



ISSN Print: 2664-9926

ISSN Online: 2664-9934

IJBS 2025; 7(2): 76-87

www.biologyjournal.net

Received: 25-11-2024

Accepted: 30-12-2024

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Impact of quinestrol on biochemical and reproductive parameters of adult male albino rats

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Abstract

This study investigates the effects of quinestrol, a synthetic estrogen, on male reproductive and biochemical parameters in sexually mature male albino rats. A total of 40 rats were divided into two groups: a control group and a treatment group receiving 2 mg/kg of quinestrol for 10 days. Body weight was measured at intervals of 0, 5, and 10 days, revealing no significant differences between groups. However, significant reductions in reproductive organ weights (testis and epididymis) were observed in the quinestrol-treated group. Sperm parameters, including motility, viability, and concentration, were adversely affected, with a notable increase in sperm abnormalities. Biochemically, quinestrol treatment led to significant decreases in total lipid and phospholipid content while cholesterol levels increased in the testis and epididymis. Additionally, total soluble protein content decreased markedly in both reproductive tissues. These findings suggest that quinestrol disrupts the hypothalamic-pituitary-gonadal axis, leading to decreased testosterone synthesis and impaired spermatogenesis, highlighting its potential as a male contraceptive.

Keywords: Quinestrol, male reproduction, sperm, hormones, contraception

Introduction

Male fertility is a complex physiological process regulated by the hypothalamic-pituitary-gonadal axis, which controls the secretion of reproductive hormones such as luteinizing hormone and follicle-stimulating hormone (Johnson *et al.*, 2018) ^[17]. These hormones stimulate the Leydig and Sertoli cells in the testes, which are essential for testosterone synthesis and spermatogenesis (Smith & Walker, 2020) ^[29]. Any disruption in this endocrine axis can lead to infertility, making it a critical target for contraceptive research.

Quinestrol, a long-acting estrogen derivative of ethinylestradiol, has gained attention as a potent antifertility agent due to its ability to alter endocrine homeostasis (Brown *et al.*, 2017) ^[6]. Initially developed for female contraception, quinestrol has also shown efficacy in male fertility suppression by disrupting testosterone production and spermatogenesis (Garcia & Patel, 2019) ^[13]. Studies on male albino rats have demonstrated that quinestrol administration leads to significant biochemical and hormonal alterations, including decreased serum testosterone levels, increased estradiol concentrations, oxidative stress, lipid peroxidation, and enzymatic dysfunction (Lee *et al.*, 2021) ^[19].

One of the primary mechanisms through which quinestrol exerts its antifertility effects is the suppression of gonadotropins. Quinestrol-induced negative feedback on the hypothalamic-pituitary-gonadal axis inhibits the secretion of gonadotropin-releasing hormone, leading to reduced luteinizing hormone and follicle-stimulating hormone production (Zhang *et al.*, 2016) ^[33]. This suppression results in decreased stimulation of Leydig cells, thereby reducing testosterone synthesis. Since testosterone is essential for spermatogenesis, its depletion leads to impaired sperm production, altered sperm morphology, and increased germ cell apoptosis (Anderson & Roberts, 2015) ^[4].

In addition to hormonal suppression, quinestrol has been found to induce oxidative stress in testicular tissues. Oxidative stress is a key factor in male infertility, as excessive reactive oxygen species can damage cellular structures, including lipids, proteins, and DNA (Gupta *et al.*, 2018; Alrubaie and Khafaji, 2024) ^[15, 20]. Studies have reported that quinestrol administration significantly reduces antioxidant enzyme activity, including superoxide

dismutase, catalase, and glutathione peroxidase, leading to increased lipid peroxidation and testicular dysfunction (Wang & Li, 2020) [31]. Elevated oxidative stress levels contribute to mitochondrial damage, disrupted cellular metabolism, and apoptosis of germ cells, ultimately resulting in reduced sperm count and motility (Singh *et al.*, 2019) [28].

Proteins, lipids, cholesterol, and phospholipids play crucial roles in the structural and functional integrity of male reproductive cells. Proteins are essential for sperm development, enzymatic regulation, and cellular signaling, while lipids and cholesterol contribute to sperm membrane fluidity and stability (Hernandez & Lopez, 2022) [16]. Phospholipids, particularly phosphatidylcholine and phosphatidylethanolamine, are key components of sperm membranes, influencing sperm motility and viability (Martinez *et al.*, 2021) [23]. Quinestrol administration has been reported to cause significant alterations in these biochemical components. Studies have shown a marked reduction in total protein content in testicular tissues, likely due to decreased biosynthesis and increased proteolysis (Chen *et al.*, 2017) [9]. This decline can impair spermatogenesis by affecting critical proteins involved in sperm maturation and function.

Similarly, lipid metabolism is disrupted in quinestrol-treated rats, with significant decreases in testicular lipid and cholesterol levels (Zhou *et al.*, 2018) [36]. Since cholesterol is vital for steroidogenesis and sperm membrane integrity, its depletion can negatively impact sperm viability and motility. Moreover, alterations in phospholipid composition have been observed, leading to reduced membrane stability and increased susceptibility to oxidative damage (Fernandez *et al.*, 2020) [11]. Increased lipid peroxidation, as indicated by elevated malondialdehyde levels, further exacerbates membrane damage and functional impairments in spermatozoa (Park & Kim, 2019) [25].

By disrupting protein, lipid, cholesterol, and phospholipid homeostasis, quinestrol contributes to male infertility through biochemical pathways that impair sperm production, motility, and structural integrity. Investigating these biochemical alterations will provide deeper insights into the mechanisms of quinestrol-induced infertility and its potential as a contraceptive agent.

Materials and methods

Procurement of animals

Sexually mature male albino rats weighing between 130-140 g were procured from the Disease-Free Small Animal House at Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, after clearance of the proposal through the Institutional Animal Ethics Committee. A total of 40 adult male albino rats were procured. After being obtained, the rats were given a 15-day acclimatization period. In the animal house, the rats were kept in polypropylene cages and were exposed to an environment similar to their natural habitat. Wheat husk was utilized as bedding. Rats were fed a diet of cracked wheat and given adequate amounts of water. Water was provided in glass bottles, and feeders were equipped with rubber stoppers. Standard laboratory conditions were maintained, including ambient temperature control (22-25°C) and a 12-hour light/dark cycle during the treatment days. Before the experiment began, the rats were divided into two groups: control (n=20) and treatment (n=20). Permission was obtained at the 63rd meeting of the IAEC, which took place on January 13, 2022, at Guru

Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, with Protocol No. (GADVASU/2022/IAEC/63/11). Male albino rats were handled according to the Indian Committee for the Purpose of Control and Supervision of Experiments with Animals standards.

Drug used for the experiment

To evaluate the effects of quinestrol, synthetic estradiol (17 alpha-Ethinylestradiol 3-cyclopentyl ether), a commercial formulation was acquired from CDH Chemicals and administered to the rats via oral intubation.

Experimental setup

Rats were acclimatized to lab conditions for 15 days before the experiment. After acclimatization, all 40 male rats were separated into two groups (n=20).

Groups	Treatment
Group 1 (n=20)	Control
Group 2 (n=20)	Quinestrol @2mg/Kg body weight

Dimethyl sulfoxide, an organic solvent, was used to dissolve quinestrol for dose preparation. The second group received treatment via oral intubation for 10 days.

Effect on body weight

After 15 days of acclimatization and 10 days of treatment, the animals' body weights were recorded at 0, 5, and 10 days and compared.

Autopsy of rats

After completing the treatment, the rats were dissected. The testicles and epididymides were removed, and the adhering tissue was carefully excised to obtain precise weights. Tissue samples were stored at -20°C for biochemical examination.

Effect on reproductive organ weight

After the 10-day treatment period, the rats were sacrificed, and the reproductive organs (testis and epididymis (caput, corpus and cauda) of rats from both groups were dissected out and weighed.

Preparation of tissue homogenates of testis and epididymal tissues

The testis and epididymis were homogenized (caput, corpus, and cauda) by combining the tissue with known with a quantity of 0.1 M phosphate buffer saline (pH 7.6). The process was carried out in a homogenizer. The entire homogenate was spun in a chilled microcentrifuge for 10 minutes at 3000 rpm (825 rcf). In tissue homogenates of the testis and epididymis, the resulting supernatant was employed for biochemical parameters.

Sperm Parameters

Sperm Motility (Salisbury *et al.*, 1978) [26]

Immediately after dissection, a drop of caput, corpus, and cauda epididymal fluid was placed on a pre-warmed glass slide and observed under a microscope at 400X magnification. The number of motile and immotile spermatozoa was counted in various fields.

Sperm Concentration (Salisbury *et al.*, 1978) [26]

Sperm concentration was determined using a hemocytometer. The diluted caput, corpus, and cauda epididymal fluid was taken into a standard red cell dilution pipette, and small air bubbles were extracted. The dilution solution (0.2% eosin) was added to the 101-mark above the bulb. The mixture was shaken for 2-3 minutes and a drop was placed under a coverslip on a Neubauer chamber. The spermatozoa were allowed to settle for 2 minutes, and then counted at 400X magnification. The total spermatozoa were averaged across five squares on both sides of the chamber. The sperm concentration was calculated using the following formula:

Sperm Viability (Campbell *et al.*, 1956) ^[37]

A drop of sperm-containing epididymal fluid was mixed with eosin/nigrosin stain and incubated at 37°C for 2 minutes before preparing smears on glass slides. At 1000X magnification, the percentage of viable (unstained) and non-viable (pink-stained) spermatozoa was determined.

Sperm Abnormality

Sperm morphology was assessed under a light microscope. The abnormalities evaluated included head, midpiece, and flagellar defects. The percentage of sperm abnormalities was calculated based on 250-300 spermatozoa per rat.

Biochemical Parameters

Quantitative Estimation of Total Soluble Protein Content: The total soluble protein content was determined using the method of Lowry *et al.* (1951) ^[38]. Tissue homogenates were prepared, and 5.0 ml of a reagent mixture was added to 0.01 ml of the sample. The mixture was allowed to stand at room temperature for 10 minutes before the addition of 0.5 ml of diluted Folin's phenol reagent. After vigorous agitation, the sample was left at room temperature for 30 minutes. Absorbance was measured at 520 nm using a UV-Visible double beam spectrophotometer. The total soluble protein content in different tissue samples was calculated using a standard curve prepared with bovine serum albumin in the concentration range of 20-200 µg/ml. The results were expressed as mg protein per gram of wet tissue.

Extraction and Estimation of Total Lipids

Total lipids were extracted from testis and epididymal tissue (caput, corpus, and cauda) using the method of Folch *et al.* (1957) ^[12]. The tissue was homogenized using phosphate-buffered saline in a homogenizer, followed by the addition of an extraction medium containing chloroform and methanol in a 2:1 ratio. The mixture was subjected to intermittent shaking for 3-4 hours to ensure thorough mixing. After filtration through a G-2 sintered glass funnel, the filtrate was mixed with 0.85% saline solution and transferred to a separating funnel, where it was left overnight for complete phase separation. The bottom layer, containing lipids, was collected in a pre-weighed crucible, while the upper layer was rinsed with chloroform to ensure maximum lipid recovery. The crucible with lipids was placed in an oven at 60°C for vaporization, and after drying, it was weighed again to determine total lipid content. The

lipid extract was further dissolved in chloroform for the quantitative analysis of lipids, cholesterol, and phospholipids. Lipid content was expressed as mg per gram of tissue weight.

Estimation of Total Phospholipids

Phospholipids were estimated in the testis and epididymal tissue (caput, corpus, and cauda) using the method of Ames (1966) ^[3]. Lipid samples and standard phosphate solutions were evaporated in a water bath at 60°C. A specific reagent was added to the test tubes containing the samples, which were then dried at 100°C in an oven. These tubes were subsequently heated over a strong flame until brown fumes ceased to emerge. Once cooled, hydrochloric acid was added, and the samples were vortexed before being placed in a boiling water bath for 15 minutes. After cooling, a color-developing reagent was added, followed by incubation at 45°C for 25 minutes. The absorbance was measured at 820 nm using a spectrophotometer, with reference to a blank reagent. A standard curve was prepared using potassium dihydrogen phosphate ranging from 2-6 µg/ml, and the phospholipid content was expressed as mg per gram of tissue weight.

Estimation of Total Cholesterol

Total cholesterol was estimated in the testis and epididymal tissue (caput, corpus, and cauda) using the method of Chiamori and Henry (1959) ^[8]. Lipid samples were evaporated at 60°C before the addition of a developing reagent. The contents were mixed thoroughly by vortexing before the addition of concentrated sulfuric acid. The samples were mixed again and left undisturbed for 10 minutes. Absorbance was measured at 560 nm using a spectrophotometer against a blank. A standard curve was created using cholesterol concentrations ranging from 20-200 µg/ml, and the results were expressed as mg per gram of tissue weight.

Results

Effect on body weight

At 0, 5, and 10 days, measurements of each rat's body weight revealed variations, as indicated in table 1 and figure 1. The body weight of rats treated with quineestrol showed no alteration in body weight at 0, 5 and 10 days when compared with control rats. There was no statistically significant difference in the average body weight of the rats after quineestrol treatment at various intervals, showing that the treatment had no adverse effects on the body weight of the rats.

Table 1: Changes in body weight (g) of rats exposed to quineestrol

Groups Days	Control	Treatment
0	130.12±0.51 ^{ab}	130.01±0.51 ^{ab}
5	130.16±0.54 ^{ab}	129.54±0.54 ^{ab}
10	130.29±0.50 ^{ab}	129.34±0.57 ^{ab}

All values are expressed as mean ± S.E, n=6

Values with no alphabetical superscript indicate non-significant difference ($p \leq 0.05$) using Tukey's test

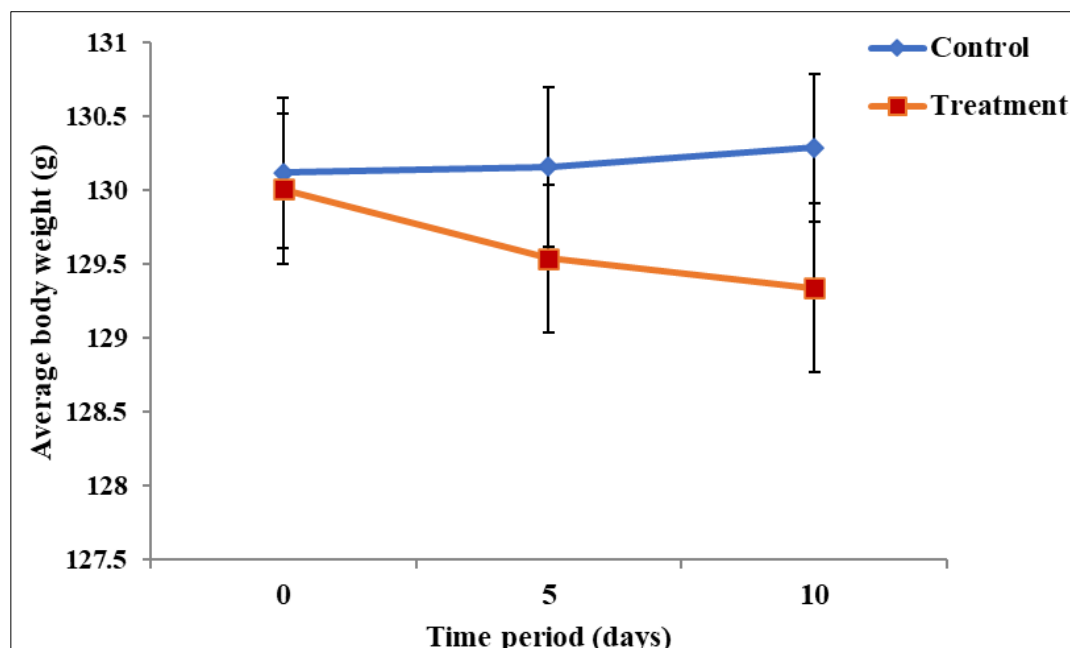


Fig 1: Changes in body weight (g) of male albino rat after treatment with quineestrol

Weight of reproductive organs

Effect on reproductive organ weight

The weight of reproductive organs, testis and epididymis of all the control and treated rats were recorded after dissection at the end of treatment. The weight of the reproductive organ is represented as g/100 g body weight (table 2 and figure 2a, 2b and 2c). The weight of testis and epididymal tissue i.e caput, corpus and cauda of treated rats showed a significant difference as compared to the control group. A remarkably significant decrease was seen in the weight of caput, corpus and cauda of rats exposed to quineestrol at the dose of 2 mg/Kg body weight for a period of 10 days as compared to control groups. Statistically, findings from the

present study indicate that all the organs were affected by quineestrol treatment.

Table 2: Changes in organ weight (g/100 g) of rats exposed to quineestrol

Groups / Organs	Control	Treatment
Testis	1.55±0.08 ^a	0.93±0.02 ^b
Caput	0.20±0.02 ^a	0.13±0.01 ^b
Corpus	0.08±0.01 ^a	0.01±0.03 ^b
Cauda	0.31±0.04 ^a	0.23±0.02 ^b

All values are expressed as mean±S.E.; n=6

Mean values with different alphabetical superscripts in column and rows indicate significant difference using ($p \leq 0.05$) Tukey's test

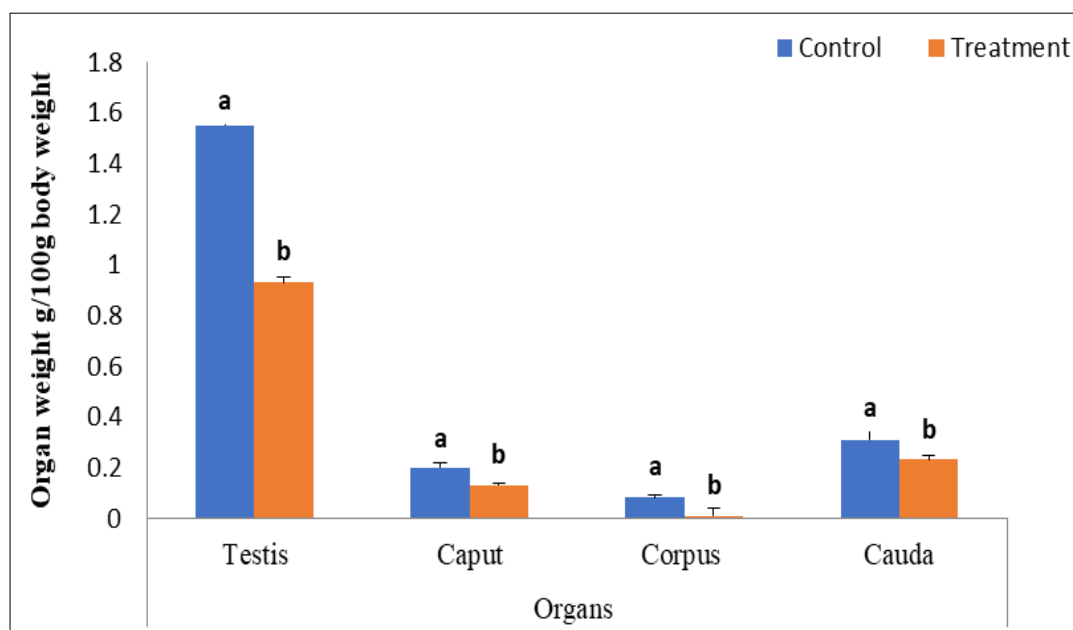


Fig 2a: Changes in weight of testis and epididymis (g/100 g) after treatment with quineestrol

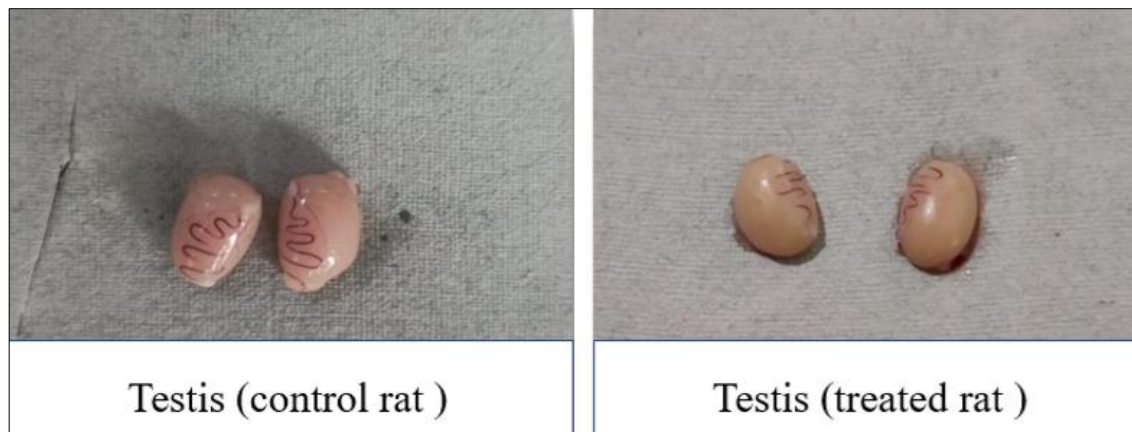


Fig 2b: Effect of quinnestrol treatment on testis of male albino rat



Fig 2c: Effect of quinnestrol treatment on epididymis of male albino rat

Sperm parameters

In the present study, the fluid from three regions of the epididymis i.e caput, corpus, cauda was taken for sperm parameters like sperm motility (%), abnormalities (%) and concentrations (millions/ml) were evaluated and results were represented in table 3-6 and figure 3-6. The sperm motility, viability and count were significantly decreased in quinnestrol treated group in comparison to control group after 10 days of treatment whereas, the sperm abnormality (figure 4a) significantly increased in quinnestrol group as compared to control group.

Table 3: Effect of quinnestrol treatment on sperm motility (%) after 10 days of treatment withdrawal

Groups / Organs	Control	Treatment
Caput	81.00±1.00 ^d	73.66±0.58 ^c
Corpus	80.66±1.54 ^d	65.00±1.00 ^a
Cauda	87.00±1.00 ^e	70.00±1.00 ^b

All values are expressed as mean ±SE; n=6

Mean values with different alphabetical superscripts indicate significant difference using ($p \leq 0.05$) Tukey's test

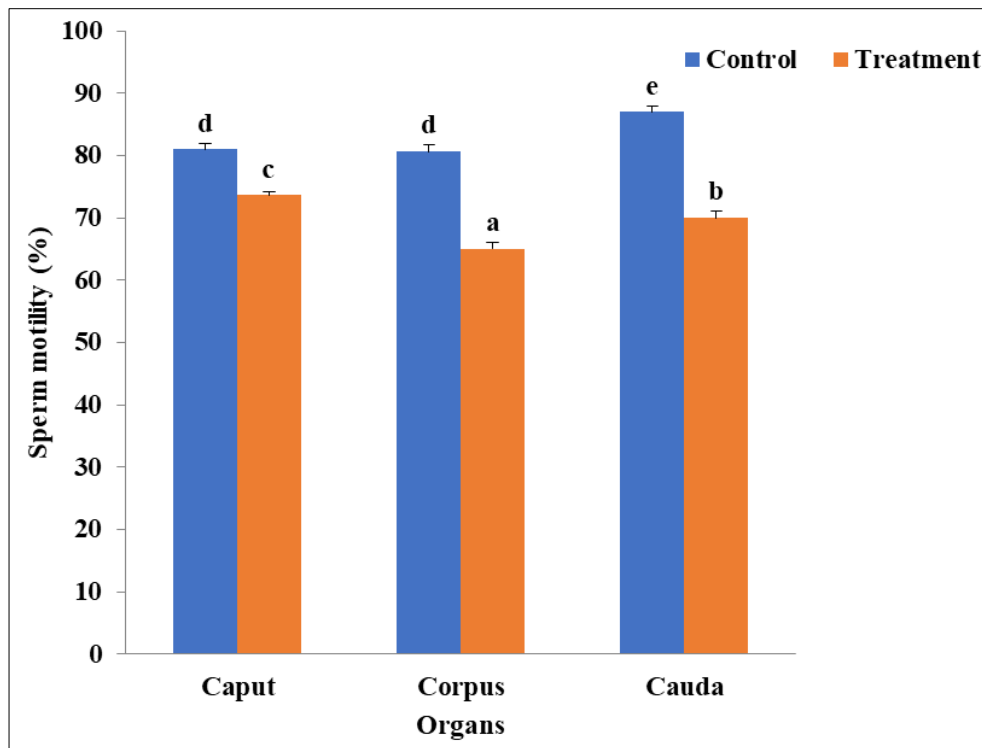


Fig 3: Sperm motility (%) of male albino rat after quineestrol treatment

Table 4: Effect of quineestrol treatment on sperm abnormality (%) after 10 days of treatment withdrawal

Groups / Organs	Control	Treatment
Caput	10.00±1.00 ^b	15.33±0.58 ^a
Corpus	9.33±0.57 ^a	14.33±0.58 ^b
Cauda	15.00±1.00 ^d	30.66±1.52 ^{bc}

All values are expressed as mean ±S.E.; n=6

Mean values with different alphabetical superscripts indicate significant difference using ($p \leq 0.05$)

Tukey's test

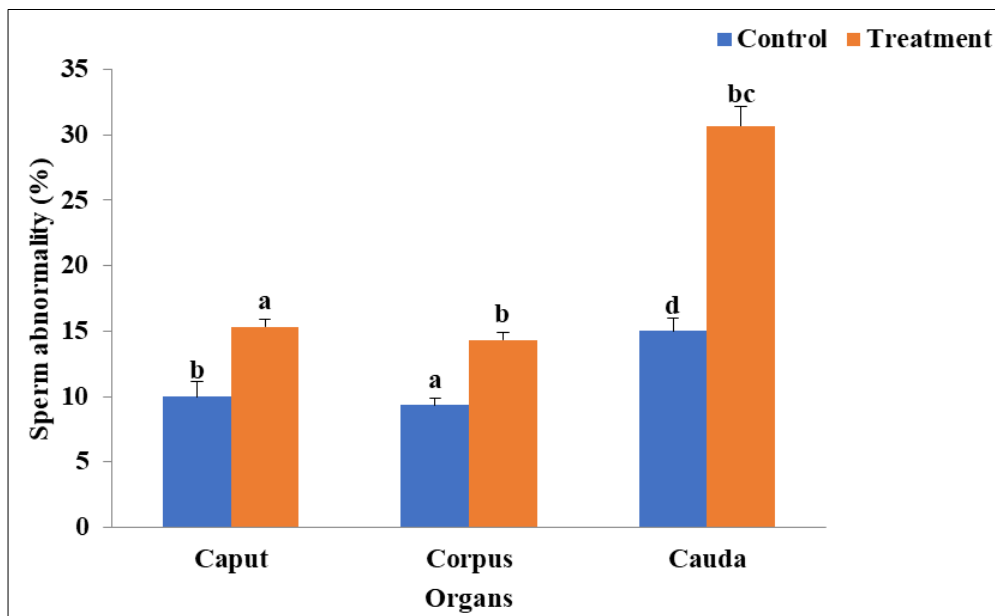


Fig 4: Sperm abnormality (%) of male albino rat after quineestrol treatment

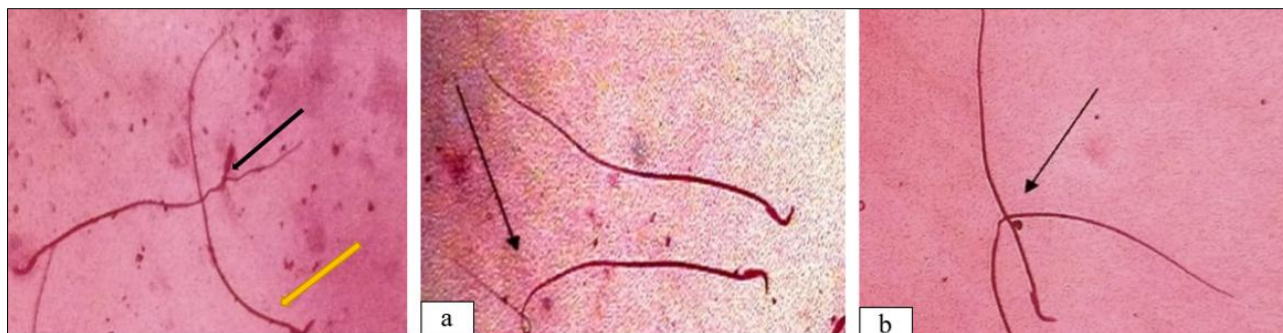


Fig 4a: Sperm abnormality of male albino rat after quinestrol treatment

- A nigrosine-eosin stained smear of cauda epididymal fluid at 400x magnification
 - The black arrow represents an abnormality in spermatozoa (tail deformity).
 - Yellow arrow represents normal spermatozoa
 - The black arrow represents an abnormality in spermatozoa
- a) curved tail
b) coiled tail

Table 5: Effect of quinestrol treatment on sperm viability (%) after 10 days of treatment withdrawal

Groups / Organ	Control	Treatment
Caput	78.66±1.00 ^a	72.33±1.52 ^a
Corpus	63.33±0.09 ^a	57.66±1.52 ^b
Cauda	82.33±1.25 ^b	79.66±0.58 ^c

All values are expressed as mean ±S.E.: n=6

Mean values with different alphabetical superscripts indicate significant difference using ($p \leq 0.05$) Tukey's test

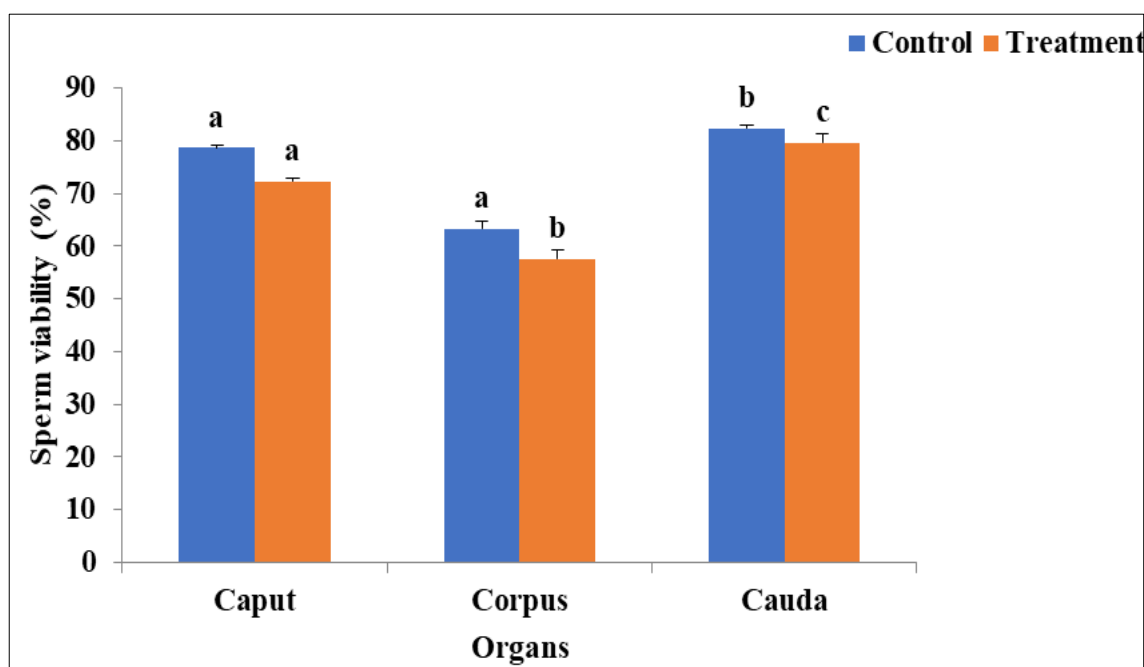


Fig 5: Sperm viability (%) of male albino rat after quinestrol treatment

Table 6: Effect of quinestrol treatment on sperm concentration (millions/ml) after 10 days of treatment withdrawal

Groups / Organ	Control	Treatment
Caput	81.00±1.00 ^a	69.00±1.54 ^b
Corpus	76.00±1.54 ^a	58.00±0.58 ^b
Cauda	89.36±1.54 ^b	71.00±1.54 ^c

All values are expressed as mean ±S.E; n=6

Mean values with different alphabetical superscripts indicate significant difference using ($p \leq 0.05$) Tukey's ztest

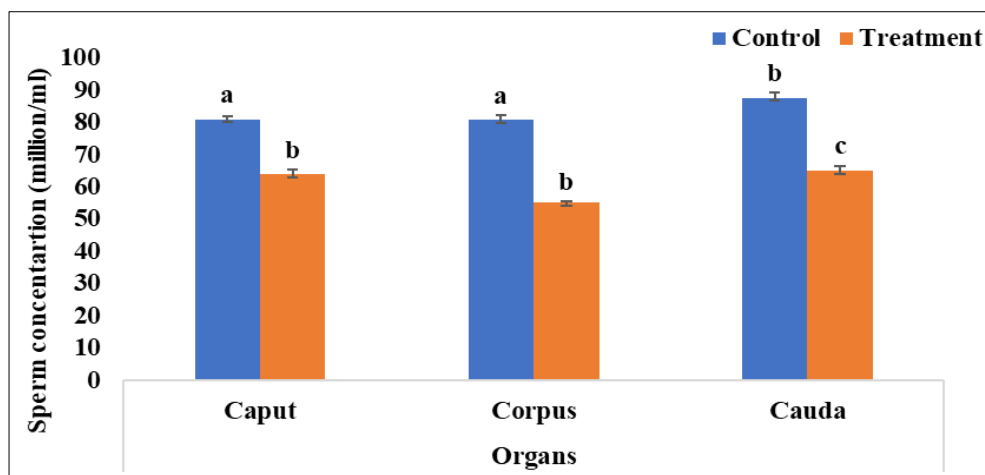


Fig 6: Sperm concentration (million/ml) of male albino rat after quineestrol treatment

Biochemical parameter in testis and epididymis

Total lipids content (TLC)

The results of TLC in testis and epididymis were presented in table 7 and figure 7. In the testis, the TLC in control rats was observed to be 16.36 ± 1.00 mg/g tissue weight whereas a significant decline was observed in the TLC in the treatment group rats i.e., 10.24 ± 0.05 mg/g tissue weight as compared to control group. Similarly, the TLC in the epididymal tissue (caput, corpus, cauda) of control rats was 25.03 ± 0.49 , 17.31 ± 0.24 , 14.89 ± 0.30 mg/g tissue weight. The TLC in the epididymis of rats treated with quineestrol were (caput, corpus, cauda) 14.35 ± 0.01 , 12.34 ± 0.01 , 6.39 ± 0.01 mg/g tissue weight. This showed that there was a significant difference between the control and rats treated with

quineestrol for dose of 2 mg/Kg body weight for a period of 10 days.

Table 7: Total Lipid content (mg/g tissue weight) in tissue homogenate of testis and epididymis

Groups / Organs	Control	Treatment
Testis	16.36 ± 1.00^{de}	10.24 ± 0.05^b
Caput	25.03 ± 0.49^f	14.35 ± 0.01^{cd}
Corpus	17.31 ± 0.24^e	12.34 ± 0.01^{bc}
Cauda	14.89 ± 0.30^{cd}	6.39 ± 0.01^a

All values are expressed as mean $\pm$ S.E.; n=6.

Mean values with different alphabetical superscripts indicate significant difference using ($p \leq 0.05$) Tukey's test

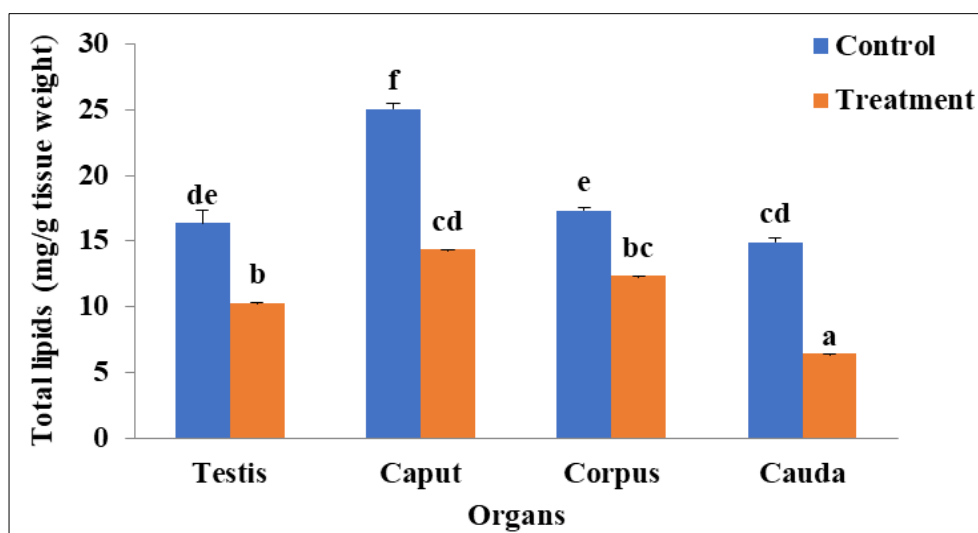


Fig 7: Total lipid content in testis and epididymis in rats after treatment with quineestrol

Phospholipid content

The phospholipid content was observed in the testis and

epididymis of rats from control and treated groups and were presented in table 8 and figure 8.

Table 8: Phospholipid content (mg/g tissue weight) in tissue homogenate of testis and epididymis

Groups / Organ	Control	Treatment
Testis	12.29 ± 0.20^e	9.07 ± 0.09^d
Caput	1.55 ± 0.27^c	0.31 ± 0.01^{ab}
Corpus	0.67 ± 0.02^{ab}	0.19 ± 0.04^a
Cauda	0.85 ± 0.05^{bc}	0.14 ± 0.02^a

All values are expressed as mean $\pm$ S.E.; n=6

Mean values with different alphabetical superscripts indicate significant difference using ($p \leq 0.05$) Tukey's test

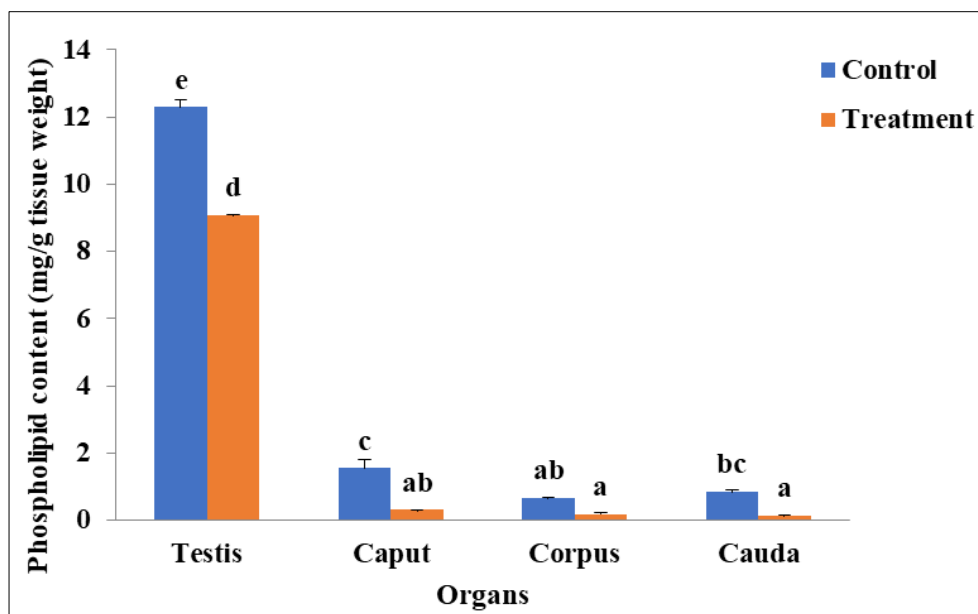


Fig 8: Phospholipid content in testis and epididymis in rats after treatment with quineestrol

In the control rat testis, the phospholipid content was observed to be 12.29 ± 0.20 mg/g tissue weight and in the rats, treated with quineestrol showed decrease in the phospholipid content i.e. 9.07 ± 0.09 mg/g tissue weight as compared to control group. The data showed that there was a significant difference between the control and rats treated with quineestrol at the dose of 2 mg/Kg for a period of days. Similarly, in the epididymis (caput, corpus, cauda) the phospholipid content was examined in the control group i.e. 1.55 ± 0.27 , 0.67 ± 0.02 , 0.85 ± 0.05 mg/g tissue weight. A significant decrease was observed in the group treated with quineestrol with dose of 2mg/Kg body weight i.e. 0.31 ± 0.01 , 0.19 ± 0.04 , 0.14 ± 0.02 mg/g tissue weight as compared to control group.

Total Cholesterol

The cholesterol content was observed in the testis and epididymis of control and treated rats and were presented in table 9 and figure 9.

In the testis, the cholesterol level in the control rats was 2.03 ± 0.10 mg/g tissue weight the treatment group showed a

significant increase in cholesterol level i.e. 6.03 ± 0.04 mg/g tissue weight as compared to control groups. Similarly, in the epididymis the cholesterol level was examined among the control group i.e. 0.68 ± 0.06 , 0.92 ± 0.05 , 0.53 ± 0.07 mg/g tissue weight. In the rats treated with quineestrol the cholesterol content was 4.30 ± 0.03 , 3.31 ± 0.08 , 2.65 ± 0.04 mg/g tissue weight which was significantly higher than control values. The results of present study indicated that after quineestrol administration the level of cholesterol increases.

Table 9: Cholesterol content (mg/g tissue weight) in tissue homogenate of testis and epididymis

Groups / Organs	Control	Treatment
Testis	2.03 ± 0.10^c	6.03 ± 0.04^g
Caput	0.68 ± 0.06^a	4.30 ± 0.03^f
Corpus	0.92 ± 0.05^b	3.31 ± 0.08^e
Cauda	0.53 ± 0.07^a	2.65 ± 0.04^d

All values are expressed as mean $\pm$ S.E.; n=6

Mean values with different alphabetical superscripts indicate significant difference using ($p \leq 0.05$) Tukey's test

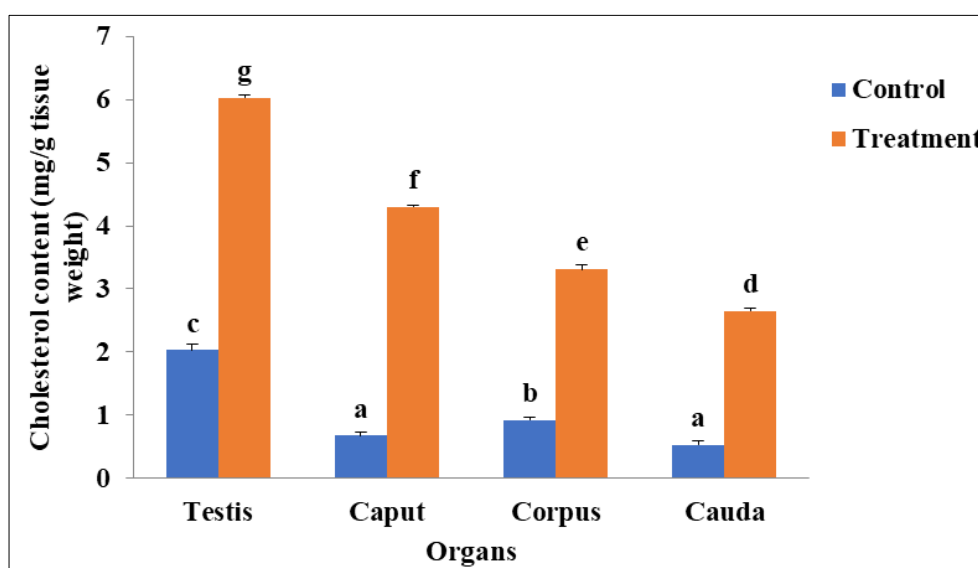


Fig 9: Cholesterol content in testis and epididymis in rats after treatment with quineestrol

Total soluble proteins content

Total soluble protein content was observed in testis and epididymis of control and treated group and were presented in shown in table 10 and figure 10. In the testis the total soluble protein content in control rats was 20.64 ± 0.06 mg/g tissue weight whereas a significant decrease was observed in total soluble protein content in the treatment group i.e. 9.33 ± 0.13 mg/g tissue weight, respectively. Similarly, in the epididymis of the control rats (caput, corpus, cauda) the total soluble protein content was 9.36 ± 0.17 , 6.23 ± 0.01 , 13.65 ± 0.03 mg/g tissue weight whereas in the group treated with quineestrol it was 4.25 ± 0.03 , 3.83 ± 0.01 and 9.22 ± 0.03 mg/g tissue weight, respectively, which showed a significant difference in the Total soluble protein content between control and treated group. From the statistical data it was

observed that all the organs were significantly affected by quineestrol.

Table 10: Total soluble protein content (mg/g tissue weight) in tissue homogenate of testis and epididymis

Groups / Organs	Control	Treatment
Testis	20.64 ± 0.06^a	9.33 ± 0.13^b
Caput	9.36 ± 0.17^c	4.25 ± 0.03^d
Corpus	6.23 ± 0.01^e	3.83 ± 0.01^f
Cauda	13.65 ± 0.03^g	9.22 ± 0.03^h

All values are expressed as mean $\pm$ S.E.; n=6

Mean values with different alphabetical superscripts indicate significant difference using ($p \leq 0.05$) Tukey's test

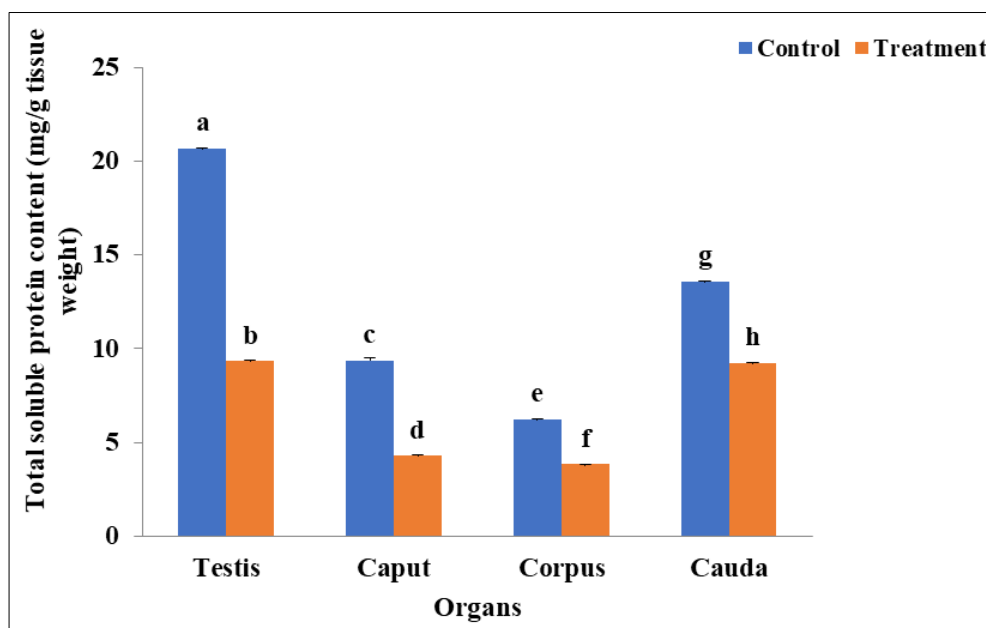


Fig 10: Total soluble protein content in testis and epididymis in rats after treatment with quineestrol

Discussion

The present study aimed to assess the impact of quineestrol treatment on body weight, reproductive organ weight, sperm parameters, total lipid content, cholesterol levels, and protein content in male albino rats. Our findings indicate that quineestrol treatment did not significantly alter the body weight of the rats throughout the study period (0, 5, and 10 days). This observation aligns with previous studies, such as those conducted by Li *et al.* (2014) [39] and Liu *et al.* (2013) [21], which reported no significant changes in body weight in quineestrol-treated male rats. However, Kang *et al.* (2022) [18] observed a decrease in body mass index in rats treated with EP-1 and E groups, suggesting species or dose-specific variations in quineestrol's effects on body weight.

Despite the lack of effect on overall body weight, quineestrol treatment significantly reduced the weight of reproductive organs, including the testis and epididymis (caput, corpus, and cauda). This reduction suggests that quineestrol disrupts normal reproductive physiology, likely through its impact on androgen production. The decrease in testis weight aligns with previous studies demonstrating the suppressive effects of estrogenic compounds on Leydig cell function and testosterone secretion (Zhang *et al.*, 2005) [32]. Similar reductions in reproductive organ weights have been reported in various rodent species, including Brandt's voles (Zhang *et al.*, 2004, 2005, 2006) [5, 34], greater long-tailed hamsters

(Zhao *et al.*, 2007) [35], and male plateau pikas (Liu *et al.*, 2011) [22]. The decline in testis weight following quineestrol treatment may be attributed to its suppressive effects on the hypothalamic-pituitary-gonadal axis, reducing luteinizing hormone and follicle-stimulating hormone secretion, thereby impairing testosterone production and spermatogenesis (Amroy *et al.*, 2006; Chen *et al.*, 2021) [1, 10]. The disruption of the hypothalamic-pituitary-gonadal axis results in negative feedback inhibition, reducing testosterone synthesis and affecting sperm maturation in the epididymis.

In addition to reductions in reproductive organ weight, sperm parameters were significantly affected by quineestrol treatment. Sperm motility and viability decreased, while sperm abnormalities increased, indicating that quineestrol disrupts spermatogenesis and leads to poor sperm quality. Carreau and Hess (2010) [7] demonstrated that excessive estrogen disrupts spermatogenesis and negatively impacts male fertility. Similarly, Adeoya-Osuguwa *et al.* (2003) [2] reported that 17- β estradiol and environmental estrogens impair mammalian sperm function. High levels of reactive oxygen species (ROS) are associated with increased apoptosis in spermatogenic cells, further contributing to poor sperm quality (Massawe *et al.*, 2018) [24]. Su *et al.* (2019) [30] also confirmed that suppression of the hypothalamic-pituitary-gonadal axis is a key factor leading to reduced sperm concentration and motility.

Furthermore, the present study demonstrated that quineestrol administration significantly affects total lipid content, cholesterol levels, and protein content in the testis and epididymis of male albino rats. A notable decline was observed in total lipid and phospholipid content in the testis and epididymis of quineestrol-treated rats compared to the control group. The reduction in total lipid content can be attributed to oxidative stress which plays a crucial role in lipid peroxidation and cellular damage. The testicular and sperm plasma membranes, rich in polyunsaturated fatty acids, are highly susceptible to reactive oxygen species which disrupt lipid integrity, leading to cell damage and decreased lipid levels. These findings align with previous studies indicating that quineestrol induces oxidative damage in male reproductive organs, affecting sperm membrane composition and function. Additionally, the phospholipid decline in treated rats may indicate the degradation of cellular membranes, signifying loss of cellular integrity and function.

Furthermore, cholesterol levels were significantly elevated in the testis and epididymis of quineestrol-treated rats. Cholesterol serves as a precursor for steroidogenesis, and its accumulation may suggest an impairment in steroid hormone synthesis due to oxidative stress-induced disruption of enzymatic activity, particularly 17 β -HSD, which plays a key role in testosterone biosynthesis. This alteration in cholesterol metabolism is consistent with findings from prior research, suggesting that exogenous estrogen administration affects hepatic cholesterol processing and steroidogenesis, leading to an increase in cholesterol levels. Moreover, total soluble protein content exhibited a marked decline in the testis and epididymis of quineestrol-treated rats. This decrease may be associated with protein oxidation caused by excessive reactive oxygen species which disrupts protein synthesis and degrades structural proteins, further impairing reproductive function. The ability of quineestrol to induce testicular atrophy, sperm cell death, and damage to the testis and epididymis suggests that its contraceptive effects result from both direct impairment of testicular function and indirect suppression of gonadotropins. Quineestrol's role in suppressing the hypothalamic-pituitary-gonadal axis has been well documented (Gill-Sharma *et al.*, 2001; Liu *et al.*, 2013) [14, 21]. It has also been shown to cause azoospermia by disrupting seminiferous tubule cytoarchitecture, leading to long-term infertility effects (Massawe *et al.*, 2018) [24]. Sidhu *et al.* (2020) [27] similarly reported significant reductions in sperm motility, viability, and density in quineestrol-treated animals, accompanied by increased sperm abnormalities.

Conclusion

In conclusion, the present study confirms that quineestrol administration significantly affects reproductive organ weight, sperm parameters, total lipid content, cholesterol levels, and total soluble protein content without altering overall body weight. The suppression of the hypothalamic-pituitary-gonadal axis leading to decreased testosterone synthesis and impaired spermatogenesis, is the likely mechanism underlying these effects. These findings reinforce quineestrol's potential as an effective male contraceptive. However, further research is required to assess the long-term effects of quineestrol treatment and its potential for reversibility in reproductive function.

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