Journal of Infertility and Reproductive Biology

Journal of Infertility and Reproductive Biology, 2025, Volume 13, Issue 4, Pages: 166-174

Co-administration of Atorvastatin with Piperine Induced Reproductive Toxicity in Male Wistar Rats: Histological, Biochemical, Hormonal and Sperm Parameter-Based Evidences

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Received: 30/08/2025 Accepted: 04/11/2025 Published: 20/12/2025

Abstract

Atorvastatin (ATR) is a synthetic competitive inhibitor of 3-hydroxy-3-methyl-glutaryl-coenzyme A. A reductase that impedes the rate-limiting phase of cholesterol production. Piperine (PIP) is an alkaloid present in the fruits of black pepper (Piper nigrum), a widely utilised and commonly employed spice. Piperine enhances the absorption of atorvastatin, and both substances are recognised for their male antifertility effects. The purpose of this study was to investigate the effects of co-administration of atorvastatin and piperine on the reproductive capacity of male Wistar rats. We acquired twenty male rats and partitioned them into four groups, each comprising five rats. Group I: control; Group II: provided atorvastatin (8 mg/kg BW); Group III: administered piperine (10 mg/kg BW); Group IV: co-administered atorvastatin (8 mg/kg BW) and piperine (10 mg/kg BW) for a duration of 28 consecutive days. The results of our investigation indicated that the co-administration of piperine and atorvastatin modified the histoarchitectural structures of the seminal vesicles, epididymis, and seminiferous tubules, and significantly reduced the weight of these reproductive organs. In rats administered atorvastatin and piperine concurrently, there was a substantial ($p < 0.05$) reduction in sperm count and viability. ELISA analysis of blood testosterone levels indicated a substantial reduction in rats co-treated with ATR and PIP. The co-administration of ATR and PIP resulted in reproductive toxicity via influencing hormonal, metabolic, histological, and sperm-related parameters.

Keywords: atorvastatin, piperine, testosterone, toxicity, fertility

1. Introduction

Male infertility is increasingly recognized as a major global reproductive health issue. Its etiology is multifactorial, involving both intrinsic and extrinsic determinants. Lifestyle-related and environmental exposures, such as tobacco use, psychological stress, excessive body weight, alcohol intake, and contact with pesticides or heavy metals, have been strongly associated with impaired male fertility [1]. In addition to these factors, internal causes including structural defects of the reproductive system, hormonal imbalances, and genetic abnormalities contribute significantly to male infertility. External influences, encompassing nutritional patterns, drug exposure, environmental conditions, and daily lifestyle choices, further exacerbate reproductive dysfunction [2]. Nevertheless, these modifiable external factors are often underemphasized during routine infertility assessments. Statins constitute a class of lipid-modifying drugs extensively prescribed for the prevention and management of cardiovascular disorders. Their primary mechanism of action involves competitive inhibition of 3-hydroxy-3-methylglutaryl–coenzyme A (HMG-CoA) reductase, leading to reduced endogenous cholesterol biosynthesis [3, 4]. Cholesterol plays a pivotal role in male reproductive physiology, as it is indispensable for steroid hormone production, normal spermatogenesis, and successful fertilization [5]. It acts as the fundamental substrate for testosterone synthesis, a hormone essential for the maintenance, proliferation, and differentiation of spermatogonial cells [6]. Moreover, cholesterol is closely involved in regulating sperm motility and capacitation [7]. Disruptions in lipid homeostasis have therefore been closely linked to compromised male fertility [8]. Atorvastatin (ATR), one of the most frequently prescribed statins, has been reported in multiple studies to exert detrimental effects on male reproductive function [9–11]. Owing to their cholesterol-lowering properties, statins are suspected to interfere with steroidogenic pathways in males [12, 13].

Piperine is a naturally occurring alkaloid derived primarily from black pepper (Piper nigrum L.), a spice consumed extensively across the globe [14]. It exhibits a broad spectrum of pharmacological activities and has been widely incorporated into traditional medicinal systems, particularly in Asian cultures [15].

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One of its notable properties is the enhancement of bioavailability of various therapeutic agents and micronutrients in both animal models and humans [16]. Piperine has also demonstrated hypolipidemic effects [17]. However, several experimental studies have highlighted its adverse influence on male reproduction. Mishra and Singh (2009) reported marked degenerative changes in testicular architecture, including the seminiferous tubules and epididymis, accompanied by altered sperm characteristics in piperine-treated male rats [18].

Similarly, administration of ethanol extracts of black pepper fruits resulted in a reduction in primary spermatocyte numbers and diminished sperm motility [19]. Earlier work by Malini et al. (1999) corroborated these antispermatogenic effects [20], and subsequent investigations have shown that piperine can induce apoptotic pathways in testicular tissue [21]. Despite the extensive consumption of phytochemicals through diet and herbal formulations, systematic investigations into their interactions with pharmaceutical agents remain limited. A deeper understanding of the interplay between drugs, dietary components, and plant-derived bioactive compounds is essential for optimizing therapeutic outcomes, improving drug responsiveness in diverse populations, and advancing personalized medicine. Herb–drug interactions (HDIs) represent a significant clinical concern, particularly when herbal products are used concomitantly with conventional medications, as such interactions may alter pharmacokinetics, efficacy, or toxicity profiles [22,23].

Although statins have been repeatedly implicated in adverse reproductive outcomes and piperine is well documented for its antispermatogenic activity, both agents share lipid-lowering properties. While previous studies have independently evaluated the reproductive toxicity of atorvastatin and piperine, evidence regarding their combined effects is lacking. Therefore, the present study aims to investigate the reprotoxic consequences of concurrent administration of atorvastatin and piperine in male rats.

2. Materials and Methods

2.1. Drugs and chemicals

Piperine and Sudan Black were procured from Sigma-Aldrich (Germany). Atorvastatin used in the present study was supplied by Lupin Ltd. (India). Enzyme-linked immunosorbent assay (ELISA) kits were obtained from Accu-Bind, Monobind Inc. (California, USA), while lipid profile assay kits were purchased from Robonik Pvt. Ltd. (India). Eosin, nigrosine, and phosphate-buffered saline (PBS) were sourced from SRL (India). All additional chemicals and reagents employed in this study were of analytical grade and of the highest available purity.

2.2 Animal Care

The study was conducted using healthy adult male Wistar rats aged approximately 70–80 days. The animals were procured from the Centre for Laboratory Animal Research and Training, West Bengal, India. Throughout the experimental period, rats were maintained under controlled laboratory conditions, including an ambient temperature of 24–26 °C, relative humidity of 50–70%, and a 12 h light/12 h dark photoperiod. Prior to experimentation, the animals were allowed to acclimatize to the laboratory environment for a period of two weeks. All experimental procedures involving animals were reviewed and approved by the Institutional Animal Ethics Committee (IEAC), University of Kalyani, in accordance with CPCSEA guidelines (Approval No. 892/GO/Re/S/01/CPCSEA).

2.3. Study design

A total of twenty male Wistar rats were included in the experimental protocol, which was conducted over a period of 28 days. The animals were randomly assigned to four groups, with five rats in each group (n = 5). The control group (C) received a standard diet without any pharmacological intervention. Rats in the second group (ATR) were administered atorvastatin at a dose of 8 mg/kg body weight. The third group (PIP) was treated with piperine at a daily dose of 10 mg/kg body weight. Animals in the fourth group (ATR + PIP) received a combined treatment of atorvastatin (8 mg/kg body weight) and piperine (10 mg/kg body weight) once daily for 28 consecutive days. All treatments were delivered via oral gavage.

2.4. Animal sacrifice and sample collection

At the end of the experimental period, all animals were anesthetized via intraperitoneal administration of ketamine (80 mg/kg) and xylazine (10 mg/kg). Following euthanasia, both testes were carefully excised and stored at −20 °C for subsequent biochemical analyses. Prior to sacrifice, blood samples were collected from each animal into heparinized tubes; serum was separated by centrifugation and preserved at −20 °C for further biochemical evaluation. Epididymal spermatozoa were collected in phosphate-buffered saline (PBS) and maintained at 37 °C in an incubator for the assessment of sperm parameters. After testicular dissection, the epididymis, seminal vesicles, and prostate glands were thoroughly cleaned of adherent tissues and weighed. All collected tissues were then fixed in Bouin's solution for histopathological examination.

2.5. Sperm count and sperm viability assessment

Following dissection, the epididymis was carefully cleared of surrounding connective tissue, and the caudal portion was isolated and sectioned into smaller fragments. Small pieces of the cauda epididymis were transferred to a Petri dish containing 2 mL of 1× phosphate-buffered saline (PBS). To release spermatozoa into the medium, the caudal tissue was gently punctured using a sterile needle. The Petri dish was then incubated at 37 °C under 5% CO_2 for 10 minutes to allow active migration of sperm from the epididymal tubules. The resulting suspension was subsequently used for the evaluation of sperm count and viability under a ZEISS Primo Star microscope at 40× magnification. Sperm concentration was determined using a Neubauer hemocytometer [24], following dilution of the epididymal suspension at a 1:5 ratio with PBS. Sperm viability was assessed using Eosin Y (0.05%) and nigrosine staining [25]. Equal volumes (20 µL each) of sperm suspension and Eosin–nigrosine stain were mixed and incubated at room temperature for 2 minutes prior to analysis.

2.6. Haematoxylin-Eosin staining of reproductive organs

Following removal of the reproductive organs (testes, epididymides, seminal vesicles, and prostate glands) from both control and treated animals, the testes were carefully isolated and fixed overnight at 4 °C in 10% neutral buffered formalin. The fixed tissues were subjected to graded dehydration using ascending concentrations of ethanol (70%, 80%, 90%, and 100%), followed by clearing in absolute xylene and embedding in paraffin wax. Serial tissue sections of approximately 7 µm thickness were prepared using a rotary microtome. Hematoxylin and eosin (H&E) staining was subsequently performed according to standard histological procedures. Stained sections were examined and imaged using a Zeiss Primo Star microscope.

2.7. Assessment of Serum Lipid Profile

Serum lipid parameters, including total cholesterol, triglycerides, low-density lipoprotein (LDL), very-low-density lipoprotein (VLDL), and high-density lipoprotein (HDL) cholesterol, were quantified using colorimetric assays with a commercially available lipid profile test kit obtained from Robonik Pvt. Ltd. (India).

2.8. Measurement of epididymal sialic acid

Cauda epididymal tissue was homogenized in phosphate-buffered saline (PBS; 0.1 M, pH 7.4) at a concentration of 50 mg/mL. The homogenates were hydrolyzed with 0.1 N sulfuric acid (H_2SO_4) and subsequently centrifuged at 3000 rpm for 10 minutes. Following incubation at 80 °C for 1 hour, 0.5 mL of the resulting supernatant was transferred to a clean test tube and mixed with 0.2 mL of sodium periodate solution (0.2 M sodium periodate prepared in 9 M phosphoric acid). The mixture was gently agitated and allowed to stand at 20 °C for 20 minutes. Thereafter, 1.0 mL of sodium arsenite solution (10% sodium arsenite in 0.1 N H_2SO_4 containing 0.5 M sodium sulfate) was added, resulting in the disappearance of the initially developed dark coloration upon mixing. Subsequently, 3.0 mL of thiobarbituric acid (TBA) reagent (0.6% TBA in 0.5 M sodium sulfate) was introduced, and the reaction mixture was heated in a boiling water bath for 15 minutes. After cooling, 4.5 mL of cyclohexanone was added, followed by centrifugation to obtain a clear pink-colored upper layer. The absorbance of the chromogen was measured at 550 nm using a spectrophotometer. Sialic acid concentration was calculated according to the method described by Warren [25] and expressed as µmol/g of tissue based on standard calibration.

2.9. Evaluation of testicular glycogen levels

Testicular glycogen content was estimated following the method described by Nicholas et al. [26]. Briefly, 50 mg of testicular tissue was homogenized in 1 mL of 5% trichloroacetic acid (TCA) and centrifuged at 3000 rpm for 15 minutes. An aliquot of 1 mL of the resulting supernatant was mixed with 5 mL of 95% ethanol in a centrifuge tube and incubated in a water bath maintained at 37–40 °C for 3 hours to facilitate glycogen precipitation. The mixture was then centrifuged at 3000 rpm for 15 minutes, after which the supernatant was carefully discarded. The glycogen pellet was allowed to drain by keeping the tubes inverted for 10 minutes and subsequently dissolved in 2 mL of distilled water to obtain the test sample. Distilled water (2 mL) served as the blank, while the standard consisted of 2 mL of a glucose solution containing 0.1 mg glucose. To each tube, 10 mL of freshly prepared anthrone reagent (0.05% anthrone, 1% thiourea, and 72% sulfuric acid, v/v) was added with thorough mixing. The tubes were first placed in cold water and then heated in a boiling water bath for 15 minutes. Following cooling to room temperature in a cold-water bath, absorbance was measured at 620 nm using a spectrophotometer. Glycogen concentration was calculated by comparing sample absorbance with that of the standard and expressed as mg/g of tissue.

2.10. ELISA for quantifying testicular testosterone

Serum testosterone levels were quantified using a commercially available enzyme-linked immunosorbent assay (ELISA) kit, following the manufacturer's protocol (Accu-Bind, Monobind Inc., California, USA).

2.11. Statistical analysis

Statistical analyses were conducted using GraphPad Prism software (GraphStats Technologies, USA). Group-wise differences were evaluated using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test for multiple comparisons. Results are presented as mean ± standard error of the mean (SEM), and differences were considered statistically significant at $p < 0.05$.

3. Results

3.1. Changes in relative weight of reproductive organs

Changes in the relative weights of reproductive organs in control and treated rats (ATR, PIP, and ATR+PIP) are presented in Table 1. A statistically significant reduction in testicular weight ($p < 0.05$) was observed in all treatment groups when compared with the control, with the most pronounced decrease detected in the ATR+PIP co-treated group. Epididymal weight was also significantly reduced in each treatment group relative to the control animals. Similarly, a marked decline in seminal vesicle weight was noted across all treated groups ($p < 0.05$). In contrast, prostate gland weight did not differ significantly in the ATR- or PIP-treated groups; however, rats receiving the combined ATR+PIP treatment exhibited a significant reduction in prostate weight ($p < 0.05$).

3.2. Sperm count and viability

Sperm count and viability were evaluated across all experimental groups, as illustrated in Fig. 1. Relative to the control group, no significant change in sperm count was observed in the ATR-treated rats; however, a significant decline in epididymal sperm viability ($p < 0.05$) was detected. In contrast, rats treated with piperine (PIP) showed a marked reduction in both sperm count and viability ($p < 0.05$) compared with controls. The combined treatment group (ATR+PIP) exhibited a significant decrease ($p < 0.05$) in sperm count as well as sperm viability when compared to the control group.

Table 1: Relative weight changes of reproductive organs

	Control	ATR	PIP	ATR+PIP
Testis weight (gm)	1.226 ± 0.020	1.096 ± 0.028*	1.016 ± 0.028*	0.856 ± 0.024*
Epididymis weight (gm)	0.436 ±0.022	0.350 ± 0.007*	0.384 ± 0.014*	0.322 ± 0.012*
Seminal vesicle weight (gm)	0.592 ± 0.015	0.326 ± 0.012*	0.468 ± 0.014*	0.302 ± 0.018*
Ventral prostate gland weight (gm)	0.144 ± 0.009	0.136 ± 0.011	0.136 ± 0.009	0.096 ± 0.006*

Values are expressed as mean ± SEM, n = 5. Significance levels are denoted as * ($p < 0.05$). All the treated groups are compared with the Control group

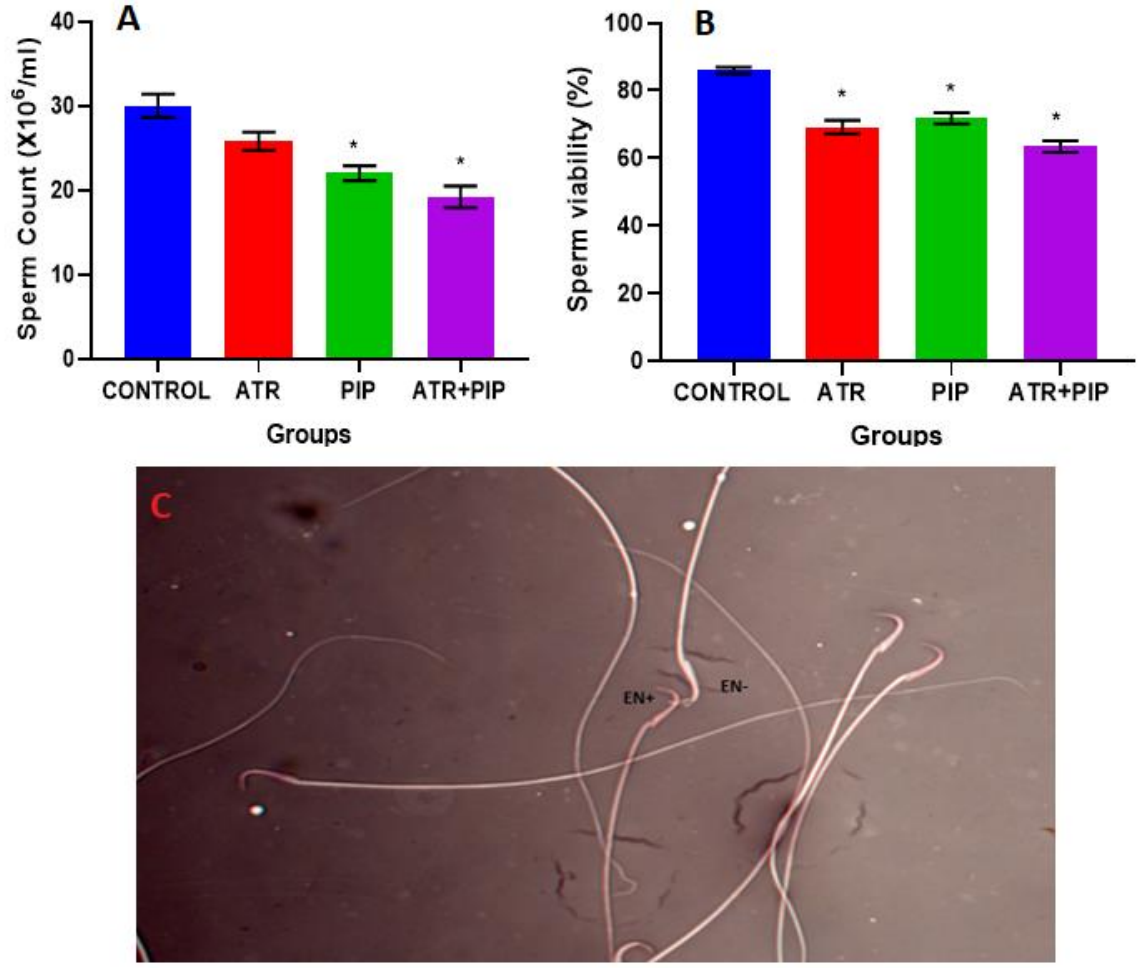

Figure 1: Sperm count and sperm viability changes. Values are expressed as mean ± SEM, n = 5. Significance levels are denoted as * ($p < 0.05$). Eosin-Nigrosin stained sperms. Live sperms are marked as EN- and dead sperms are marked as EN+.

3.3 Alterations in the histopathology of reproductive organs

Histological examination of testicular sections from control animals revealed normal architecture, characterized by well-organized seminiferous tubules containing spermatogonia, Sertoli cells, Leydig cells, and intact vascular structures. In contrast, rats receiving combined ATR and PIP treatment exhibited mild atrophy of the seminiferous tubules along with slight interstitial edema. Enlargement of the seminiferous tubular lumen was evident in all treatment groups when compared with the control group (Fig. 2). Epididymal sections from control animals displayed normal morphology, with compactly arranged tubules and a dense accumulation of spermatozoa within the lumen of the cauda epididymis. However, rats treated with ATR alone or in combination with PIP demonstrated a marked reduction in luminal sperm content (Fig. 2). Histopathological analysis of seminal vesicle sections from the control group showed typical structural features, including a columnar epithelial lining and abundant luminal secretions. In treated animals, particularly those receiving combined ATR and PIP,

moderate epithelial hyperplasia was observed. Examination of the ventral prostate in control rats revealed tubules with normal epithelial height, whereas a reduction in epithelial thickness was noted across all treatment groups. Interstitial edema was most pronounced in the ATR+PIP group and was comparatively less evident in the ATR- and PIP-treated groups relative to controls (Fig. 2). Additionally, the ATR+PIP co-treated group exhibited mild contraction of prostatic acini accompanied by partial disruption of the epithelial lining.

3.4. Alterations in serum lipid composition

Analysis of the serum lipid profile revealed treatment-related changes in cholesterol and triglyceride concentrations across all experimental groups. Serum total cholesterol levels were significantly decreased ($p < 0.05$) in rats receiving combined ATR and PIP treatment, as well as in animals treated with ATR or PIP alone. Triglyceride concentrations were also significantly reduced ($p < 0.05$) in all treatment groups when compared with the control group. In contrast, high-density lipoprotein (HDL) levels remained unchanged across the treated groups relative to controls (Table 2).

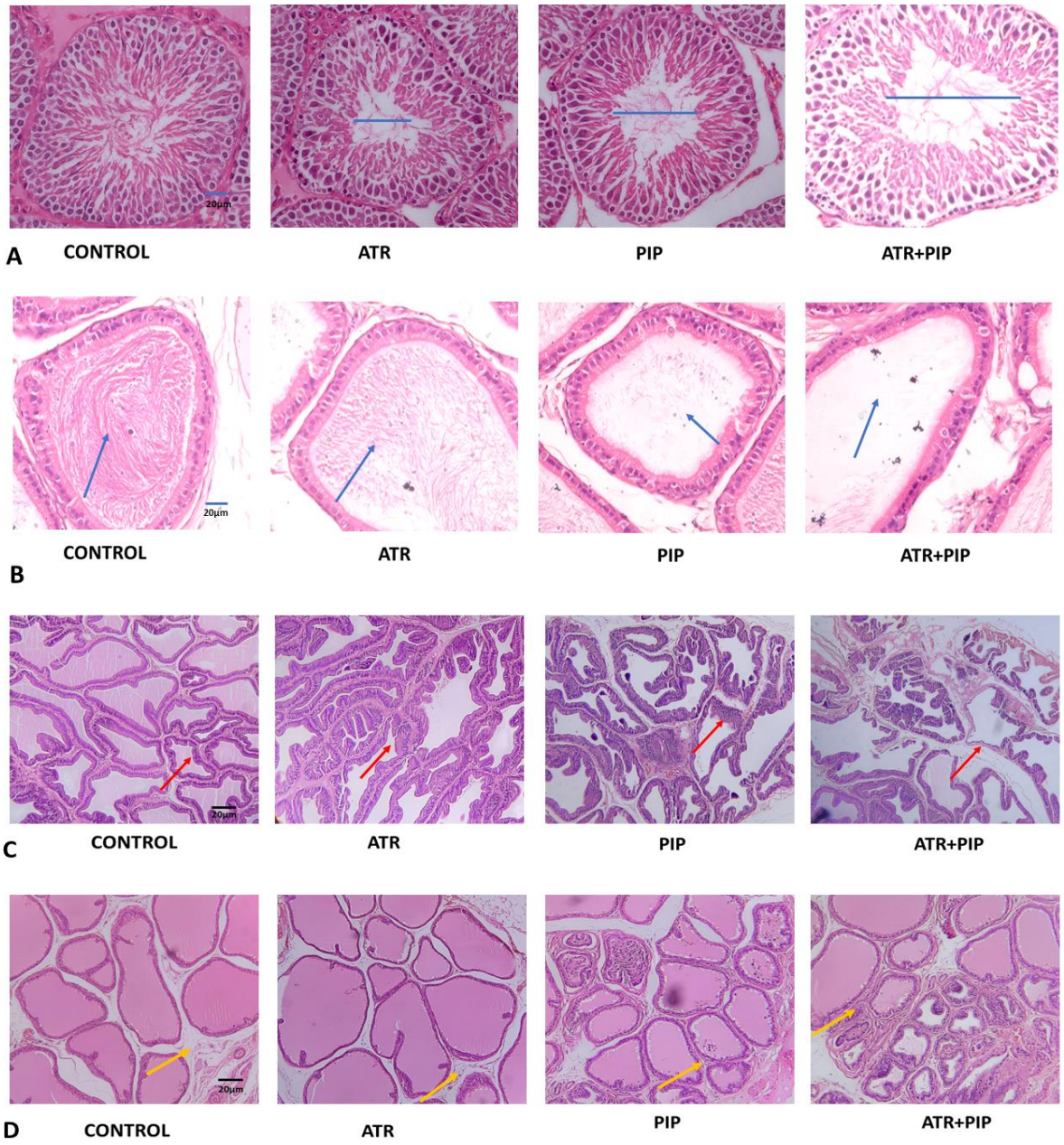

Figure 2: H&E staining of reproductive organs of all experimental groups. A. Testis (blue arrows showing tubular lumen diameter changes), B. Epididymis (arrows showing gradual decrease in lumen spermatozoa), C. Seminal vesicle (red arrows for showing changes in epithelium wall), D. Prostate glands (yellow arrows showing changes in inter luminary spaces). Histological observations done in 40X magnification.

Table 2: Changes in lipid profile

	Control	ATR	PIP	ATR+PIP
Cholesterol (mg/dl)	109.4 ± 5.74	82.4 ± 5.52*	89.2 ± 2.85*	64.4 ± 2.60*
Triglycerides (mg/dl)	118.2 ± 5.15	81.2 ± 2.87*	84.8 ± 5.05*	56.6 ± 2.50*
HDL (mg/dl)	33.8 ± 3.86	32.0 ± 2.28	24.2 ± 1.53	23.8 ± 1.85
VLDL (mg/dl)	23.6 ± 1.03	16.2 ± 0.58*	17.0 ± 0.95*	11.4 ± 0.51*
LDL (mg/dl)	60.8 ± 6.54	33.0 ± 3.94*	43.6 ± 4.81	21.4 ± 2.60*

Values are expressed as mean ± SEM, n = 5. Significance levels are denoted as * ($p < 0.05$).

3.5. Reduced levels of epididymal sialic acid and testicular glycogen

Testicular ascorbic acid levels were reduced in all treated groups except the ATR-only group when compared with the control animals (Fig. 3). A statistically significant decrease ($p < 0.05$) in ascorbic acid concentration was observed in the ATR+PIP co-treated group. With respect to sialic acid content in the cauda epididymis, no significant change was detected in the PIP-treated group, whereas a slight reduction was noted in the ATR-treated group. In contrast, rats receiving combined ATR and PIP treatment showed a marked decrease ($p < 0.05$) in cauda epididymal sialic acid levels.

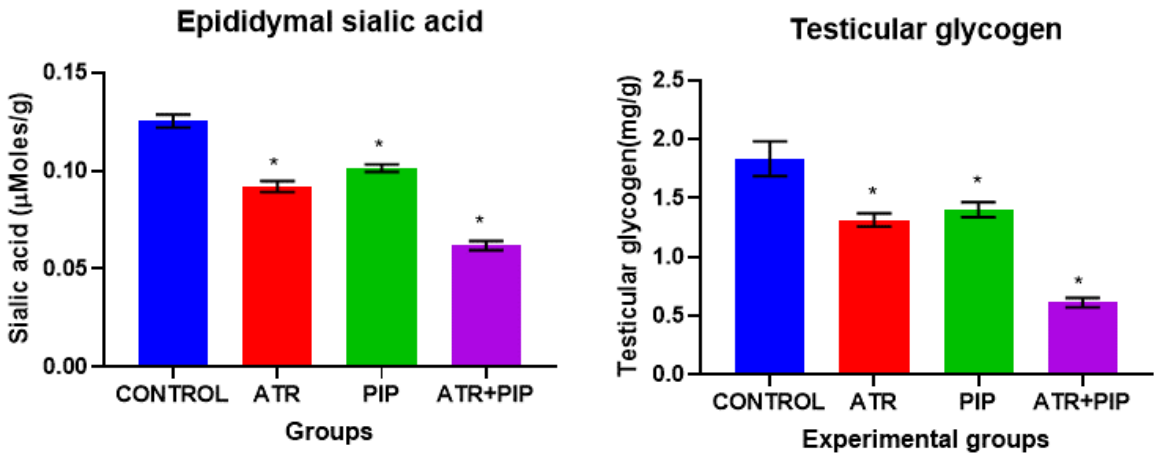

Figure 3: Changes in testicular glycogen and epididymal sialic acid levels in all the experimental groups. Values are expressed as mean ± SEM, n = 5. Significance levels are denoted as * ($p < 0.05$).

3.6. Decrease in serum testosterone concentrations

Fig. 4 illustrates the effects of the treatments on testosterone levels. Administration of ATR ($p < 0.01$) or PIP alone resulted in a significant decline in testicular testosterone concentrations when compared with the control group. A more pronounced reduction ($p < 0.001$) was observed in animals receiving the combined ATR+PIP treatment. Similarly, serum testosterone levels were significantly decreased in the ATR-treated ($p < 0.01$), PIP-treated ($p < 0.001$), and ATR+PIP co-treated ($p < 0.001$) groups relative to controls.

4. Discussion

Reproductive organs are highly vulnerable to a variety of external stressors, including heavy metals, xenobiotics, electromagnetic radiation, nanomaterials, and pharmacological agents [27,28]. Alterations in the mass of reproductive organs are widely regarded as reliable indicators of reproductive toxicity.

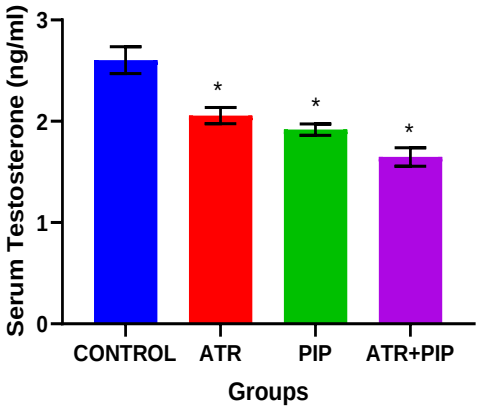

Figure 4: Changes in serum testosterone levels in all the experimental groups. Values are expressed as mean ± SEM, n = 5. Significance levels are denoted as * ($p < 0.05$).

Changes in testicular weight often reflect pathological conditions such as interstitial edema or disruption of seminiferous tubule architecture, whereas variations in epididymal weight may indicate inflammatory responses or reduced sperm production, making it a sensitive marker of spermatogenic impairment [29,30]. In the present investigation, changes in the weights of the testes, epididymis, seminal vesicles, and ventral prostate were systematically evaluated (Table 1). The findings demonstrate a consistent reduction in the weights of the testes, epididymis, and seminal vesicles across all treated groups when compared with controls. In contrast, ventral prostate weight remained largely unaffected in rats treated with ATR or PIP alone, but showed a significant decline in the ATR+PIP co-treated group. These observations are in agreement with the report of Klinefelter et al. (2014), who documented reduced testicular weights following high-dose atorvastatin exposure [10]. Similarly, D'Cruz and Mathur (2005) reported alterations in epididymal, seminal vesicle, and prostate weights in rats administered piperine [31]. Notably, the combined administration of ATR and PIP exerted a more pronounced impact on reproductive organ mass than either treatment alone, suggesting a potential synergistic effect. A reduction in organ weight is often associated with tissue atrophy and degeneration and is therefore considered a hallmark of reproductive toxicity [32].

Testicular weight is largely influenced by the abundance of developing germ cells; hence, a decrease in mature spermatids and germ cell density may account for the observed reduction in testicular mass. Likewise, impaired spermatogenesis can lead to diminished sperm storage, ultimately resulting in reduced epididymal weight [33]. The arrest of spermatogenesis and reduction in seminiferous tubule size observed in ATR+PIP co-treated rats may explain the marked decrease in testicular weight, given that seminiferous tubules constitute approximately 90% of the wet weight of the rat testis [34]. Furthermore, reduced lipid content—accounting for nearly 30% of total testicular weight—observed in the ATR+PIP group may have contributed to the decline in testicular mass [35]. Histopathological examination revealed significant enlargement of both the tubular lumen and interstitial spaces following ATR+PIP exposure. This was accompanied by a reduction in seminiferous tubule diameter, epithelial height, and tunica albuginea thickness. In addition, marked depletion of spermatogonia, primary and secondary spermatocytes, and spermatids was evident. Such morphological features—characterized by reduced germ cell populations and tubular atrophy—are well-established indicators of spermatogenic failure [36]. Earlier studies have shown that piperine suppresses spermatogenesis in adult male rats following prolonged exposure [20,31,37], while atorvastatin has also been reported to possess antispermatogenic properties [9]. Consistent with these findings, the present study demonstrates that combined ATR and PIP exposure markedly disrupts spermatogenesis. Histological evaluation of the epididymis in control animals showed intact columnar epithelium, cilia, smooth muscle layers, and luminal spermatozoa. In contrast, epididymal sections from ATR+PIP co-treated rats revealed ducts with sparse or completely absent spermatozoa. Seminal vesicle sections from control animals displayed typical architecture, including columnar epithelial lining and dense luminal secretions, whereas combined ATR and PIP treatment resulted in mild epithelial hyperplasia. These findings collectively indicate substantial damage to androgen-dependent accessory reproductive organs, including the epididymis, seminal vesicles, and prostate gland. Androgens are essential for maintaining secretory activity and structural integrity of these organs [38].

Serum lipid profiling revealed treatment-associated alterations in total cholesterol, HDL, LDL, and triglyceride levels (Table 2). While ATR and PIP alone produced only modest reductions in total cholesterol, the ATR+PIP co-treated group exhibited a significant decrease. Vijaykumar et al. (2006) similarly reported reduced serum cholesterol levels following piperine administration in rats [39]. The pronounced hypocholesterolemic effect observed with combined ATR and PIP treatment aligns with previous reports evaluating the lipid-lowering actions of these agents [40]. Sperm count and viability were markedly compromised in rats co-treated with ATR and PIP. Comparable reductions in sperm parameters following ATR [11] and PIP [31] administration have been documented previously. The pronounced decline observed in the combined treatment group suggests that atorvastatin-associated reproductive toxicity may be exacerbated by concurrent piperine intake, either pharmacologically or through dietary exposure. This reduction in sperm quality may be attributed, at least in part, to decreased testosterone bioavailability, as epididymal function and sperm fertilizing capacity are highly dependent on testicular androgens.

Spermatogenesis is tightly regulated by luteinizing hormone (LH) and follicle-stimulating hormone (FSH). FSH primarily acts on Sertoli cells, whereas LH regulates steroidogenesis in Leydig cells, which synthesize testosterone and estrogens essential for germ cell development [41]. The present study revealed a significant decline in serum testosterone levels across treatment groups, particularly in the ATR+PIP co-treated rats. These findings are consistent with previous reports demonstrating reduced testosterone levels following atorvastatin [9] and piperine [20] exposure. The concordance between our results and earlier studies [6,9,11,42] suggests that co-administration of ATR and PIP may disrupt testosterone synthesis by modulating the activity or expression of key steroidogenic enzymes. It is plausible that piperine enhances the bioavailability of atorvastatin [22–24], thereby amplifying its inhibitory effects on steroidogenesis within Leydig cells.Sialic acid plays a critical role in maintaining epididymal sperm membrane integrity and facilitating sperm maturation. A significant reduction in epididymal sialic acid content was observed in the ATR+PIP co-treated group [43], likely reflecting androgen and gonadotropin insufficiency. Such depletion may contribute to impaired spermatogenesis, reduced sperm motility, and diminished fertilizing capacity [44]. Additionally, testicular glycogen serves as an important energy reserve required for normal testicular function and spermatogenic activity. The observed reduction in testicular glycogen levels following ATR+PIP treatment may be indicative of compromised energy metabolism. Similar reductions in glycogen content associated with testosterone dependency and

testicular dysfunction have been reported by Kamal et al. [45].

5. Conclusion

In conclusion, our research indicates that the co-administration of atorvastatin and piperine reduces serum testosterone levels. The epididymal sperm count and sperm viability were observed to deteriorate in rats co-treated with ATR and PIP. The weights of the testis, epididymis, and seminal vesicles were considerably reduced in rats concurrently fed atorvastatin and piperine. Histopathological examinations of the seminiferous tubules, cauda epididymis, and seminal vesicles revealed degenerative conditions. Animals administered ATR+PIP exhibited a reduction in sialic acid levels in the cauda epididymis and glycogen levels in the testis. Consequently, our study's findings indicate that the co-administration of piperine and atorvastatin may enhance reproductive damage via influencing cholesterol and testosterone production.

Acknowledgement

The author conveys profound appreciation to the Head of the Department of Zoology at the University of Kalyani and the Principal of Rishi Bankim Chandra College for their invaluable assistance and resources that facilitated the execution of this research.

Conflict of interest

The author asserts the absence of any recognised financial conflicts or personal affiliations that might have seemingly impacted the findings presented in this paper.

Ethics approval

This study was conducted in compliance with the established guidelines for the care and use of laboratory animals. All experiments were conducted in compliance with the guidelines set forth by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India (No. 892/GO/Re/S/01/CPCSEA), and received approval from the Institutional Animal Ethics Committee (IAEC), University of Kalyani.

Declaration on the use of AI

The author declares that AI tools were used only for language refinement and grammatical assistance.

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