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Testicular toxicological effects of combination antiretroviral therapy (cART): An x-ray on the therapeutic potential of bioflavonoids

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Abstract
The advent of combination antiretroviral therapy (cART) has improved the quality of life of people living with HIV/AIDS, however, the side effects of cART on the testes has been of great concern because of the reproductive desires of these people. This study was designed to determine the modulating and curative roles of bioflavonoids on cART-induced testicular disorders. Thirty-five (35) adult male rats were randomly divided into 7 groups and treated with; 1ml of distilled water; 24mg/kg cART (3TC, 300mg+TDF, 300mg+EFV, 600mg); 1% v/v DMSO; 50mg/kg Naringenin (N), 50mg/kg Quercetin (Q), 50mg/kg of Q and N co-administered differently with 24mg/kg cART. There was marked depletion of cells of the spermatogenic series in the seminiferous tubules in animals that received cART, while those that received bioflavonoids showed well-structured seminiferous tubules. cART-only treated group had significantly increased MDA levels as well as significantly reduced SOD and GSH levels when compared with control ($p<0.05$). Hormonal analysis showed a significant increase in TT and LH in the H/N treated group and H/Q group respectively compared with cART-only group ($p<0.05$) while a significant decrease was observed in FSH in cART-only and other treated groups (except N and H/Q) compared with control ($p<0.05$). 3β HSD expression was significantly reduced in the cART-only treated group ($p<0.05$) compared with control while the co-treatment with quercetin significantly ($p<0.05$) increased 3β HSD expression. This study demonstrates the curative potential of selected bioflavonoids in mitigating testicular cART-induced effects.

Keywords: Combination Antiretroviral Therapy (cART); oxidative stress; testis; 3β HSD; Naringenin; Quercetin

Introduction

Combination Anti-Retroviral Therapy (cART) is a combination therapy of three or more HIV suppressing drugs that have significantly improved the immunological status of the infected population, making it a manageable illness (Miguez *et al.*, 2011).This drug has greatly expanded and improved the quality of life and optimism that HIV can as well be managed (Kayode *et al.*, 2011; Gudina *et al.*, 2017) with a significant reduction in HIV-associated morbidity and mortality and improvement in the

quality of life of people living with HIV (Mayer and Venkatesh, 2010).

The first-line of cART guidelines for initial HIV therapy are those consisting of tenofovir disoproxil fumarate (tenofovir) and efavirenz combined with either lamivudine or emtricitabine. This makes the drug a combination of two nucleoside reverse transcriptase inhibitors as the backbone combined with a non-nucleoside reverse transcriptase inhibitor (WHO, 2016). Owing to the replicative speed of HIV and the incapability of cART to annihilate this infection, HIV-patients have to use these drugs continuously for the rest of their lives. This makes HIV a chronic disease and infected patients completely dependent on cART.

Despite the advantageous effects of cART, its side effects continue unabated (Horne *et al.,* 2019). In addition, the role of oxidative stress in the progression of the disease has become more complicated since cART itself reportedly leads to several organo-toxicities (O'Brien *et al*., 2003; Njuguna *et al*., 2013; Gudina *et al*., 2017). Azu *et al.,* (2014) reported that cART causes depletion of Sertoli cells and leads to distortion in testicular structure. Research has shown that one of the most important limiting factors to the success of cART is its adverse drug reaction (Shet *et al*., 2014; Yunihastuti *et al*., 2014). It has recently been discovered to be responsible for co-morbidities which include testicular dysfunction (Azu *et al*., 2014; Adana *et al*., 2018). Hence continuous exposure to antiretroviral drugs leaves one with few options of either decreasing the dosage of antiretroviral drugs and causing impairment on the drug's ability to produce desired results, or withdrawing the particular drug causing injury/toxicity and replacing it with another drug, or simply treating the disorders emanating as side effects. Whether HIV infection *per se* and/or therapy (from cART) directly contributes to the deleterious effects of cART on the testis remains to be elucidated. However, various authors have described oxidative stress as a major pathway responsible for the adverse effects of most Nucleoside/Nucleotide Reverse Transcriptase Inhibitors (NRTI) class of cART on tissues/cells (Andries *et al.,* 2000; Day and Lewis, 2004; Montessori *et al.,* 2004). Indeed, oxidative stress is one of the factors inducing infertility in males because plasma membranes of spermatozoa contain large quantities of polyunsaturated fatty acids (PUFAs) and their cytoplasm contain low concentrations of scavenging enzymes (Agarwal *et al.,* 2014). Hence, oxidative stress leads to lipid peroxidation and DNA fragmentation which disrupts motility of the sperm cells and their ability to fertilize and produce normal embryo (Aitken and Roman, 2008, Akang *et al.,* 2017).

In light of the emanating toxic effects of cART, suggestions have been made in the course of using micronutrients as a low cost immune-modulating intervention (cite a reference here). Micronutrients such as vitamin A, C, D, E and phytochemicals (e.g. flavonoids) are essential for maintaining proper immunologic functions in the body (Nahak *et al*., 2014).When these micronutrients are used together with the cART regime, it may reduce oxidative stress and improve the function of the immune system (Drain *et al.,* 2007). Hence, the effectiveness of these phytochemicals could be used to reverse or mitigate the toxic effects of cART on the testes as well. In addition, these phytochemicals are cheap and easily derived from vegetables and fruits; hence, HIV- infected patients living in low income countries can have easy access to it. Also, research has indicated the beneficial effects of phytochemicals such as flavonoids (Narigenin and Quercetin), derived from fruits in reproduction (Patel *et al.,* 2014; Abarikwu *et al*., 2016).

Flavonoids are naturally occurring polyphenolic compounds containing two benzene rings linked together with a heterocyclic pyran or pyrone ring, they are normal constituents of the human diet and are known for a variety of biological activities which involve acting as enzyme inhibitors and antioxidants, and also contain anti-inflammatory properties. They are the major constituents of fruit, vegetables and beverages and are structurally similar to steroid hormones (Metodiewa *et al*., 1997; Walker *et al*., 2000; Dewick, 2001; Panche *et al*., 2016).

Flavonoids such as quercetin and naringenin have been found to possess strong antioxidant and anti-inflammatory activities (Tripoli *et al*., 2007). Quercetin belongs to the flavonols class of flavonoids which are found in plants such as onions, leeks, berries, grapes, shallots and so on while dietary flavanones which include naringenin are found predominantly in citrus fruits and tomatoes (Khaki *et al.,* 2010; Kelly, 2011; Patel *et al*., 2014). Flavonoids have been shown to scavenge for free radicals such as superoxide and hydroxyl radicals, because they are chelators of metal ions (Khaki *et al*., 2011). Research has also shown that quercetin in particular inhibits reverse transcriptase, which is part of the replication process of retroviruses (Kaul *et al*., 1985; Khaki *et al*., 2010; Kelly, 2011).

In line with the promising properties of bioflavonoids, naringenin and quercetin have been chosen as the phytochemicals of choice to be investigated as the supplementary therapy for use with cART in this study.

Materials and Method

Chemicals and reagents

Natural Naringenin, Quercetin, Dimethyl Sulfoxide (DMSO) were obtained from Sigma-Aldrich (South Africa), while cART containing Efavirenz (EFV, 600mg), Lamivudine (3TC, 300mg), Tenofovir (TDF, 300g) (Rao *et al*., 2017) was obtained from AIDS Preventive Initiative of Nigeria (APIN) Clinic, University of Lagos Teaching Hospital (LUTH), Lagos, Nigeria. Therapeutic dose of cART was adjusted for animals using human therapeutic dose equivalent for rat model.

Animals and treatment

Thirty-five (35) adult male Wistar rats with a mean body weight of 230±10g were obtained from the animal house, College of Medicine, University of Lagos, Lagos, Nigeria and kept in the animal *house* of the Department of Anatomy, College of Medicine of the University of Lagos. This research was approved by the Health Research Ethics Committee of the College of Medicine of the University of Lagos with protocol number CMUL/HREC/03/17/113. The animals were left to acclimatize for a period of two weeks before the experiment commenced. The animals were allowed proper access to food and distilled water *ad libitum*, their cages were designed to allow proper ventilation with wire gauge netting and kept where prevalent temperature range during the study was 28^0C to 30^0C with constant humidity (40 to 70%) and 12 h/12 h light/dark cycle.

The animals were randomly divided into 7 groups (n=5) and treated with 1 mL of distilled water, cART (H) 24 mg/kg (3TC, 300mg+TDF, 300mg+EFV, 600 mg); 1% v/v dimethyl sulfoxide (DMSO), 50 mg/kg Naringenin (N), 50 mg/kg Quercetin (Q), 50 mg/kg of both Q and N combined with 24 mg/kg of H respectively. Drugs were orally administered daily for 56 days. Animals were euthanized on the 57th day.

Animal sacrifice and collection of samples

Animals were euthanized using urethane. 2.5 g of urethane was dissolved in 10 mL of warm water. Testes were surgically excised through lower abdominal incision and separated from the cauda epididymis. Each testis was weighed using sensitive weighing scale and the average of the two testes was recorded for each animal. Testicular volume was obtained using Archimedes principle of water displacement. Testis was fixed in Bouin's fluid for histological analysis (Akang *et al.,* 2019)

Sperm motility, sperm count, and sperm morphology

This was done by the modification of the method reported by Adana *et al.* (2018). The caudal epididymides from each animal were minced in 5 mL of normal saline and used for determination of sperm count, sperm motility, and sperm morphology. Sperm motility was determined using the procedure by WHO (2010). A drop of prepared epididymal fluid was collected on a glass slide and covered with a coverslip (22 x 22 mm). It was immediately examined under the light microscope (X400 magnification). The field was methodically scanned, and the motility of spermatozoa carefully

assessed and graded as progressive, non-progressive, and immotile.

The epididymal sperm counts were obtained by the standard hemocytometer method. The epididymal fluid was diluted with 2 mL of normal saline and 20µl of this diluted specimen was transferred to slides of Bio-Rad counting chambers and counted with a Bio-Rad automated cell counter. Both sides of the counting chamber were used for each specimen and the average recorded to the nearest millions/millilitre.

Sperm morphology was accessed by staining dry smeared diluted epididymal fluid on a glass slide with 1% eosin staining and observed under a DMLS light microscope (ICC50 HD Leica Microsystems, Wetzlar, Germany) with DM750 (Leica) image pick-up and Leica Application Suite version 4.1 imaging software at 400x magnification. The number of normal, abnormal heads, abnormal tail, and abnormal mid-piece spermatozoa was recorded in percentage.

Testicular histopathology

The histology of the testes was done by modification of method reported by Akang *et al.* (2015). The organs were harvested and fixed in Bouin's fluid for 24 h and thereafter transferred to 70% alcohol for dehydration. The tissues were put through 90% alcohol, absolute alcohol and xylene for different durations before they were transferred into two changes of molten paraffin wax. They were subsequently embedded and serial sections cut using rotary microtome at 5 microns. The tissues were picked up with albumenized slides and allowed to dry on hot plate. The slides were dewaxed with xylene and passed through absolute alcohol (2 changes); 70% alcohol, 50% alcohol respectively and then to water for 5 min. The slides were then stained with haematoxylin and eosin. The slides were mounted in DPX. Photomicrographs were taken at a magnification of X100 and X400.

Immunohistochemistry

Testes tissues were fixed in 10% buffered formalin and transferred to 70% ethanol (Latendrese *et al.*, 2002). They were then processed using a graded ethanol series and embedded in paraffin. The paraffin embedded sections were cut into 4 µm-thick slices using a microtome (Microm HM 315 microtome, Walldorf, Germany). Immunohistochemistry was performed using Santa Cruz 3β HSD primary antibody and Dako Envision FLEX kit. The processed and sectioned tissues were dewaxed with 2 changes of xylene and hydrated with decreasing grades of alcohol, and water. The sections were placed in diluted Envision FLEX Target solution for 20 minutes at 95-99^{o}C. Tissue sections were washed in wash buffer, blocked with peroxidase and incubated with diluted 3β HSD (1:150) from Santa Cruz for 30 minutes and with HRP for 20 minutes, DAB and counterstained in hematoxylin, washed in wash buffer, dehydrated, cleared and mounted on DPX. The sections were viewed and photographed using a Leica microscope (DM 750, Germany) and an ICC HD 50 camera. Images were captured at a magnification of X400.

Percentage immunoreactivity

Image analysis and capturing was done using Image analysis and capturing was done using ImageJ 1.51J8 software (NIH, USA). At least six fields of view per slide were randomly selected and captured using X40 objective (Akang *et al.*, 2019).

Assay of oxidative stress markers

Using tissue homogenizer, 0.5g of the tissue was homogenized in 4.5 ml of 0.4M sodium phosphate buffer (pH 7.0), centrifuged at 3500rpm for 10min and the supernatant removed for the assay. Oxidative stress markers including catalase (CAT), superoxide (SOD), reduced glutathione (GSH) and malonidialdehyde (MDA) were determined by a modified method of Quinlan *et al.,*(1994), Marklund and Marklund (1974); Tappel (1978); Wright *et al.* (2003) respectively.

Morphometry Analysis

The primary aim was to estimate the volumes of seminiferous tubular epithelium (seminiferous epithelium) and interstitium in the testis. This was done in accordance with a modified method used by Akang *et al.* (2015). Volume densities of testicular ingredients were determined by randomly

superimposing a transparent grid comprising 70 test points arranged in a quadratic array. Test points falling on a given testis and its ingredients were added over all fields from all sections. The total number of points hitting on a given ingredient (lumen (EL), epithelium (EE), interstitium (EI)), were divided by the total number of points hitting on the testis sections (ET) multiplied by 100, provided an unbiased estimate of its % volume density/volume fraction.

Statistical analysis

Variables were tested for normality using Shapiro–Wilk test. All parametric data were analyzed with one-way ANOVA, followed by Turkey's multiple test while the non-parametric data were analyzed with Kruskal–Wallis 1-way ANOVA paired comparisons test. Significant values were adjusted by Tukey's multiple comparison tests using Graph pad prism version 5. Level of statistical significance was set at $p< 0.05$.

Results

Evaluation of testicular sperm count, sperm motility and sperm morphology

In this experiment there was no record of death of any of the animals used. There was no statistically significant difference (p=0.5923) in the sperm morphology of all treated groups when compared against control (Fig 1).

There was a statistically significant decrease (p=0.0212) in the sperm count of cART and DMSO groups compared to control, N, Q, H/Q and H/N (Fig 2d).

There was a statistically significant increase (p=0.0056) in progressive motility of spermatozoa in the H/Q group when compared with the cART group, while the quercetin and naringenin only treated groups showed a significant decrease(p=0.0056) in progressive sperm motility when compared with the control (Fig 2a). Non-progressive motile spermatozoa showed no statistically significant difference (p=0.3032) in all treated groups when compared with the control and cART only groups (Fig 2b). While immotile spermatozoa increased significantly (p=0.0070) in the N and Q groups when compared with the control (Fig 2c).

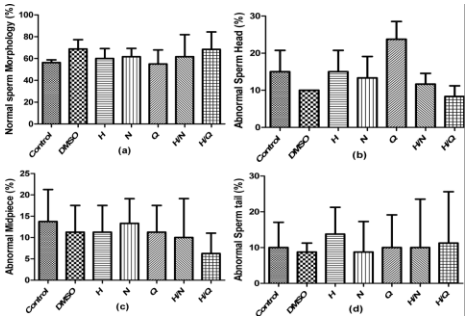

Figure 1: Sperm morphology (%).

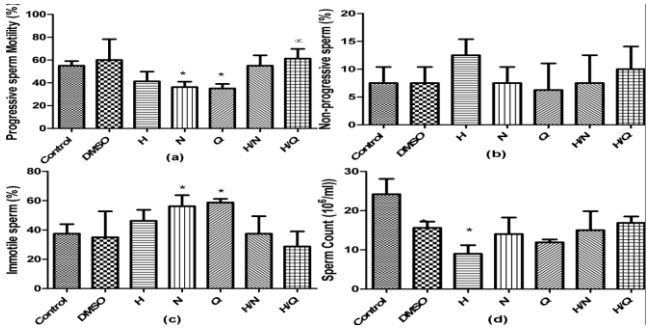

Figure 2: Sperm motility% and sperm count *p<0.05 compared to control, $^{\alpha}p$<0.05 compared to HAART. Role of cART on Oxidative Stress markers

There was a statistically significant increase in MDA level in the cART (p=0.0016) and DMSO treated (p= 0.0002) groups when compared with the control. However, when compared with the cART group, there was significant reduction in MDA level in the groups treated with a combination of cART with naringenin (p=0.0029) and quercetin (p=0.0471) (Fig 3d). GSH was significantly reduced (p=0.0242) in the cART group when compared with the control (Fig 3b); SOD level reduced significantly p=0.0044, 0.00001, 0.0231 in the DMSO, cART, and N groups when compared with the control, while groups H/N and H/Q increased significantly (p=0.00001, 0.0007 respectively) when compared with the cART treated group (Fig 3a). There was no significant change (p=0.7181) in CAT level when cART was compared with other groups and with control (Fig 3c).

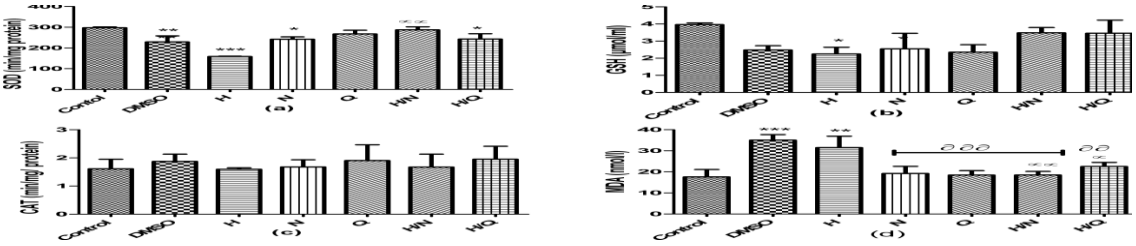

Figure 3: Oxidative stress markers. (a) Superoxide dismutase (SOD), (b) Reduced glutathione (GSH), (c) Catalase (CAT), (d) Malondiadehyde (MDA). *p<0.05, **p<0.01, ***p<0.001 compared to control, $^{\alpha}p$<0.05, $^{\alpha\alpha}p$<0.01, $^{\alpha\alpha\alpha}$ p<0.001 compared to HAART. $^{\delta\delta}p$<0.01, $^{\delta\delta\delta}p$<0.001 compared to DMSO.

Histopathology and immunohistochemistry of the testes

The cross section of testis from rats in the control and DMSO treated groups showed a normal cellular composition in their germinal epithelium with sperm cells in the lumen and a normal interstitial space. However, testes of the cART (H) group showed a marked depletion of cells of the spermatogenic series in the seminiferous tubules, significant distortion of the germinal layer and sloughing/degeneration of spermatogenic cells. A general mark of testicular dysfunction was observed and an onset of atrophy. The group treated with HN and HQ showed well-arranged seminiferous tubules similar to the control, while tiny vacuoles were observed within the germinal layer of the HQ group. The testes of the singly-treated bioflavonoids groups (Q and N) displayed well-structured seminiferous tubules as well (Fig 4).

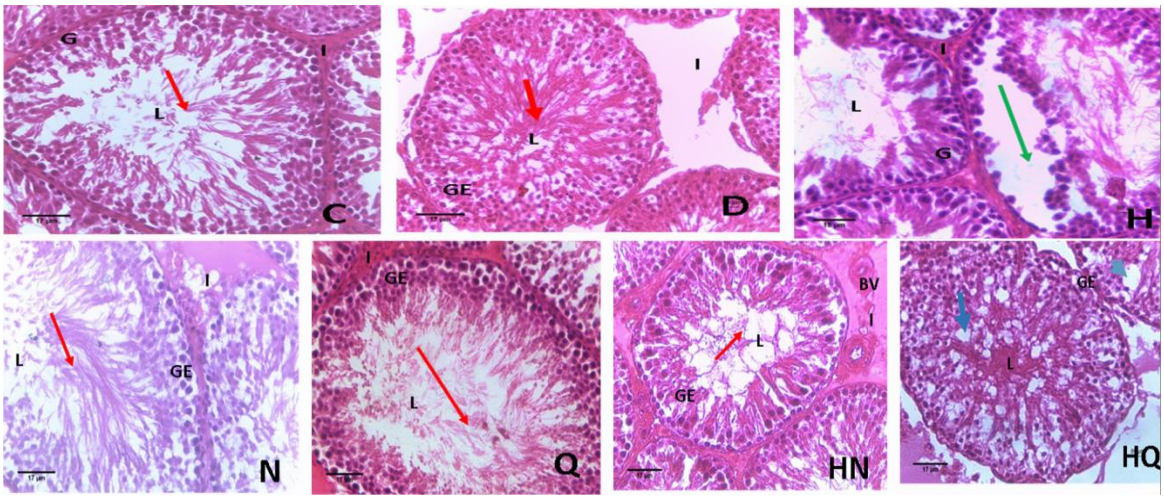

Figure 4: Photomicrographs of testis in HE stain of control (c) and treated rats x400. D-DMSO; H-cART; N-Naringenin; Q-Quercetin; H/N-cART+Naringenin ; H/Q-cART+Quercetin.

All photomicrographs (Fig 5) showed varying degrees of expression of 3β HSD in the interstitial cells of Leydig. However, quantification of the expression of 3β HSD showed a significant reduction of 3β HSD in the cART-only treated group when compared with the control (p=0.0263), while quercetin-only treated group reduced significantly when compared with control and DMSO (p=0.0001, 0.0022 respectively). The group treated with cART+Q however showed a significant increase in the expression of 3β HSD when compared with the cART-only treated group (p=0.0468) (Fig 5).

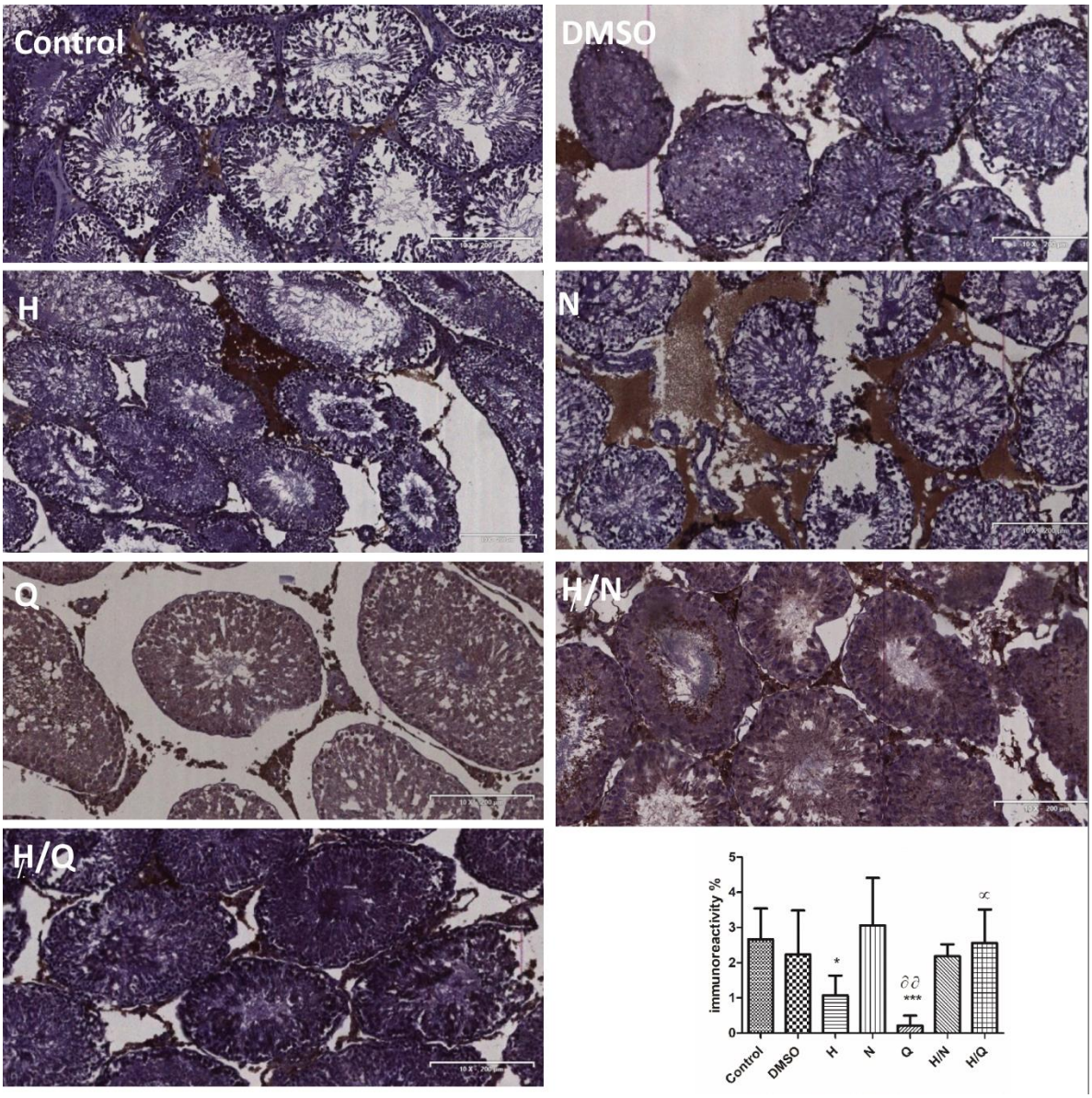

Figure 5: Photomicrographs of testis showing immunohistochemistry of 3 beta hydroxysteroid dehydrogenase (3β HSD) in the interstitium of testis of control and treated rats x100. H-cART; N-Naringenin; Q-Quercetin; H/N-cART+Naringenin; H/Q-cART+Quercetin. Chart shows mean area percentage of 3 beta hydroxysteroid dehydrogenase (3β HSD) immunohistochemical stains in the interstitium of the testis. *p<0.05 compared to control, ***p<0.001 compared to control, $^{\alpha}p$<0.05 compared to HAART. $^{\delta\delta}p$<0.01 compared to DMSO.

Morphometric (stereological) analysis

There was a significant increase in luminal volume of the testes treated with quercetin only when compared with control, and DMSO (p=0.0066, 0.0134 respectively) while only the groups treated with DMSO, Naringenin and Quercetin alone showed a significant increase (p=0.0017, 0.00001, 0.0437 respectively) in interstitium when compared with the control. (Fig 6).

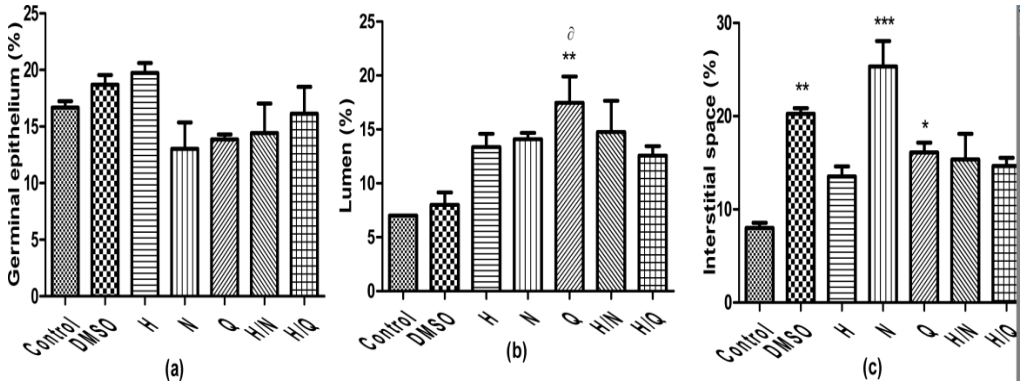

Figure 6: Relative volume fraction (%) of germinal epithelium, lumen and the interstitial space of testis. *p<0.05, **p<0.01, ***p<0.001 compared to control, $^{\delta}p$<0.05 compared to DMSO.

Fig 7: Reproductive hormones: Testosterone, Luteinizing hormone, Follicle stimulating hormone (FSH). *p<0.05, **p<0.01, ***p<0.001 compared to control, $^{\alpha}p$<0.05 compared to HAART.

Effect of cART on testicular hormones

Testosterone and LH levels were significantly increased in the H/N (p=0.0375) and H/Q (p=0.0370) groups respectively. FSH however, showed a significant decrease in the groups treated with DMSO, cART, Q, and cART+N (p=0.0284, 0.0014, 0.0103, 0.0052) when compared with control (Fig 7).

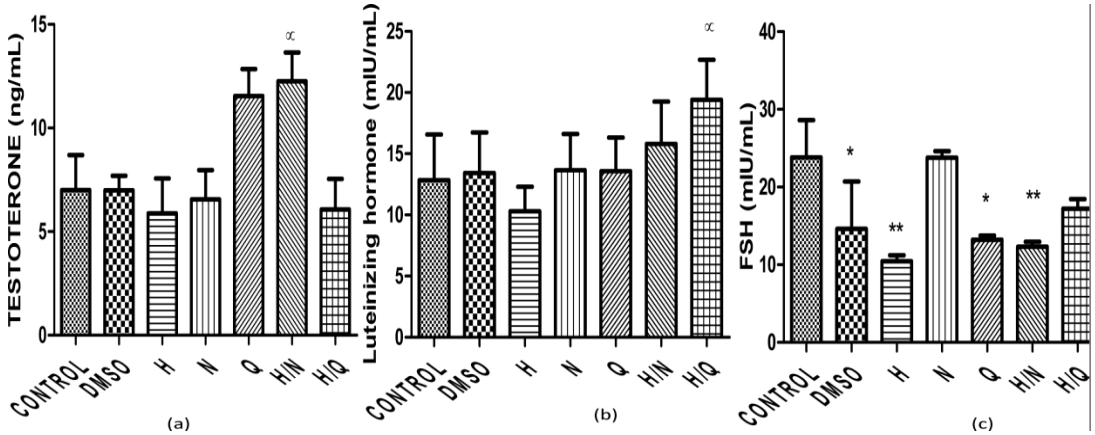

Figure 7: Reproductive hormones: Testosterone, Luteinizing hormone, Follicle stimulating hormone (FSH). *p<0.05, **p<0.01, ***p<0.001 compared to control, $^{\alpha}p$<0.05 compared to HAART.

Discussion

The aim of this study was to find out the possible modulating and curative role of bioflavonoids in reducing the toxic effects of cART on the testes.

The improvements observed in the prevention and management of HIV infection is as a result of the long-term use of cART. This has also significantly reduced the incidence of AIDS and death rates in Africa and other parts of the World (The Antiretroviral Therapy Cohort Collaboration, 2008; Liu, 2016; Adana *et al*., 2018). Our study shows that cART treatment caused decrease in sperm count, marked depletion of cells of the spermatogenic series, increased luminal width and interstitium. This is in agreement with the findings of earlier reported studies (Azu *et al*., 2014; Adana *et al*., 2017; Adana *et al*., 2018), where abnormalities in the semen parameters as well as depletion of cells of the spermatogenic series and distortion of germinal

epithelium were described. It has been reported that many of the cART drugs are culprits of oxidative stress-facilitated mechanisms (Azu *et al.,* 2018). In particular, Efavirenz a non-nucleoside reverse transcriptase inhibitor (NNRTI) has been shown to cause cellular degeneration, vacuolation, lipid disturbances and oxidative stress (Adjene and Igbigbi, 2010). As stated earlier, cART's ability (especially NRTIs) to increase reactive oxygen species production may result in decreased mitochondrial transmembrane potential which ultimately leads to the release of cytochrome C. The release of this protein then subsequently initiates programmed cell death (Sergerie *et al*., 2004). This may further explain the degeneration of spermatogenic cells together with the depletion of the germinal epithelium observed following ART administration. In addition, many authors believe that NRTIs such as Telofovir, target the mitochondria and reputably compromise the energy reserve of spermatozoa consequently leading to cell death (Dulioust *et al*., 2002; van Leeuwen *et al*., 2008; Lambert-Niclot *et al*., 2011; Azu and Naidu, 2018).

Indeed, research has shown that an increase in oxidative stress with a concomitant decrease in the level of antioxidants is one of the negative effects of cART (Mandas *et al*., 2009). This is agreement with the results from the present study which showed that the level of MDA was significantly increased following cART administration while SOD and GSH were reduced significantly. The plausible explanation for this can be that cART may induce (i) an increase in the generation of oxidants (ii) a decrease in the protective potentials of antioxidants (iii) a failure to repair oxidative stress damage (Ngandi *et al.,* 2006). While cART may induce a reduction in the protective potentials of antioxidant defence system as suggested by Ngandi *et al.* (2006) and supported by our study, it has been reported that cART may specifically reduce GSH level by reducing GSH synthesis, enhancing its utilization or limiting the intracellular reduction of its oxidized form [GSSG] (Sen, 2000). Hence the resultant GSH insufficiency as seen in the present study may compromise a number of related functions such as spermatogenesis, spermiogenesis, and stereodogenesis, ultimately leading to testicular dysfunction.

Efavirenz has been reported to increase morphological alterations of the mitochondria, ultimately leading to mitochondrial dysfunction and induction of oxidative stress (Polo *et al.,* 2015; Ganta *et al.,* 2017). Our study shows a significant decrease in the expression of 3β HSD in the cART-only treated group compared with control. Wang *et al.* (2015) previously reported that alterations in testicular mitochondrial antioxidants adversely affect 3β HSD expression and Leydig cell stereodogenesis. Indeed these effects could be observed because normal stereodogenesis is promoted with a normal functioning mtochondrial pH, membrane potential and ATP synthesis (Park *et al.,* 2014). Treatment with bioflavonoids however, as revealed by the present study attenuated the deleterious effects of cART on 3β HSD expression possibly through the oxidant-antioxidant pathway. However, the group treated with Quercetin alone showed a significant reduction in 3β HSD expression. Research has shown that flavonoids like Quercetin can generate ROS by auto-oxidation and redox-cycling (Ranwat and Bakshi, 2017). Hence, this effect is also possibly acting through the oxidation pathway.

Quercetin and Naringenin are bioflavonoids abundantly present in fruits and vegetables (Formica and Regelson, 1995; Yáñez *et al.,* 2008). These bioflavonoids in addition to possessing antioxidant properties also have the unique ability to act as pro-oxidants (Watjen *et al.,* 2005; Ranwat *et al.,* 2013; Ranwat and Bakshi 2017). The protective potentials of these bioflavonoids can be attributed to their ability to prevent antioxidant enzyme inactivation by pro-oxidants, scavenge free radicals and prevent excessive lipid peroxidation (Roy *et al.,* 2013; Adana *et al.,* 2017). From the present study, combination of cART and Quercetin as well as combination of cART and Naringenin resulted in a better improvement in sperm count and motility as well as the histomorphometry of the testes when compared

with administration of cART alone. Interestingly however as stated earlier, the pro-oxidant potential of both bioflavonoids was also observed in the motility of the spermatozoa where treatment with naringenin or quercetin alone showed significant decrease in progressive motile sperm while treatment with combination of cART and Quercetin showed a significant increase. The study also revealed that the concomitant addition of bioflavonoids together with cART significantly reduced the level of MDA. The plausible explanation for this observation can be hinged on the report that under stress conditions, Naringenin and Quercetin have the potential to chelate ions and scavenge reactive oxygen species (Mostafa *et al.,* 2016). Hence they attenuate cellular damage, and restore normal cellular homeostasis and physiological function within tissues (De Oliveira *et al.,* 2016)

While cART did not significantly reduce the level of testosterone and LH, it significantly reduced the level of FSH in the present study. FSH is known to support spermatogenesis. This may further explain the reduced sperm count as well as the depletion of spermatogonia and distortion of the germinal epithelium observed in the cytoarchitecture of the testes. The concomitant administration of quercetin with cART was able to prevent the significant reduction of FSH however. Osawe and Farombi (2018) in their study reported that quercetin significantly reversed alterations in plasma gonadotropins. They reasoned that the antioxidant capacity of quercetin enables it to protect the testicular sterodogenic enzyme machinery against oxidative stress which is in agreement with the present study. In addition, it was observed that combination of cART and Naringenin showed an increase in the level of testosterone when compared with control and other treated groups. This agrees with the findings of Adana *et al.* (2018) who reported similar findings in serum testosterone level. Reports on the role of Naringenin on testosterone activity in males are quite limited. However, it is possible that Naringenin inhibits a key steroid metabolizing enzyme UDP-glucuronosyltransferase, hence reducing the rate of testosterone glucuronidation (Jenkinson *et al.,* 2012) and thereby increasing the availability of testosterone.

Conclusion

The present study suggests that bioflavonoids, if used together with cART have the potential to ameliorate the deleterious effects of cART on the testes and improve the quantity and quality of the spermatozoa.

Conflict of Interest

Authors declare that there are no conflicts of interest

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