

Testicular microanatomical and hormonal alterations following use of antiretroviral therapy in *Sprague Dawley* rats: Role of Naringenin

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Abstract

Human immunodeficiency virus-infected man may require assisted reproductive technology not just for safer conception but also due to subfertility. The study investigated the effect of antiretroviral drugs on the fertility potentials of males and the possible protective role of Naringenin, using Sprague Dawley rats. Thirty adult male Sprague Dawley rats were grouped into—A: Distilled water; B: Highly Active Antiretroviral Therapy (HAART); C: Naringenin 40 mg/kg; D: Naringenin 80 mg/kg, E: HAART + Naringenin 40 mg/kg; F: HAART + Naringenin 80 mg/kg. The rats were euthanised after 10 weeks. Results showed a significant decrease in sperm count in group B when compared to the control and other groups. Spermatozoa with normal morphology also reduced significantly in the B group and progressive sperm motility reduced when compared to the control, D and the F group. The serum testosterone was not significantly different between groups A and B, however the groups C and D displayed significant increase when compared to groups A and B. The serum luteinising hormone was significantly higher in group B when compared to groups A, E and F. Our data suggest that Naringenin improves the male reproductive anatomy and function, therefore, it promises to be a beneficial adjuvant for mitigating HAART testicular and reproductive perturbations.

KEYWORDS

antiretroviral therapy, hormones, male infertility, semen parameters, Sprague Dawley, testis

1 | INTRODUCTION

Even though infection with Human Immunodeficiency Virus (HIV) depresses the health status of men, it does not eliminate the desire for future fertility (Cooper et al., 2009; Heys, Kipp, Jhangri, Alibhai, & Rubaale, 2009; Iliyasu et al., 2009; Tesfaye, Admassu, Getachew, & Sharma, 2012; Wekesa & Coast, 2014) yet testicular toxicity has been a major complication associated with Highly Active Antiretroviral Therapy (HAART). The Nucleoside reverse transcriptase inhibitors (NRTIs) are particularly a major culprit. Reports

suggest that most people living with HIV and Acquired Immune Deficiency Syndrome (AIDS) (PLWHA) are in their reproductive age (Azu et al., 2014; Lewis, Day, & Copeland, 2003; van Leeuwen et al., 2008). The vigorous efforts directed to reducing and treating HIV infection has recorded success in improving the life expectancy and aspirations among PLWHA (Iliyasu et al., 2009; Nakagawa et al., 2012). Reports of The Joint United Nations Program on HIV/AIDS (UNAIDS) suggests that approximately 1.8 million people were newly infected in 2016. The number of people accessing antiretroviral has increased steadily over the years (UNAIDS, 2016).

An estimated 19 million people were accessing antiretroviral drugs globally as at the end of 2016 (UNAIDS, 2016). According to this report, South Africa had the highest figure of 3.4 million people on treatment. HAART has been associated with deleterious organ toxicity especially as it affects the gonads (Azu et al., 2014; Bujan et al., 2007; Semprini et al., 2013).

HAART-induced toxicity has been reported to cause deleterious histopathological changes in the testes. Changes observed include tubular atrophy with altered morphometric indices (Adana et al., 2017; Azu et al., 2014). Autopsy studies on individuals on HAART revealed testicular histomorphologic abnormalities, and testicular atrophy in more than 95% of cases (Da Silva et al., 1990). Earlier study carried out by researchers equally reveal that HAART-induced alterations result in significantly decreased total sperm count, progressive motility, and increased numbers of abnormal forms (Nicopoullos, Almeida, Vourliotis, & Gilling-Smith, 2011). In addition, structural integrity of the testis is essential for maintenance of the hormonal equilibrium including testosterone production. Therefore, with altered androgen levels linked to HAART protocols (Adana et al., 2017; Muthiah, Kallikadavil, Shivaswamy, & Menon, 2016), fertility issues become very rife in men on HAART.

An intact testicular microanatomical structure is essential for normal testosterone production. The exact mechanism for HAART-induced organ toxicity is still unclear however the most popularly proposed pathway is through initiation of a cascade of events involving the oxidant-antioxidant pathway (Azu et al., 2014; Olaniyan, Maduagwu, Akintunde, Oluwayelu, & Brai, 2015). Earlier studies have reported that increased activities of reactive oxygen species (ROS) overwhelm tissue's total antioxidant status leading to oxidative stress (Aitken & Curry, 2011; Aitken & De Iulii, 2007; Aitken & Roman, 2008). The overall effect is reduced fertility of men on HAART. This calls for an intervention strategy to protect against declining fertility.

Currently, the most specialised form of treatment of infertility is assisted reproductive techniques especially in vitro fertilisation. However, availability and access to these techniques are hindered by the relatively high cost (Chambers, Sullivan, Ishihara, Chapman, & Adamson, 2009) and availability of trained personnel. In addition, failure rates are high even when the male appears to have normal spermograms (Liu & Baker, 2000; Younes et al., 2016). Therefore there is need for continued search for alternative effective, affordable and easily accessible therapies.

The use of medicinal plants for the treatment of diseases is gaining more popularity in the 21st century especially in Africa (Kayode & Kayode, 2011; Mahlangeni, Moodley, & Jonnalagadda, 2014). This is consequent on their various biologically active phytochemicals (Krishnaiah, Sarbatly, & Nithyanandam, 2011; Rahmatullah et al., 2010). Flavonoids mitigate free radical/oxidative stress induced tissue damage (Chen et al., 2015; Salem et al., 2016).

Bioflavonoids are known for their immense health benefits in many human tissues (Çetin, Çiftçi, & Otlı, 2016; Ma, Zhang, & Jiang, 2017; Shoskes, 1998; Tirkey, Pilkhwal, Kuhad, & Chopra, 2005) and particularly in the reproductive functions in males

(Ciftci, Ozdemir, Aydin, & Beytur, 2012; Jamalana, Ghaffari, Hoseinzadeh, Hashemitabar, & Zeinali, 2016). Naringenin, a bitter colourless flavone extract from citrus species (Felgines et al., 2000; Yáñez et al., 2008) has been reported to have a wide variety of bioactivity. Naringenin and closely related bioflavonoids are reported to be beneficial for protecting and ameliorating testicular toxicity (Ganaie, 2015; Jamalana et al., 2016; Roy, Rahaman, Ahmed, Metya, & Sannigrahi, 2013; Sahin, Ozkaya, Cuce, Uckun, & Yologlu, 2017). This has been attributed to their free radical scavenging properties (Ganaie, 2015; Roy et al., 2013). This study was designed to quantify testicular damage associated with use of HAART and investigated the effect of naringenin on these changes. This was carried out by assessing the semen characteristics, serum biochemical parameters, pregnancy occurrences and testicular histoarchitectural changes in *Sprague Dawley* rats following long-term use of HAART.

2 | MATERIALS AND METHODS

2.1 | Materials

Thirty (30) adult male and 18 female *Sprague Dawley* (Aitken & Roman, 2008) rats, weighing 200–220 g (Jegade, Ofor, Onanuga, Naidu, & Azu, 2017), were obtained from the animal house of the Biomedical Resource Unit, University of KwaZulu–Natal, South Africa. They were housed in standard cages under controlled environmental conditions (25°C and a 12-hr light/dark cycle). The animals had free access to pulverised standard rat pellet food and tap water. The approval of the Animal Research Ethical Committee (AREC), UKZN, South Africa was sought and gotten with reference number AREC/046/016D. A fixed-dose combination (FDC) of HAART containing 600 mg of Efavirenz (EFV), 200 mg of Emtricitabine (FTC) and 300 mg of Tenofovir (TDF) [TDF/FTC/EFV] was administered daily. The therapeutic dose of the FDC was adjusted for animal weight using the human therapeutic dose equivalent for a rat model (7.5/5/15 mg/kg) (Meintjes et al., 2014). The drug was procured from Pharmicare Ltd, Port Elizabeth, South Africa. Naringenin was procured from Sigma Aldrich Avengelee.

2.2 | Experimental design

Thirty (30) disease-free adult male SD rats were randomly divided into 6 equal number groups. Group A served as control and was given distilled water, B was given the FDC of HAART containing TDF/FTC/EFV, C had naringenin, 40 mg/kg, D had naringenin, 80 mg/kg (Adana et al., 2017). The last two groups, E and F had TDF/FTC/EFV and naringenin 40 and 80 mg/kg respectively.

All administrations were through the oral route and were performed between 8:00 a.m. and 10:00 a.m. for a period of 10 weeks (March 13th to 23rd of May 2017). Animals were examined and weighed on daily for signs of toxicity and dose adjustments. Weights were taken between 8:00 and 10:00 a.m.

2.3 | Determination of fertility index

At the end of the 10-week period of drug administration to male rats, eighteen female animals in the proestrous or oestrus phase were randomly divided into 6 groups of 3 animals each and labelled according to the male animal grouping (A¹, A², A³; B¹, B², B³; C¹, C², C³; D¹, D², D³; E¹, E², E³; F¹, F², F³) described above. They were then introduced to the corresponding males for copulation to take place. The morning on which spermatozoon is found in the vagina lavage was designated gestational day zero (gd 0) (Hafez 1970). Female animals were studied at day 20 of pregnancy. The number of pregnancies and subsequently number of litters per group were noted. Fertility index (FI) was calculated as follows:

$$FI = (\text{number of pregnancies}) / (\text{number of mated females}) \times 100$$

(Hood, 2016).

2.4 | Animal sacrifice and collection of samples

Five days after mating, each male SD rat was euthanised under excess halothane anaesthesia and blood samples were collected via transcardial puncture. Five millilitre (5 ml) of blood sample was obtained from each rat and divided into two: One half in a plain bottle and the other half in an ethylenediaminetetraacetic acid (EDTA) bottle. Plasma and serum were obtained by centrifugation at 1401 × g for 20 min. The testes were excised, weighed and fixed in Bouin's fluid for histological analysis. The cauda epididymis was gently minced and assayed for sperm count, sperm motility and sperm morphology. The relative testicular weights were calculated using the following formula:

$$\text{Organ weight} / \text{Final body weight} \times 100$$

(El-Aziz, El-Fark, & Hamdy, 2016; Patrick-Iwuanyanwu, Udowelle, & Okereke, 2016)

2.5 | Sperm characteristics

Sperm count, motility and morphology were assessed as described by Amniattalab and Razi (2015) and Jalali, Najafi, Hosseini, and Sedighnia (2015). In brief, Epididymal spermatozoa were collected from the cauda epididymis in 1 ml of human tubular fluid (HTF) solution and incubated for 10 min at 37°C in an atmosphere of 5% CO₂ incubator to allow sperm to swim out of the epididymal tubules.

2.5.1 | Motility

To assess the sperm motility, one drop of sperm suspension was placed on a microscope slide, and a coverslip was placed over the droplet. Ten (10) microscopic fields were observed at 400x magnification using a phase contrast microscope.

2.5.2 | Count

The epididymal sperm counts were obtained by the standard hemocytometric method using the Biorad automated counting chamber and equipment. In brief, after dilution of epididymal spermatozoa to 1:20 in HTF medium, approximately 10 microlitre (μl) of this diluted specimen were transferred to each of the counting chambers for automatic counting.

2.5.3 | Morphology

Sperm morphology was accessed by staining dry smeared spermatozoa on glass slide with eosin-nigrosin staining. Morphology was described as normal, abnormal head, abnormal midpiece, and abnormal tail (Adana et al., 2017; Azu et al., 2014).

2.6 | Determination of reproductive hormones

The serum levels of luteinising hormone (Hegazy, Ali, & Sabry, 2016), and free testosterone were determined by Elabscience rat specific kits with catalogue numbers: E-EL-R0026 and E-EL-R0389 respectively. Procedure was carried out in accordance with manufacturers' instructions.

2.7 | Histomorphometric studies

For histopathological examination, fixed testicular tissues were dissected. Samples were then processed using a graded ethanol series and embedded in paraffin. The paraffin sections were cut into 5 μm-thick slices using a microtome (microm HM 315 microtome, Walldorf, Germany) and stained with haematoxylin and eosin for histological examination. The sections were viewed and photographed using an Olympus light microscope (Olympus BX51, Tokyo, Japan) with an attached camera (Olympus E-330, Olympus Optical Co. Ltd., Japan).

A ten millimetre (10 mm) grid was placed on the 20 μm haematoxylin and eosin stained testicular tissue sections. Eleven sections per animal were selected using the Leica slidePath gateway LAN software. Selections were carried out using the systematic random sampling method with a random start. Four blocks per testes were used. The same number of grid points was allowed on all sections. The number of grid points falling on each component of the seminiferous tubules (ST)- germinal epithelium (GE), lumen (L) and interstitium (I) were noted. The volume fraction (Vv) of each component was calculated as follows:

$$Vv(\text{Component} / \text{Ref Volume}) =$$

$$\frac{\sum \text{Points component}}{\sum \text{Points reference}} \times 100$$

(Bordbar, Esmailpour, Dehghani, & Panjehshahin, 2013; Howard & Reed, 2004).

2.8 | Statistical analysis

The data were presented as mean ± SEM. Variables were subjected to Shapiro-Wilk test to check for normality. The differences were

TABLE 1 Body weights (BW) and relative testicular weights (TW) in animals in groups A (control), H (HAART), C (Naringenin 40 mg/kg), D (Naringenin 80 mg/kg), E (HAART + Naringenin 40 mg/kg) and F (HAART + Naringenin 80 mg/kg) after 10 weeks of treatment. Bars indicate mean $\pm$ SD

Group	Initial BW	Final BW	Weight gain	Relative TW
A (control)	147.40 $\pm$ 7.24	341.60 $\pm$ 15.16	194.20 $\pm$ 15.89	1.02 $\pm$ 0.04
B (HAART)	164.40 $\pm$ 1.33	339.80 $\pm$ 15.50	175.4 $\pm$ 15.56	1.01 $\pm$ 0.04
C (naringenin 40 mg/kg)	161.20 $\pm$ 8.31	359.60 $\pm$ 14.50	198.40 $\pm$ 13.73	0.97 $\pm$ 0.02
D (naringenin 80 mg/kg)	165.00 $\pm$ 7.19	370.80 $\pm$ 9.32	205.80 $\pm$ 9.69	1.04 $\pm$ 0.02
E (HAART + Naringenin 40 mg/kg)	152.20 $\pm$ 3.99	361.00 $\pm$ 11.15	208.80 $\pm$ 13.95	1.03 $\pm$ 0.03
F (HAART + Naringenin 80 mg/kg)	149.80 $\pm$ 5.01	355.80 $\pm$ 5.68	206.00 $\pm$ 2.26	1.03 $\pm$ 0.03

Group	Sperm count ($\times 10^6$)	Progressive motility (%)	Abnormal spermatozoa (%)
A (Control)	29.28 $\pm$ 0.66	84 $\pm$ 2.92	5.5 $\pm$ 1.50
B (HAART)	20.05 $\pm$ 1.58**	58 $\pm$ 3.74**	12.26 $\pm$ 5.03
C (naringenin 40 mg/kg)	25.56 $\pm$ 1.78†	62 $\pm$ 3.74**††	4.64 $\pm$ 1.18
D (naringenin 80 mg/kg)	26.12 $\pm$ 1.64	76 $\pm$ 2.92††	8 $\pm$ 3.78
E (HAART + Naringenin 40 mg/kg)	24.92 $\pm$ 2.06	65 $\pm$ 2.24**††	5 $\pm$ 0.77
F (HAART + Naringenin 80 mg/kg)	25.04 $\pm$ 1.17	74 $\pm$ 2.92††	5.6 $\pm$ 1.17

Note. Semen parameters in animals in groups A (control), H (HAART), C (Naringenin 40 mg/kg), D (Naringenin 80 mg/kg), E (HAART + Naringenin 40 mg/kg) and F (HAART + Naringenin 80 mg/kg) after 10 weeks of treatment. Bars indicate mean $\pm$ SD.

* $p \leq 0.05$ between the group and control. ** $p \leq 0.001$ between the group and control. † $p \leq 0.05$ between the group and HAART group while. †† $p \leq 0.001$ between the group and HAART group.

TABLE 2 Semen parameters

then evaluated using one-way analysis of variance (Ciz et al., 2012) followed by Fisher's Least Significant Difference (LSD) procedure for multiple comparisons. $p < 0.05$ was considered statistically significant and <0.01 , very significant using Statistical Package for Social Sciences (SPSS) version 23.

3 | RESULTS

3.1 | Body weight and relative testicular weight

There were no significant differences in the weight gain [ANOVA $p = 0.472$] and relative testicular weights [ANOVA $p = 0.506$] across the groups (Table 1).

3.2 | Semen parameters

Sperm count was significantly different between the groups [ANOVA $p = 0.019$]. There was a significantly lower count in group B (HAART) compared to A (control) [$p = 0.01$] and C [$p = 0.032$]. There were significantly lower progressive spermatozoa in group B when compared to A [$p < 0.0001$], C [$p = 0.001$], D [$p = 0.00$], E [$p = 0.00$] and F (HAART + Naringenin 80 mg/kg) [$p = 0.001$]. Group C [$p = 0.001$] and E [$p = 0.002$] also displayed significantly lower progressive motility when compared to A (Table 2).

3.3 | Fertility index

Statistical comparison was not made for fertility index. However, the fertility index was higher in the A group than in the B and C groups. The number of pups per group was also higher. The average number of pups per pregnant female were lower in the B and C groups (Table 3).

3.4 | Reproductive hormones

Serum testosterone levels were not significantly different [$p = 0.344$] between groups A and B. Groups that were administered only naringenin- C and D displayed significant increase [$p = 0.025$] and [$p = 0.006$], respectively, when compared to groups A. Testosterone level were higher in the D was compared to B group [$p = 0.051$]. The serum luteinising hormone was significantly higher in group B when compared to groups A [$p = 0.027$], E [$p = 0.017$] and F [$p = 0.018$] Figure 1a,b.

3.5 | Histomorphometry

There were significant differences in the volume fractions of the seminiferous epithelium (SE) [ANOVA $p = 0.027$] and lumen [ANOVA $p = 0.024$]. The volume fraction of SE was higher in the B

TABLE 3 Fertility index in animals in groups A (control), H (HAART), C (Naringenin 40 mg/kg), D (Naringenin 80 mg/kg), E (HAART + Naringenin 40 mg/kg) and F (HAART + Naringenin 80 mg/kg) after 10 weeks of treatment. Bars indicate mean $\pm$ SD

Group	No of pregnancies	Fertility index (FI)	No of resorptions	No of pups
A (control)	5	100.00	0.67 $\pm$ 0.58	8.67 $\pm$ 2.08
B (HAART)	3	60.00	0.67 $\pm$ 1.15	6.67 $\pm$ 5.77
C (naringenin 40 mg/kg)	3	60.00	0.33 $\pm$ 0.58	6.00 $\pm$ 5.57
D (naringenin 80 mg/kg)	5	100.00	0.00	9.67 $\pm$ 1.53
E (HAART + Naringenin 40 mg/kg)	5	100.00	0.67 $\pm$ 0.58	9.33 $\pm$ 1.15
F (HAART + Naringenin 80 mg/kg)	5	100.00	0.33 $\pm$ 0.56	9.33 $\pm$ 1.15

group than all groups except the C group which was significantly higher [$p = 0.017$]. The lumen in group B was significantly smaller than those of D [$p = 0.007$], E [$p = 0.023$], F [$p = 0.002$] (Table 4).

3.6 | Histomorphology

The seminiferous tubules (ST) of the control animals displayed the normal progression of cells of the spermatogenic series with sperm cells in the lumen and Leydig cells in the interstitium (Figure 2). Group b had apparently smaller ST with scanty cells in the lumen of some of the ST, whereas the c, d, e, and f displayed larger ST with the normal progression of cells of the spermatogenic series. Leydig cells in the interstitium and mature sperm cells in the lumen were similar to control (Figure 2).

4 | DISCUSSION

Long-term use of HAART has become the cornerstone of the success achieved in the prevention and management of HIV infections. It is associated with decline in AIDS incidence and death rates across different parts of the world (Antiretroviral Therapy Cohort Collaboration, 2008; Kitahata et al., 2009; Liu, 2016; Liu & Baker, 2000). However, it is also associated with myriads of biochemical, physiological and structural changes and their attendant complications (Azu et al., 2014; del Valle, Hernández, & Ávila, 2013; Mudie et al., 2014). This study investigated the effects of HAART on the male reproductive potential and the protective role of naringenin in animals.

4.1 | Semen parameters and fertility rate

This study reports that HAART-induced alterations in semen parameters in rats. This was evident from the decrease in the sperm count, sperm motility and an increase in abnormal forms with the use of TDF/FTC/EFV combination. This is in tandem with the findings of earlier studies (Azu et al., 2014; Kehl et al., 2011; Ogedengbe et al., 2016) who also reported semen abnormalities. The effect on sperm parameters could explain the lesser number of pregnancies and subsequently pups in the rats on TDF/FTC/EFV combination. The co-administration of naringenin (80 mg/kg) and HAART restored

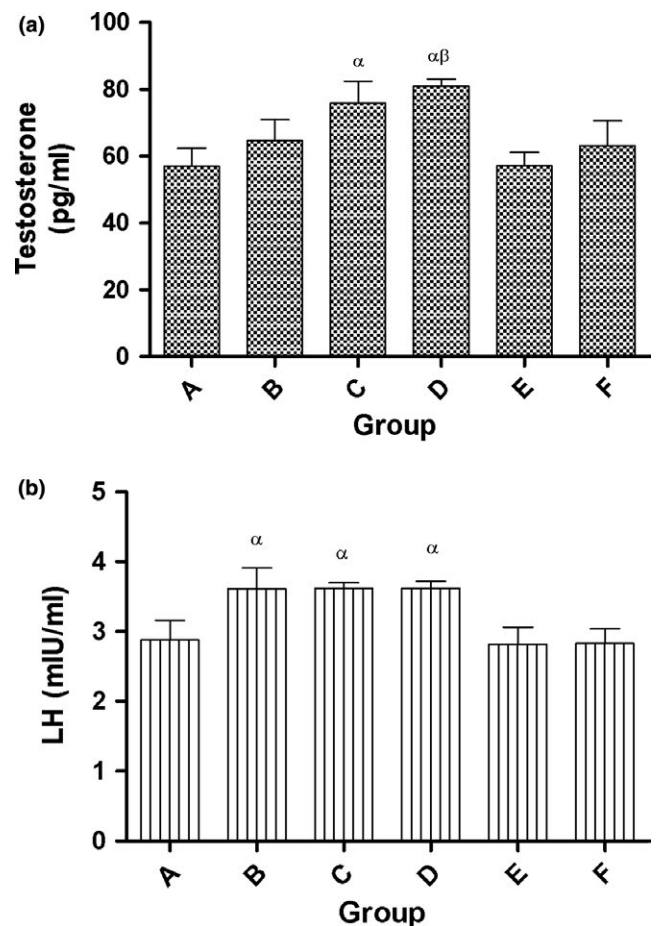


FIGURE 1 (a) Serum Testosterone and (b) Luteinising hormone in groups A (control), B (HAART), C (Naringenin 40 mg/kg), D (Naringenin 80 mg/kg), E (HAART + Naringenin 40 mg/kg) and F (HAART + Naringenin 80 mg/kg) after 10 weeks of treatment. Bars indicate mean $\pm$ SD. ^α $p \leq 0.05$ between the group and control. ^β $p \leq 0.05$ between the group and HAART group

the fertility rate and did prevent alterations in the semen parameters as they were comparable to those of the control. This suggests that naringenin may protect against HAART-induced testicular toxicity. Roy et al., 2013 earlier reported the ameliorative properties of naringenin in diabetes-induced testicular toxicity (Roy et al., 2013). The protective property of naringenin has been attributed to its ability to prevent antioxidant enzyme inactivation by potential

Group	Vol. fraction of seminiferous epithelium (%)	Vol. fraction of lumen (%)	Vol. fraction of interstitium (%)
A (control)	56.67 ± 1.52	16.67 ± 2.91	25.00 ± 1.96
B (HAART)	57.23 ± 2.86	11.11 ± 1.76*	31.67 ± 2.58
C (naringenin 40 mg/kg)	61.66 ± 2.04	15.00 ± 2.07	23.33 ± 1.11
D (naringenin 80 mg/kg)	57.22 ± 1.67**	21.67 ± 1.36**	21.11 ± 1.11
E (HAART + naringenin 40 mg/kg)	56.67 ± 2.99**	18.89 ± 1.84**	24.44 ± 2.22
F (HAART + naringenin 80 mg/kg)	48.33 ± 1.88**	20.55 ± 2.99**	31.11 ± 4.60

TABLE 4 Volume fraction of the germinal epithelium lumen and interstitium

Note. Volume fraction of in groups A (control), B (HAART), C (Naringenin 40 mg/kg), D (Naringenin 80 mg/kg), E (HAART + Naringenin 40 mg/kg) and F (HAART + Naringenin 80 mg/kg) after 10 weeks of treatment. Bars indicate mean ± SD.

*Represents significant difference ($p < 0.05$) between the group and control. **Represents significant difference between the group and B group.

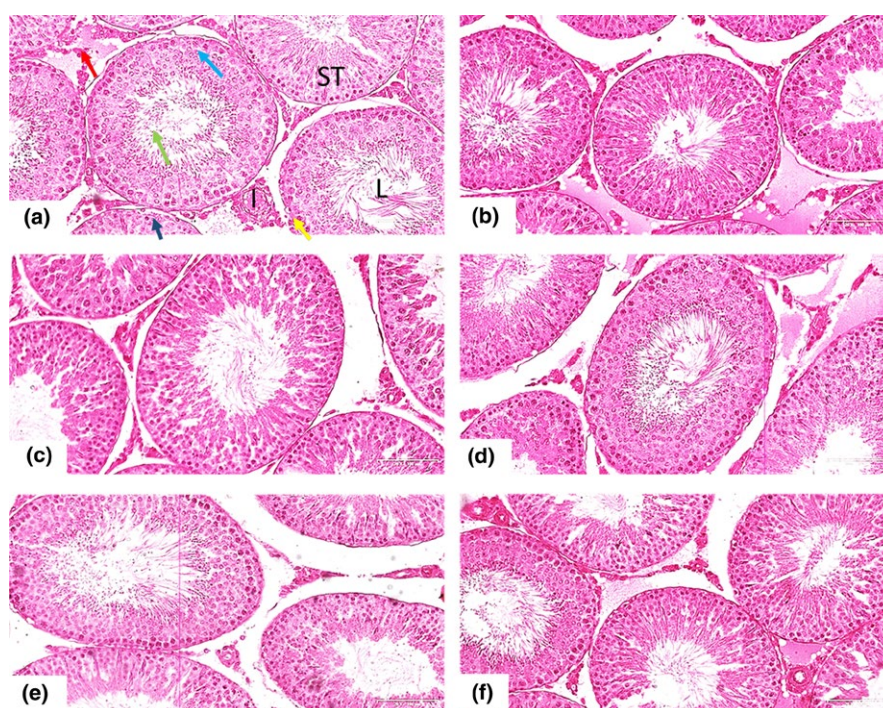


FIGURE 2 Haematoxylin and eosin stained histological sections in groups (a; control), (b; HAART), (c; Naringenin 40 mg/kg), (d; Naringenin 80 mg/kg), (e; HAART + Naringenin 40 mg/kg) and (f; HAART + Naringenin 80 mg/kg). The seminiferous tubules (ST) of the control animals displayed the normal progression of cells of the spermatogenic series with sperm cells in the lumen and Leydig cells in the interstitium. Group (b) had apparently smaller ST with scanty cells in the lumen, whereas the (c), (d), (e), and (f) displayed larger ST with the normal progression of cells of the spermatogenic series. Leydig cells in the interstitium and mature sperm cells in the lumen were similar to control

prooxidants, prevention of excessive lipid peroxidation, and prevention of structural and ultrastructural tissue changes induced by toxic agents (Hermenean et al., 2013). The ability to scavenge free radical was explained by its 5-hydroxy and 4-carbonyl groups in the C ring, which may interact with Cu and Fe ions (Podder, Song, & Kim, 2014). Naringenin has also been shown to be effective in inducing the expression of many antioxidant-related genes (Renugadevi &

Prabu, 2009) and inhibit the activity of ROS-forming enzymes such as NADPH oxidase (Ciz et al., 2012).

4.2 | Reproductive hormones

In the present study, serum testosterone levels were reduced with exposure to HAART. Sperm function and hence the fertility potential

of a male is highly dependent on the state of his hypothalamopituitary testicular axis (HPT) (Gandhi et al., 2017). This can be reflected by the circulating level of the reproductive hormones including testosterone and the gonadotropins. A well balanced HPT axis is essential for a successful reproduction; however, the balance can be offset by several factors such as the existing medical or surgical conditions and drugs used (Whirlledge & Cidlowski, 2010).

This is contrary to the findings of Collazos, Mayo, Martínez, & Ibarra, 2002 who recorded an increase in testosterone levels. This conflict could be attributed to the difference in class of drug and mechanism of organ toxicity (Collazos et al., 2002). The earlier study associated a use of protease inhibitor with an increase in circulating testosterone (Collazos et al., 2002). This was however accompanied by sexual dysfunction. Another study reported a similar reduction in testosterone levels (Rochira et al., 2011). Naringenin conversely, induced a significant increase in serum levels of testosterone when compared to the control and HAART administered animals. The combination of naringenin and HAART used in this study has minimal effects on the serum levels of testosterone. This may explain the maintenance of the testicular weight and suggests other mechanisms of altered sperm function.

Naringenin significantly increased the level of testosterone in a dose-related manner. This may explain the high sperm count and motility (although not better than control) and the less abnormal forms observed in the naringenin administered group. This is similar to the findings from other studies (Roy et al., 2013; Sahin et al., 2017) and contrary to others (Papiez, 2003; Ranawat & Bakshi, 2017). In study examining the effects of naringenin on diabetes, naringenin-treated diabetic rats showed an improved histological appearance, sperm parameters and serum testosterone levels (Roy et al., 2013). An earlier study revealed that naringenin also caused minor changes in metabolic processes in the testes of the rats but did not significantly affect the synthesis of androgens (Papiez, 2003).

The serum luteinising hormone levels increased significantly in HAART administered animals. A normal serum level and intratesticular concentration are required for optimal testicular weight and function (Harada et al., 2016).

4.3 | Histomorphometry

Structural changes were observed across the groups. We observed increase in the volume fractions of the interstitium with the use of HAART. The seminiferous tubules (ST) of the control animals displayed the normal progression of cells of the spermatogenic series with sperm cells in the lumen and Leydig cells in the interstitium. This was also observed in the HAART administered group but there was an apparently smaller ST with scanty cells in the lumen of some of the ST. On the other hand, the other experimental groups displayed larger ST with the normal progression of cells of the spermatogenic series. Leydig cells in the interstitium and mature sperm cells in the lumen were similar to control. Minimal changes were detectable in the tubular structure of the testis associated with HAART use in this study, although a

reduced intertubular space may suggest oedema of the testes induced by tissue inflammation. This, according to prior studies, preceded changes in the seminal epithelium (Chiquoine, 1964; Heuser, Mecklenburg, Ockert, Kohler, & Kemkowski, 2012; Hijazi, Ahmed, UI Haq, Naqvi, & Parveen, 2016). Likely mechanism of toxicity is a disturbance of fluid homeostasis (Heuser et al., 2012). These changes may be better delineated using transmission electron microscope studies. The study made use of viral free animal models. The co-administration of naringenin and HAART is intended for HIV infected men. The immunological reaction to the virus may alter the observed interactions.

5 | CONCLUSION

The study has shown that use of the FDC-TDF/FTC/EFV induced changes in serum reproductive hormones, local tissue inflammation and disturbed fluid homeostasis in *Sprague Dawley* rats. These changes resulted in reduced sperm count and motility as well as an increase in abnormal forms, and subsequently reduced fertility index. Naringenin demonstrated some protection against these changes. Further studies on the testicular ultrastructure may be able to authenticate the beneficial role of this bioflavonoid as an adjuvant in the management of men on HAART.

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