraceptive drug development. Examples include 2-[piperidinoethoxyphenyl]-3-[4-hydroxyphenyl]-2H-benzo(b)pyran (1), analogs of pyrimethamine (2), organoantimony(III) compounds (3), derivatives of Schiff bases (4), and 2-(2"-chloroacetamidobenzyl)-3-(3'-indolyl) quinoline (5).

Despite the availability of various contraceptive methods, current options still result in a high rate of unintended pregnancies. Approximately 50% of all pregnancies are unintended at conception, and 50% of these occur among the 94% of sexually active couples who report using some form of contraception (6). The only male-specific contraceptive methods currently available are withdrawal, condoms, and vasectomy.

Hence, there is a pressing need for biologically active, eco-friendly fertility-regulating agents that effectively interfere with natural reproductive processes without adverse environmental effects.

In the present investigation, five compounds were chemically synthesized, characterized, and evaluated for their antifertility and antispermatogenic activities in male albino rats.

Material and method

All the compounds were synthesized following established procedures and characterized using IR, ^{1}H NMR, and ^{13}C NMR spectroscopy to confirm their structures.

3'-(2-Methylphenyl)spiro[3H-5-methyl benzo[b]thiophene-3,2'-thioazolidine]-2,4'-dione (1)

Benzothiazolidinone was synthesized by refluxing the imino compound (0.53 g, 2 mmol) with mercaptoacetic acid (0.18 g, 2 mmol) in toluene (25 mL) using a Dean-Stark apparatus. After 6 hours, the reaction mixture changed color from reddish to colorless. The product (1) precipitated, was filtered, washed with cold petroleum ether, and recrystallized from a methanol-ethanol mixture (3:1) to give a pale yellow solid (0.41 g, 62% yield), m.p. 148–152°C.

5-Methyl-3-(2-oxo-2-o-tolyl-ethylidene)-3H-benzo[b]thiophen-2-one (2)

2-Methylacetophenone (0.67 g, 5 mmol) and diethylamine (0.36 g, 5 mmol) were added with stirring to 5-methylthioisatin (0.89 g, 5 mmol). The mixture was refluxed for 15 hours. The resulting precipitate was filtered and recrystallized from ethanol. The hydroxy derivatives obtained were dehydrated in the presence of an AcOH-HCl mixture to yield chalcones (2). The concentrated reaction mixture was allowed to stand overnight, and the crude product was purified using column chromatography on silica gel, eluting with solvents of increasing polarity. Compound (2) was isolated from the petroleum ether-chloroform (1:3) fraction as a white powder (1.00 g, 68% yield), m.p. 195–196°C.

3-(2-Methylphenyl)-6-methyl benzo[b]thiophene[2,3-d][1,2]benzodiazocine (3)

o-Phenylenediamine (0.05 g, 0.50 mmol) was added to a solution of chalcone (2) (0.15 g, 0.50 mmol) in absolute ethanol (25 mL) at room temperature. The reaction progress was monitored using TLC. After 20 hours, the reaction mixture changed from dark red to a lighter color,

indicating completion. The resulting product was filtered, washed with cold petroleum ether, and recrystallized from chloroform to yield a faint brown solid (3) (0.11 g, 62% yield), m.p. 198–200°C.

3-(2-Chlorophenyl)-6-methyl benzo[b]thiophene[2,3-d][1,2]benzodiazocine (4)

o-Phenylenediamine (0.05 g, 0.50 mmol) was added to a solution of chalcone (0.16 g, 0.50 mmol) in absolute ethanol (25 mL) at room temperature. TLC monitoring showed the reaction was complete after 21 hours, as the reaction mixture changed color from dark red to light yellow. The product was filtered, washed with cold petroleum ether, and recrystallized from a petroleum ether-chloroform mixture (1:1) to yield a yellow powder (4) (0.12 g, 63% yield), m.p. 210°C.

Animals

All experiments were conducted on Rattus norvegicus (albino strain) weighing approximately 150–200 g. The rats were bred in a standard animal house and housed in polypropylene cages under environmentally controlled conditions. The room was maintained at a temperature of approximately 25°C with a 12:12-hour light-dark cycle. They were fed a standard pellet diet and provided tap water ad libitum.

Before the experiments, the animals were allowed to acclimatize to the laboratory environment for 1 hour. All procedures were carried out in a quiet laboratory setting with ambient illumination and a temperature comparable to that of the animal housing conditions.

Study plan

All the animals were divided into six groups containing five animals in each group.

Group I is served as Control and the animals in this group received 0.5 ml/day of the vehicle, i.e. olive oil. **Group II** animals were treated with **A** at the dose level 5 mg/kg b wt/rat/day, **Group III a**nimals were treated with **B** at the dose level 5 mg/kg b wt/rat/day, **Group IV** animals were treated with **C** at the dose level 5 mg/kg b wt/rat/day, **Group V** animals were treated with **D** at the dose level 5 mg/kg b wt/rat/day and **Group VI** animals were treated with **E** at the dose level 5 mg/kg b wt/rat/day.

All groups are treated for 30 days and after withdrawal of treatment animals were autopsied and tissue and blood was collected for further study.

Ethical Approval

This research approve by Departmental ethical committee of Department of Zoology, University of Rajasthan, Jaipur

Fertility test:- Male rats were cohabited with female in 1:3 ratio before and after treatment for 5 days for fertility test, allow them to complete gestation period and number of liters delivered were also counted.

Body and Organ weight:-Body weight was recorded at time interval of 15 days and organ weight observed after autopsy and calculated as mg/100 g b. wt.

Sperm motility and count:-Sperms were obtained by small cuts in Cauda epididymis. Sperm density and percent motility was calculated using Neubauer counting chamber. (7). Sperm morphological analysis was done by prepare semen smear slides.

Hormonal Assay:-Serum testosterone (8)., Folicular Stimulating Harmon (FSH) and Luteinizing Harmon (LH) (9) was done by RIA method.

Biochemical parameter:-

Superoxide dismutase (SOD) (10), Reduced glutathione (GSH) (11), Lipid peroxidation (LPO) (12), Cholesterol (13), Glycogen (14), Protein (15) were done in tissues.

Hematology:-Total RBC Count (16), Total WBC Count (16), Hemoglobin % (17), Hemetocrit Value (18) done in blood.

Histology of testes:- Testes of all groups rats were fixed in 10% formaldehyde and after microscopic slide preparation Hematoxylin Eosin double staining were used.

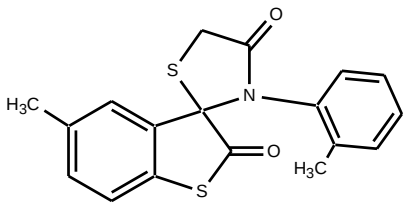
Statistical analysis:-

Results obtained were expressed as the mean SD. Means were analyzed using one way analysis of variance, followed by student's *t*- test to determine significant differences between pairs. Differences with values of $p<0.001$ $P<0.01$ and $P<0.05$ were considered statistically significant.

RESULT AND DISCUSSION:-

Chemical synthesis of all compound have been done. The structural formula and elementary estimation was prepared by IR and NMR studies of the synthetic compound.

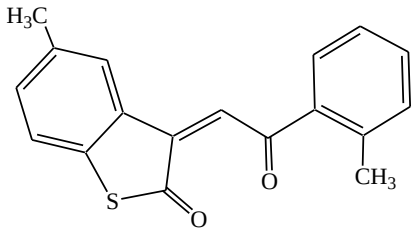
***3'-(2-Methylphenyl)spiro[3H-5-methyl benzo [b]thiophene-3,2'-thioazolidine]-2,4'-dione* (1)**



Analysis Found: C, 63.36; H, 4.20; N, 4.25; S, 19.22% Calcd. for $C_{18}H_{15}NO_2S_2$: C, 63.32; H, 4.43; N, 4.10; S, 18.78%

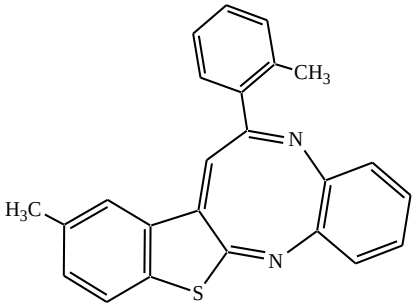
IR(ν_{max}) KBr	3132-3000 (Ar-H), 1738 (>C=O), 1722 (>C=O), 1608 (C=C_{arom}), 609 (-C-S) cm^{-1}.
^{1}H NMR 300 MHz, $CDCl_3$	δ 7.52-6.21 (m, 7H, Ar-H), 3.56 (s, 2H, CH_2), 2.10 (s, 3H, CH_3), 1.92 (s, 3H, CH_3) ppm.
^{13}C NMR 75.45 MHz, $CDCl_3$	δ 184.20 (>C=O), 161.43 (>C=O), 142.20-121.20 (Ar-C), 82.2 (spiro C), 21.2 (CH_3) ppm.

5-Methyl-3-(2-oxo-2-o-tolyl-ethylidene)-3H-benzo[b]thiophen-2-one (2)



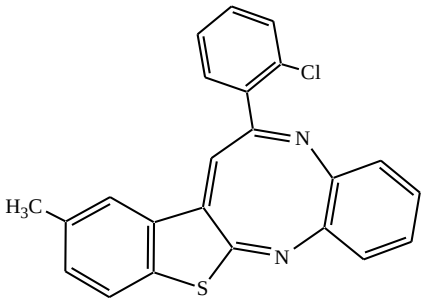
Analysis	Found: C, 73.62; H, 4.44; S, 10.74% Calcd. for $C_{18}H_{14}O_2S$: C, 73.44; H, 4.79; S, 10.89%
IR(ν_{max}) KBr	3020-3005 (Ar-H), 1686 (>C=O), 1651 (>C=O), 625 (-C-S) cm^{-1}
^{1}H NMR 300 MHz, $CDCl_3$	δ 7.71-7.30 (m, 7H, Ar-H), 4.98 (s, 1H, C=CH), 2.36 (s, 3H, CH_3), 2.12 (s, 3H, CH_3) ppm.
^{13}C NMR 75.45 MHz, $CDCl_3$	175.62 (>C=O), 161.24 (>C=O), 138.00-127.00 (Ar-C), 119.57 (C=CH), 23.65 (CH_3), 18.12 (CH_3) ppm.

3-*(2-Methyl phenyl)-6-methyl benzo [b]thiophene[2,3-d][1,2]benzodiazocine* **(3)**



Analysis	Found: C, 78.20; H, 4.88; N, 7.81; S, 8.69% Calcd. for $C_{24}H_{18}N_2S$: C, 78.66; H, 4.95; N, 7.64; S, 8.75%
IR(ν_{max}) KBr	3070-3010 (Ar-H), 1510, 1565 (C=N), 1530-1490 (C=C_{arom}) cm^{-1}
^{1}H NMR 300 MHz, $CDCl_3$	δ 7.60-6.80 (m, 11H, Ar-H), 5.70 (s, 1H, C=CH), 2.42 (s, 3H, CH_3), 2.55 (s, 3H, CH_3) ppm.
^{13}C NMR 75.45 MHz, $CDCl_3$	δ 158.70 (C=N), 154.60 (C=N), 146.20-123.60 (Ar-C), 22.70 (CH_3), 20.30 (CH_3) ppm.

3-*(2-Chloro phenyl)-6-methyl benzo[b] thiophene[2,3-d][1,2]benzodiazocine* **(4)**

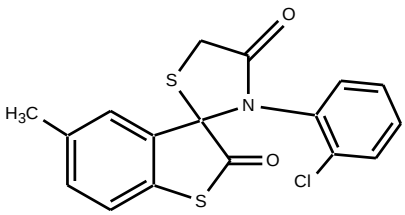


Analysis	Found: C, 71.12; H, 3.71; Cl, 9.19; N, 7.68; S, 8.17% Calcd.

	for $C_{23}H_{15}ClN_2S$: C, 71.40; H, 3.91; Cl, 9.16; N, 7.24; S, 8.29%
IR(ν_{max}) KBr	3060-3030 (Ar-H), 1560, 1535 (C=N), 1590-1570 (C=C_{arom}) cm^{-1}.
^{1}H NMR 300 MHz, $CDCl_3$	δ 7.53-6.77 (m, 11H, Ar-H), 5.40 (s, 1H, C=CH), 2.36 (s, 3H, CH_3) ppm.
^{13}C NMR 75.45 MHz, $CDCl_3$	δ 160.24 (C=N), 156.70 (C=N), 139.98-123.22 (Ar-C), 21.35 (CH_3) ppm.

3'-(2-Chlorophenyl)spiro[3H-5-methylbenzo[b]thiophene-3,2'-thiazolidine]-2,4'-dione

(5)



Analysis	Found: C, 56.21; H, 3.43; Cl, 9.67; N, 3.75; S, 17.88% Calcd. for $C_{17}H_{12}ClNO_2S_2$: C, 56.42; H, 3.34; Cl, 9.80; N, 3.87; S, 17.72%
IR(ν_{max}) KBr	3134-3000 (Ar-H), 1740 (>C=O), 1720 (>C=O), 1600 (C=C_{arom}), 608 (-C-S) cm^{-1}.
^{1}H NMR 300 MHz, $CDCl_3$	δ 7.35-6.21 (m, 8H, Ar-H), 3.52 (s, 2H, CH_2), 2.21 (s, 3H, CH_3) ppm.

^{13}C NMR	δ 185.5 (>C=O), 162.5 (>C=O), 135.90-123.20 (Ar-C), 85.4
75.45 MHz, $CDCl_3$	(spiro C), 20.1 (CH_3) ppm.

Final body weight showed no significant change in all groups as compared to control that's indicating good health of all animals and no toxic effect of treatment in animals. (Table:-1) (19).

Table:-1: Final body weight

Parameter Group	Body weight (g)	Organ weight (mg\100g)								
		Testes	SV	VD	Prost	EPI	Liver	Heart	Kidney	AD
I	252.53 ±14.13	1123.19 ±13.58	558.25 ±19.22	182.11 ±9.29	252.01 ±18.38	382.81 ±15.11	3809.96 ±32.73	328.71 ±16.32	282.98 ±5.15	20.21 ±2.13
II	235.98 ns 3.77	867.67 b 9.68	487.57 a 5.87	146.56 a 1.45	207.45 a 5.24	318.56 a 5.54	3796.05 ns 43.56	345.68 ns 6.57	306.97 ns 3.65	20.06 ns 0.09
III	243.13 ns ±6.19	776.67 b 11.56	435.87 b 5.34	132.54 b 2.34	198.43 b 3.54	301.54 b 4.55	3987.56 ns 37.67	320.76 ns 18.50	278.68 ns 6.47	19.57 ns 0.75
IV	267.78 ns	746.79 b	409.77 b	121.43 b	175.55 b	287.56 b	3756.47 ns	302.78 ns	267.77 ns	19.39 ns 0.08

	6.78	15.65	4.70	1.09	2.54	5.41	36.78	11.73	4.78	
V	285.56 ns ±9.18	576.67 c 6.78	358.56 c 7.45	109.45 c 1.54	157.46 c 3.54	254.53 c 3.55	3698.67 ns 17.98	325.87 ns 4.78	296.57 ns 7.45	18.76 ns 0.29
VI	287.65 ns 9.65	478.67 c 5.98	345.65 c 7.33	99.43 c 1.01	145.25 c 2.45	231.45 c 2.89	3836.87 ns 47.75	345.78 ns 5.47	270.57 ns 3.87	19.98 ns 0.17

Ns:- Non- significant, a P≤0.05, b P≤0.005, P≤0.001

The weight of reproductive organ in treated groups was observed highly significantly reduction which shows adverse effect on reproductive physiology of the treatment but no reduction found in vital organ weight in comparison to control in all treatment groups indicate safe use as a drug because do not have any side effect. (Table:- 1) (20). Fertility test showed that females that cohabitated with control animal have 100% fertility but in treated group II, III, IV, V and VI showed respectively 55.56, 44.44%, 22.22%, 11.11% and 0% fertility after treatment. (Table 2)

Table 2:- Fertility Index of treatment

Parameter Group	No of Male	No of Females	Pregnant	% fertility	Litters
I	3	9	9	100	42
II	3	9	5	55.56	22
III	3	9	4	44.44	13
IV	3	9	2	22.22	8

V	3	9	1	11.11	4
VI	3	9	0	0	0

Ns:- Non- singnificant, a P≤0.05, b P≤0.005, P≤0.001

Decrease fertility rate in treated groups shows antifertility activity of the synthesized compound. Fertility rate reduction may be due to altered antioxidant level and hormone level. (21)

Sperm morphological observation shows altered structure of sperm. In treated groups most of sperm were have these alterations i.e. head only, Tail without head, Banded tail, Raptured head, degenerated tail. (fig:- 1)

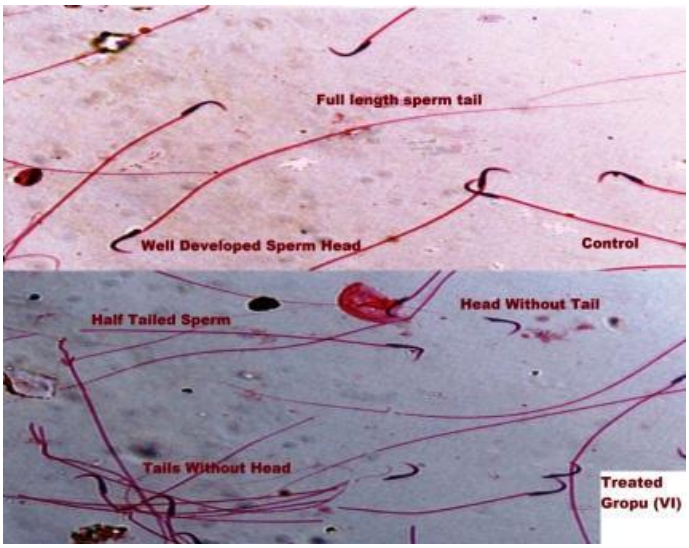


Figure. 1. Control and treated (Group VI) rats sperm at 400X

Sperm morphology is main important feature for fertilization possibilities, altered sperms are unable to move to ovum and unable to fertilized it (22). Sperm density and motility decrease highly significantly in all treated groups in comparison to control. (Table 3)

Table 3:- Hormone level and sperm dynamics of rats.

Parameter Group	Testosterone	FSH	LH	Sperm density (Million\ml)	Sperm Motality (%)
I	4.1 0.01	5.1 0.03	4.3 0.02	54.33 ±2.14	70.54 ±4.04
II	3.2 [b] 0.01	4.2 [c] 0.02	3.1 [b] 0.01	35.12 [b] 1.25	40.53 [c] 2.50
III	2.9 [c] 0.02	4.1 [c] 0.01	2.8 [c] 0.02	27.56 [c] 1.95	34.48 [c] 1.54
IV	2.8 [c] 0.02	3.6 [c] 0.01	2.4 [c] 0.02	22.38 [c] ±3.08	30.18 [c] ±2.`12
V	2.6 [c] 0.03	3.2 [c] 0.02	2.2 [c] 0.03	20.35 [c] 2.45	27.46 [c] 2.88
VI	1.8 [c] 0.03	3.4 [c] 0.03	2.1 [c] 0.01	18.13 [c] ±1.18	20.18 [c] ±1.72

Ns:- Non- singnificant, a P≤0.05, b P≤0.005, P≤0.001

Reduction in sperm dynamics support altered biochemical parameter (23) and reduced Testosterone, LH and FSH level in serum. (Table 3) (24)

Protein is a main part of all animal's cell and enzyme system, reduction in total protein level in testes indicated cell degradation and physiology alteration. (25) Cholesterol level increase in significant manner was also potent information of physiological alteration because cholesterol

is a millstone component of cellular architecture and a precursor of many steroids hormone. (Table 4) (26)

Table 4 :- Tissue biochemistry of treated rats

Group	Cholesterol (Mg\g)			Protein (Mg\g)			Glycogen (Mg\g)		
	T	EPI	SV	T	EPI	SV	T	EPI	SV
I	14.67 ±1.43	15.46 ±0.96	18.93 ±0.86	434.22 ±8.24	359.54 ±11.03	411.91 ±10.07	2.92 ±0.18	3.93 ±0.27	3.29 ±0.13
II	28.56 [b] 2.45	32.55 [c] 1.46	29.56 [c] 2.56	378.98 [b] 13.70	302.66 [b] 3.89	340.73 [b] 6.87	2.45 [b] 0.14	3.22 [b] 0.03	2.67 [b] 0.05
III	23.18 [b] ±2.92	39.31 [c] ±1.93	35.85 [c] ±8.67	358.13 [b] ±6.71	298.18 [b] ±4.13	332.18 [b] ±6.73	2.06 [b] ±0.09	2.91 [c] ±0.11	2.28 [c] ±0.16
IV	35.67 [c] 4.48	36.65 [c]	36.78 [c] 7.34	312.56 [c] 3.87	256.72 [c] 7.78	310.67 [c] 8.24	1.87 [c] 0.05	2.58 [c] 0.11	2.10 [c] 0.09
V	35.28 [c]	41.07 [c] ±1.53	37.63 [c] ±4.58	305.81 [c] ±9.03	258.73 [c] ±6.18	307.61 [c] ±10.13	1.68 [c] ±0.09	2.49 [c] ±0.07	1.99 [c] ±0.17

	±2.76								
VI	43.56 [c] 2.56	42.98 [c] 4.78	39.54 [c] 2.67	290.39 [c] 5.87	236.28 [c] 4.78	301.33 [c] 7.57	1.56 [c] 0.06	2.16 [c] 0.05	1.78 [c] 0.07

Ns:- Non- singnificant, a P≤0.05, b P≤0.005, P≤0.001

Antioxidant parameter alteration in testes also shows pathophysiological support of antifertility activity.

Table 5:- Antioxidant parameters showing effect of treated rats

Parameter Group	SOD µ mole \mg protein			LPO n mole MDA\mg protein			GSH		
	Liver	Testes	Kidney	Liver	Testes	Kidney	Liver	Testes	Kidney
I	3.92 ±1.37	1.39 ±0.04	1.19 ±0.53	5.21 ±0.41	1.89 ±0.15	3.73 ±0.74	3.50 ±0.37	2.25 ±0.06	2.38 ±0.25
II	3.13 [ns] ±0.19	0.41 [c] ±0.03	0.78 [a] ±0.07	5.45 [ns] ±0.21	0.91 [c] ±0.12	3.88 [ns] ±0.07	3.25 [ns] 0.06	1.30 [c] 0.04	1.98 [ns] 0.07
III	3.58 [ns] ±0.03	0.85 [b] ±0.08	0.71 [ns] ±0.12	5.18 [ns] ±0.19	1.51 [b] ±0.08	3.19 [ns] ±0.11	3.12 [ns] 0.18	1.76 [b] 0.09	2.08 [ns] 0.07
IV	3.78 [ns]	0.57 [c]	1.05 [ns]	4.67	1.24 [c]	3.29 [ns]	3.01 [ns]	1.48	2.43

	0.87	0.06	0.18	ns 0.29	0.11	0.07	0.09	c 0.07	ns 0.09
V	3.98 ns 0.16	0.94 b 0.05	1.23 ns 0.09	5.78 ns 0.28	1.37 b 0.09	3.58 ns 0.17	3.89 ns 0.14	1.79 b 0.04	2.76 ns 0.15
VI	3.57 ns 0.34	0.38 c 0.02	1.15 ns 0.05	4.78 ns 0.27	0.86 c 0.05	3.22 ns 0.21	3.67 ns 0.15	1.22 c 0.02	2.02 ns 0.04

Ns:- Non- singnificant, a P≤0.05, b P≤0.005, P≤0.001

Altered antioxidant level was disrupting the histological architecture of reproductive organs. Testes histopathological studies reveal that reduced seminiferous tubule diameter, degraded interstitial cell and fatty deposition in seminiferous tubules. (Figure 2)

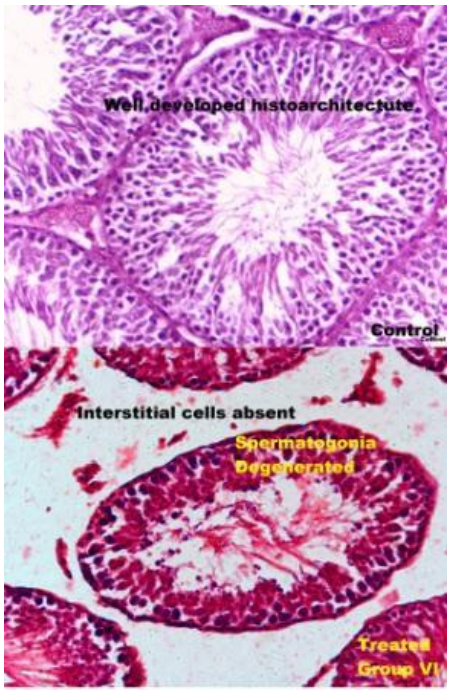


Figure. 2. Control and treated (Group VI) Rats Testes TS at 200X.

Altered histological architecture may be due to altered biochemical parameter, antioxidant level and reduced hormone level (27). All altered reproductive parameter effect on physiology of reproductive organ and produced antifertility activity.

Histology of testes:- The testes and epididymides from each rat were separately fixed in 10% formaldehyde. A thin section of the tissue was made and finally stained with haematoxylin and eosin dye. Each slide was clean-blotted and mounted in Canada balsam under a cover slip.

Table 6:- Hematology of treated Table rats.

Parameter Group	Hb (g\dl)	TLC (Thousand\mm3)	PCV %	TRBC (Million\cu mm)
I	13.80 ±1.08	11.11 ±1.69	40.00 ±2.01	5.65 ±0.52
II	12.80 ns 0.95	10.6 ns 0.52	42.85 ns 1.56	5.44 ns 0.23
III	11.70 ns ±0.91	11.0 ns ±1.12	40.18 ns ±2.78	5.19 ns ±0.93
IV	12.00 ns 1.01	10.0 ns 0.81	45.45 ns 2.25	5.75 ns 0.41
V	11.30 ns ±0.57	11.4 ns ±1.62	41.66 ns ±3.19	5.11 ns ±0.29
VI	13.00 ns 0.56	11.60 ns 0.21	30.76 ns 5.13	5.69 ns 0.15

Ns:- Non- singnificant, a P≤0.05, b P≤0.005, P≤0.001

Conclusion:-

On the basis of IR and NMR the structural formula was conformed of all synthesized compound. In the biological activity reduced reproductive organ weight, sperm density, sperm motility, testicular glycogen, testicular protein and hormone level in serum indicated adverse effect of compound on reproductive physiology of male rats. Altered fertility test, antioxidant level and morphological analysis were shows antifertility activity of compounds. Altered sperm morphology also shows antispermatogenic nature of compounds. But compound E has more potential activity other than all other compound. So it may be concluded that all five compound have potent antifertility and antispermatogenic activity however, 3'-(2-Chlorophenyl)spiro[3H-5-methylbenzo[b]thiophene-3,2'-thiazolidine]-2,4'-dione (**5**) have highest effect. They may be used as a contraceptive drug for male but further higher animal studies needed.

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Conflict of interest

On behalf of all authors, the corresponding author states that there is no conflict of interest

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