SEQUENTIAL ADMINISTRATION OF FEBUXOSTAT, AMLODIPINE AND VITAMIN E ATTENUATE OXIDATIVE STRESS AND IMPROVE SPERMATOGENESIS IN TESTICULAR ISCHEMIA-REPERFUSION INJURY IN WISTAR RATS.

ABSTRACT

This study investigated the effects of sequential administration of febuxostat, amlodipine, and vitamin E on oxidative stress and spermatogenesis following testicular ischemia-reperfusion injury (TIRI) in Wistar rats. Ninety male rats (120-150g) were divided into 9 groups (n=10 each): sham operation (SO), torsion-detorsion (TD), and seven treatment groups receiving different combinations of the drugs. The treatment groups included torsion + febuxostat + detorsion (TFD), torsion + detorsion + amlodipine (TDA), torsion + detorsion + vitamin E (TDV), and combinations thereof (TFDA, TFDV, TDAV, TFDAV). TIRI was induced by 720° clockwise testicular torsion for 1 hour followed by detorsion. Febuxostat (5 mg/kg) was administered 30 minutes after torsion, amlodipine (2.5 mg/kg) immediately upon detorsion, and vitamin E (10 mg/kg) 30 minutes after detorsion.

Rats were sacrificed 56 days post-reperfusion. Testicular tissue was analyzed for antioxidant enzymes (superoxide dismutase, catalase), lipid peroxidation (malondialdehyde), total protein, inflammatory markers (serum nitrite, IL-1β), and spermatogenesis indices (testicular biopsy score, Leydig cell count).

Results showed that TIRI significantly decreased antioxidant enzymes, increased lipid peroxidation and inflammatory markers, and impaired spermatogenesis. In the TD group, superoxide dismutase (SOD) and catalase (CAT) activities decreased, while malondialdehyde (MDA) increased compared to the SO group ($p<0.01$). Serum nitrite and IL-1β levels in the TD group were also increased ($p<0.001$). Furthermore, the testicular biopsy score and Leydig cell count in the TD group were decreased in this study ($p<0.01$).

Sequential administration of febuxostat, amlodipine and vitamin E, particularly when all three were used (TFDAV group), significantly attenuated these changes. In the TFDAV group, SOD and CAT activities were improved, while MDA decreased compared to the TD group ($p<0.05$). Serum nitrite and IL-1β levels in the TFDAV group were also decreased ($p<0.001$). In addition, the testicular biopsy score and Leydig cell count in the TFDAV group increased in comparison with the TD group ($p<0.001$).

The study concludes that this sequential multi-drug approach shows promise in mitigating the long-term detrimental effects of TIRI on testicular function and fertility. The combination of febuxostat (a xanthine oxidase inhibitor), amlodipine (a calcium channel blocker), and vitamin E

(an antioxidant) appears to provide protection against oxidative stress and inflammation induced by TIRI, thereby preserving spermatogenesis.

INTRODUCTION

Testicular ischemia-reperfusion injury (TIRI) is the primary pathophysiological event in testicular torsion repair (Shimizu *et al*., 2011). It is caused by reoxygenation of the testis after episode of ischemia (Eltzschig and Eckle, 2011). It is a common urological emergency in male of all ages (George and Nick, 2018). TIRI has been reported to cause male infertility via activation of oxidative stress (Martinon, 2010) which occur as a result of imbalance between pro-oxidants and antioxidants thereby resulting in cell or tissue injury (Cay *et al*., 2006). Oxidative stress have been documented to disrupt the capacity of the germinal epithelium to differentiate into normal sperm cells (Kalogeris *et al*., 2012).

Based on previous studies, testicular ischemia-reperfusion injury induced testicular damage is via two mechanisms; direct action on testicular DNA, protein, lipids and carbohydrate (Kalogeris *et al*., 2012) and indirect action via non-radical oxidants such as hydrogen peroxide, modulator via molecular bond, oxidative or nitrosative principle regulatory proteins. In addition to this, TIRI-induced oxidative stress has been reported to increase lipid peroxidation process, deplete antioxidant enzymes, activate oxido-inflammatory response and increase the rate of mitochondria mediated apoptosis in germ cells (Lysiak *et al*., 2003).

During testicular ischemia-reperfusion injury, reactive oxygen species (ROS) is generated during the ischemic phase, early phase of reperfusion and minutes after reperfusion (Granger and Kviety, 2015). In the ischemic phase, ROS is generated via xanthine oxidase (XO) build-up, in the early phase of reperfusion via calcium-mediated ROS-production while minutes after reperfusion ROS burst is generated by leukocyte recruitment to the site of injury (Turner *et al*., 2004). This generated ROS is capable of forming peroxynitrite radicals which can interfere with cellular structures such as proteins, lipids and DNA to cause severe oxidative damage to the testes (Antonuccio *et al*., 2006; Kalogeris *et al*., 2012; Minutoli *et al*., 2015). The ROS generated minutes after reperfusion is capable of activating germ cell apoptosis thereby resulting in late organ damage in the long-run (Granger and Kvietys, 2015).

Based on the multifactorial nature of TIRI pathway before and after repair of torsion of the testes (Kalogeris *et al*., 2012), it is necessary to prevent TIRI-induced oxidative stress damage to the testes urgently by blocking sources of ROS in the ischemic phase, early phase of reperfusion and minutes after reperfusion. In this study, febuxostat was used to block xanthine oxidase (XO) driven ROS production during TT to reduce oxidative stress induced testicular damage (Wang *et al*., 2015) in the ischemic phase, amlodipine (Ferrari, 1997; Mason, 2002) was administered immediately on detorsion to prevent Calcium-mediated ROS production (Lysiak *et al*., 2001; Berkels *et al*., 2004; Dogan *et al*., 2015) while vitamin E was administered minutes after detorsion to reduce intratesticular ROS production that can trigger cell death pathways (Turner *et al*., 2004).

Febuxostat is a non-purine xanthine oxidase (XO) inhibitor (Wang *et al*., 2015; Granger and Kviety, 2015; Khan *et al*., 2017). It has been reported to reduce ischemia-reperfusion injury via it suppressive effect on xanthine oxidase-reactive oxygen species (XO-ROS) production. Amlodipine has an antioxidant features effective in reducing vascular ischemia-induced damage (Dogan *et al*., 2015; Javanmardi *et al*., 2018) while Vitamin E, is a commonly consumed lipid-soluble antioxidant previously reported to improve spermatogenic function after TIRI induction in rats (Adekeye *et al*., 2014; Zubair, 2017). This study therefore investigates the effect of sequential administration of febuxostat, amlodipine and vitamin E on attenuating oxidative stress induced testicular damage following TIRI.

Materials and Methods

Experimental animal design and treatment

Male Wistar rats 120- 150 g were fed with pelletized feed obtained from commercial dealer in Ogbomoso and watered *ad libitum*. They were kept throughout the experiment in well aerated plastic cages in the Animal House of Ladoke Akintola University of Technology (LAUTECH), Ogbomoso, Oyo State, Nigeria before the onset of the study. Ethical approval was obtained from the ethical research committee of Faculty of Basic Medical Sciences, LAUTECH with reference number: ERCFBMSLAUTECH: 033/05/ 2024.

Drugs and reagents

All drugs and reagents used were of high analytical grade. Febuxostat and carboxymethylcellulose (CMC) solution were purchased from TCI chemicals, India (product number: FO840) and LOBA Chemie Pharmaceutical, Ltd. India: Product number: 0253000100. Alpha-Tocopheryl Acetate (Vitamin E) (Gujarat Liqul Pharmacaps Pvt. Ltd, Gujarat, India) and Amlodipine tablets (Swiss Pharmaceutical Pvt. Ltd. Ahmedabad-382445, Gujarat, India).

Experimental Design

Ninety (90) male Wistar rats were divided in 9 groups (n=10) as follows:

Group 1: Sham; Rats in this group underwent surgery, without TIRI induction.

Group 2: Torsion + Detorsion (TD); Rats underwent left unilateral testicular torsion (TT) for one hour and testicular detorsion followed immediately to induce reperfusion for 56days.

Group 3: Torsion + Febuxostat + Detorsion (TFD); Rats received 5 mg/kg of Febuxostat after 30 minutes of testicular torsion and testicular detorsion followed 30 minutes later, thereafter reperfusion was allowed for 56 days.

Group 4: Torsion + Detorsion + Amlodipine (TDA); Rats received 2.5 mg/kg of amlodipine immediately on detorsion that is, one hour after testicular torsion was induced.

Group 5: Torsion + Detorsion + Vitamin E (TDV); Rats received 10 mg/kg of vitamin E 30 minutes after detorsion, that is one hour ,thirty minutes after torsion was induced.

Group 6: Torsion + Febuxostat + Detorsion+ Amlodipine (TFDA); Rats received 5 mg/kg of Febuxostat after 30 minutes of testicular torsion and 2.5 mg/kg of amlodipine immediately on detorsion.

Group 7: Torsion + Febuxostat + Detorsion+ Vitamin E (TFDV); Rats received 5 mg/kg of Febuxostat after 30 minutes of testicular torsion and 10 mg/kg of Vitamin E 30 minutes after detorsion.

Group 8: Torsion + Detorsion + Amlodipine + Vitamin E (TDAV); Rats received 2.5 mg/kg of amlodipine immediately on detorsion and 10 mg/kg of vitamin E 30 minutes after detorsion.

Group 9: Torsion + Febuxostat + Detorsion + Amlodipine + Vitamin E (TFDAV): Rats received 5 mg/kg of Febuxostat after 30 minutes of testicular torsion and 2.5 mg/kg and 10 mg/kg of Vitamin E immediately on detorsion and 30 minutes after detorsion respectively.

Experimental induction of testicular ischemia-reperfusion injury

The rats were fasted for 12 hours prior to the experiment. They were weighed and anaesthetized with Ketamine (50 mg/kg) and Xylazine (10 mg/kg) intraperitoneally (Lorenzini *et al*., 2012;

Afolabi *et al*., 2022).The rats were restrained on the dissecting board. The left scrotal, perineal and inguinal areas of the rats were shaved and cleaned with methylated spirit. The left testis was firmly grasped and the caudal epididymis was located and used as a reference point. A high left scrotal incision was made to slightly open up the tunica vaginalis to locate the testis. The edges of the tunica vaginalis was clamped with toothed dissecting forceps to produce a tissue plain. The essence of this is to enhance easy returning of the testes back into the scrotum. A gentle pressure was applied to push the left testes out. The gubernaculum testes was located and cut off to free the left testes. The freed left testis was twisted at 720^0 in a clockwise direction to induce ischemia for one hour. A pouch was created in the scrotum with a long surgical scissors into which an anchoring suture was passed from outside into the inside and attached to the tuft of tissue in-between the testes and epididymis and then passed outward and pulled down to ensure the testes is returned into the scrotum to remain in a twisted state. The incision site was closed up with 2-0 chromic suture. After one hour of torsion, the rats were opened up to untwist the testes to induce reperfusion which lasted for 56 days. This procedure was explained as described by Afolabi *et al*. (2022).

Animal sacrifice, blood and serum collection

Fifty-six (56) days after reperfusion, the rats were anaesthetized with ketamine (50 mg/kg). Blood was collected through retro-orbital sinus using heparinized capillary tube and introduced into the plain bottles. The blood collected into the plain bottles were allowed to clot for 15 minutes and then centrifuged at 2500 revolutions per minutes for 15 minutes to obtain serum. The serum was collected into Eppendorf bottles with Pasteur pipettes and refrigerated for further analysis.

Tissue collection and preparation of testicular homogenate

Testicular tissue were harvested and cleared of adherent tissue. The tissue were cut longitudinally such that one section was fixed inside Bouin's fluid for histological examination of testicular biopsy score and Leydig cell count while other section were homogenized and centrifuged for assay of biochemical parameters.

Biochemical Analysis

Testicular homogenates was used to assess superoxide dismutase (SOD) activity spectrophotometrically using the protocol of Misra and Fridovich, (1972). Catalase activity was assessed spectrophotometrically at 570-610 nm using the method of Sinha, (1972), MDA concentration was evaluated according to the method of Adegunlola *et al*. (2012). Total protein concentration was assessed by the method of Ellam, (1959). Serum nitrite concentration was assessed by checking the nitrite level as described by Fox and Suhre (1985) while interleukin-1B was assessed using ELISA kit as described by the manufacturer.

Assessment of Testicular biopsy score and Leydig cell count

After staining the sections with haematoxylin-eosin stain, they were viewed under light microscope (Omax 40x-2, 500x LED binocular Lab compound Microscope, M82EZ-C50S, China) and the image were observed x 100 magnification. All the cells were counted manually in about 5-10 seminiferous tubules per tissue section or per photomicrograph with the assistance of an expert. The testicular biopsy score count, an index of complete spermatogenesis was scored using Johnsen's scoring system as previously described by (Afolabi *et al*., 2022). Each seminiferous tubule evaluated was scored between 1 and 10 depending on the presence or absence of germ cells while large round cells with 1-3 nucleoli in the interstitial space present outside the seminiferous tubules were counted as Leydig cells.

Statistical analysis

Data were expressed as mean ± standard error of mean (Mean ± SEM). Analysis of variance with Graph Pad Prism version 7.0 (Graph Pad statistical software, Inc., USA) was used to compare within groups and Tukey's Post-hoc test was used for multiple comparison.

RESULTS

From the result obtained, the testicular biochemical parameters such as SOD, CAT and total protein were decreased during torsion + Detorsion (Reperfusion injury) while MDA was increased during torsion + Detorsion. Tissue SOD ($p<0.01$), CAT ($p<0.01$), MDA ($p<0.01$) and total protein ($p<0.01$) were decreased in torsion + Detorsion group rats when compared with rats in the sham group while MDA Tissue SOD was significantly increased ($p<0.05$) in TFD, TDV, TFDA, TFDV and TFDAV only, CAT was significantly increased in TFD, TDV, TFDV and TFDAV only ($p<0.05$), total protein was significantly increased in all the treated groups (TFD, TDA, TDV, TFDA, TFDV, TDAV & TFDAV) ($P<0.05$, 0.01, 0.001) while testicular MDA was significantly decreased in all the groups as well (TFD, TDA, TDV, TFDA, TFDV, TDAV & TFDAV) (febuxostat and vitamin E ($p<0.01$, $p<0.001$) (Fig. 1A, B, C, & D).

IL-1β and serum nitrite were increased ($p<0.001$) in the Torsion + Detorsion group when compared to sham group but all the treated groups significantly reduced IL-1β and serum nitrite ($p<0.05$; 0.01; 0.001) (Fig.2A-B).

Indices of spermatogenesis such as testicular biopsy score and Leydig cell count were reduced in Torsion + Detorsion rats when compared to sham group while all the febuxostat administered group (TFD, TFDA, TFDV & TFDAV) were significantly increased both testicular biopsy score and Leydig cell counts (P<0.01;0.001) (Fig. 3A-B).

DISCUSSION

This study shows that long-term testicular ischemia-reperfusion injury (TIRI) after torsion repair can cause infertility in male via impact of oxidative stress and inflammation on spermatogenesis indices. This study also demonstrates the ameliorative effect of sequential administration of febuxostat, amlodipine and vitamin E on the oxido-inflammatory effect of TIRI.

Antioxidants investigated include superoxide dismutase (SOD) and catalase (CAT). In this study, the observed decrease in SOD, and catalase activities in the TD group is an indication of oxidative stress caused as a result of increase depletion of these antioxidants by free radicals generated during reperfusion injury. These enzymes are crucial for maintaining the redox balance in the body. SOD is an antioxidant enzyme that catalyses the dismutation of superoxide radicals (O^{-2}) to generate oxygen (O_2) and hydrogen peroxides (H_2O_2) (Olufunmilayo *et al*., 2023). The antioxidant activity of catalase is dependent on the degree of SOD activity because it breaks down hydrogen peroxide (H_2O_2) into water and oxygen (Hamam *et al*., 2022).

Administration with only febuxostat or vitamin E, and combination of these treatments (TFD, TDV,TFDA, TFDV, and TFDAV), resulted in a significant increase in SOD activity compared to the Tor + Det group. Also, catalase activity was significantly increased in the groups treated with febuxostat and vitamin E, singly or combined, and the group that received all three treatments. This suggests that these treatments preserved the antioxidant capacity in the testicular tissue.

In this study, the observed reduction in testicular total protein concentration in the TD is also indicative of oxidative stress. Proteins are essential biomolecules involved in various cellular processes, including structural integrity, enzymatic catalysis, signalling pathways, and transport mechanisms (Battelli *et al*., 2014; Bhattacharyya *et al*., 2014). The depletion of protein level suggests a disruption in protein synthesis, increased protein degradation, or a combination of both, as a consequence of the oxidative stress and cellular damage associated with IRI (Ayala *et al*., 2014; Granger and Kvietys, 2015).

Ischemia-reperfusion injury induces ER stress, which is characterised by accumulation of misfolded or unfolded proteins in the ER lumen (Granger and Kvietys, 2015). Prolonged ER stress, as may be the case in this study 56 days after TIRI, can lead to cell death and tissue injury. Also, there can be disruption of cellular energy metabolism, leading to a depletion of ATP levels and subsequent impairment of energy-dependent processes (Bhattacharyya *et al*., 2014). Moreover, oxidative stress and damage to ribosomes and transcriptional machinery can further compromise protein synthesis (Battelli *et al*., 2014; Ayala *et al*., 2014). Proteins, which are susceptible to oxidation by ROS, can form reversible or irreversible oxidative modifications, such as disulfide bonds, sulfenic acids, and sulfinic/sulfonic acids (Ghezzi, 2013). These modifications can alter protein structure and function, contributing to cellular dysfunction and tissue injury (Ghezzi, 2013; Ayala *et al*., 2014). Sequential administration of febuxostat, amlodipine and vitamin E probably due to their combined antioxidant effect showed better potential in preserving protein levels in the tissue. Febuxostat may reduce oxidative modifications and degradation of proteins (Kim *et al*., 2020). Moreover, it may indirectly promote proper protein folding, contribute to the preservation of protein levels by suppressing ER stress. Amlodipine on the other hand, prevents calcium overload to preserve mitochondrial function and integrity, and thus reduce the production of ROS and subsequent oxidative stress (Park *et al*., 2019).Vitamin E neutralises ROS generation, thereby preventing the depletion of NPSH and the oxidation of PSH, contributing to the maintenance of antioxidant defences and protein integrity.

Malondialdehyde (MDA) is an established marker of oxidative stress in TIRI. This study corroborates this fact as reflected in their significantly higher levels in the TD group. MDA, a marker of lipid peroxidation emanates from the degradation of polyunsaturated fatty acids in cellular membranes (Ayala *et al*., 2014), Malondialdehyde (MDA) is one of the by-products of lipid peroxidation (Kalogeris *et al.*, 2012); hence increased MDA levels in the testis homogenate indicated the occurrence of membrane lipid peroxidation and damage via repeated radical chain reactions. Upon reperfusion, XO catalyzes the generation of ROS which can lead to lipid peroxidation and oxidative stress. Administration of febuxostat caused inhibition of XO which attenuate the production of uric acid and consequently ROS generation, thereby mitigating lipid peroxidation and preserving endothelial function (Granger and Kvietys, 2015; Kim *et al*., 2020), this may be responsible for the observed reduction in MDA level in the group than TDAV. Amlodipine and vitamin E also help attenuate oxidative stress by scavenging ROS, which in turn preserve the normal levels of MDA (Ganafa *et al*., 2004; Traber and Stevens, 2011). Similarly, by maintaining proper calcium levels, amlodipine can preserve mitochondrial function and integrity, reducing the production of ROS and subsequent lipid peroxidation (Peng and Jou, 2010).

One of the main features associated with IRI is inflammation, which greatly contributes to tissue damage and compromised function. Serum nitrite levels, which is a storage form of nitric oxide (NO), has been reported to be involved in inflammatory response following TIRI (Reddy *et al*., 2006). NO is produced from the oxidation of L-arginine in the vascular endothelium under the catalytic action of endothelial nitric oxide synthase (eNOS) (Fawzy and Aisel, 2023). Nitric oxides (NO) interact with oxygen radicals (O^{2-}) to form reactive nitrogen species such as peroxynitrites which are more reactive and injurious *(*Piacenza et al., 2022*)*. Hence, high level of nitrite 56 days after post-reperfusion suggests the occurrence of oxidative damage by reactive nitrogen species interactions. When TIRI occurs, the inflammatory response includes the release of pro-inflammatory cytokines like interleukin-1 beta (IL-1β) (Kalogeris *et al*., 2012). During reperfusion, the pro-inflammatory cytokines cause recruitment of neutrophils from blood to the site of injury. This results in the production of reactive oxygen species (ROS), reactive nitrogen species (RNS) and proteolytic enzymes that are highly toxic into the cell and therefore cause tissue damage (Liu *et al.,* 2023). In this study, the observed increase in the level of serum NO and IL-1beta suggest heightened inflammatory state resulting from ischemia and subsequent reperfusion injury, and is consistent with previous studies demonstrating their upregulation in ischemia-reperfusion injury (IRI) (Reddy *et al*., 2006; Chen *et al*., 2017).

The decrease in IL-1β level in the groups treated with febuxostat, amlodipine, and vitamin E, either alone or in combination suggest potent anti-inflammatory abilities of these drugs. The anti-inflammatory action of vitamin E can contribute to the attenuation of the inflammatory response associated with TIRI. It has been suggested that xanthine oxidase plays an important role in inflammatory processes and febuxostat can modulate the inflammatory responses by inhibiting ROS production which may contribute to opening of mitochondrial permeability transition pore (mPTP) (Kalogeris *et al*., 2012). Increased mitochondrial calcium level will lead to activation of calcium pyrophosphate which eventually results in increased release of interleukin-1-B (Kalogeris *et al*., 2012; Amirshahrokhi, 2019).

Spermatogenesis indices measured include Leydig cell count and testicular biopsy score count. The testicular biopsy score provides a comprehensive assessment for the evaluation of various cells as they are arranged in the seminiferous tubules of the testes (Alukal *et al*., 2009). These cells are present in the seminiferous tubules of the testes for the process of sperm production. The observed decrease in Leydig cell count and testicular biopsy score count in TD indicates disruption of differentiation of germinal epithelium (spermatogonia) into spermatozoa, germ cell loss, impaired spermatogenesis and disruption of Leydig cell steroidogenic function attributed to increased ROS production during TIRI which is equally responsible for reduction in testicular biopsy score count observed. Previous studies have demonstrated that testicular ischemia-reperfusion injury results in permanent loss of spermatogenesis (Turner and Brown, 1993; Mestrovic *et al*., 2014) and ROS generated is the major cause of spermatogenic abnormalities.This finding is consistent with a previous study (Al-saleh *et al*., 2020) that reported TIRI-induced degeneration of germ cells and impaired spermatogenesis.

Elshaari *et al*. (2011) also demonstrated that TIRI-induced ROS was capable of disrupting the steroidogenic function of the Leydig cells. Careful investigation into the treatment groups showed that febuxostat administered in the ischemic phase (TFD, TFDA, TFDV, TFDAV) improved Leydig cell count and testicular biopsy score count 56 days after TIRI. This may be due to suppressive effect of febuxostat on xanthine oxidase-driven ROS production in the ischemic phase of TT as well as its anti-inflammatory effect against inflammatory responses that raised the intratesticular ROS to triggers germ cell apoptosis. Also, administration of febuxostat in the ischemic phase of TT may be responsible for the observed improvement in spermatogenesis indices in all the febuxostat administered rats when compared to those that received amlodipine only (TDA), vitamin E only (TDV), amlodipine and vitamin E administered only (TDAV) combined. Since male infertility has been connected to rapidly increased ROS production as reported by Aitken and Baker, (2004), the ROS has to be removed from the testicular environment rapidly, it is important to note that maximum protection against ROS is important to sustain Leydig cell and germ cells' integrity.

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Biochemical parameters

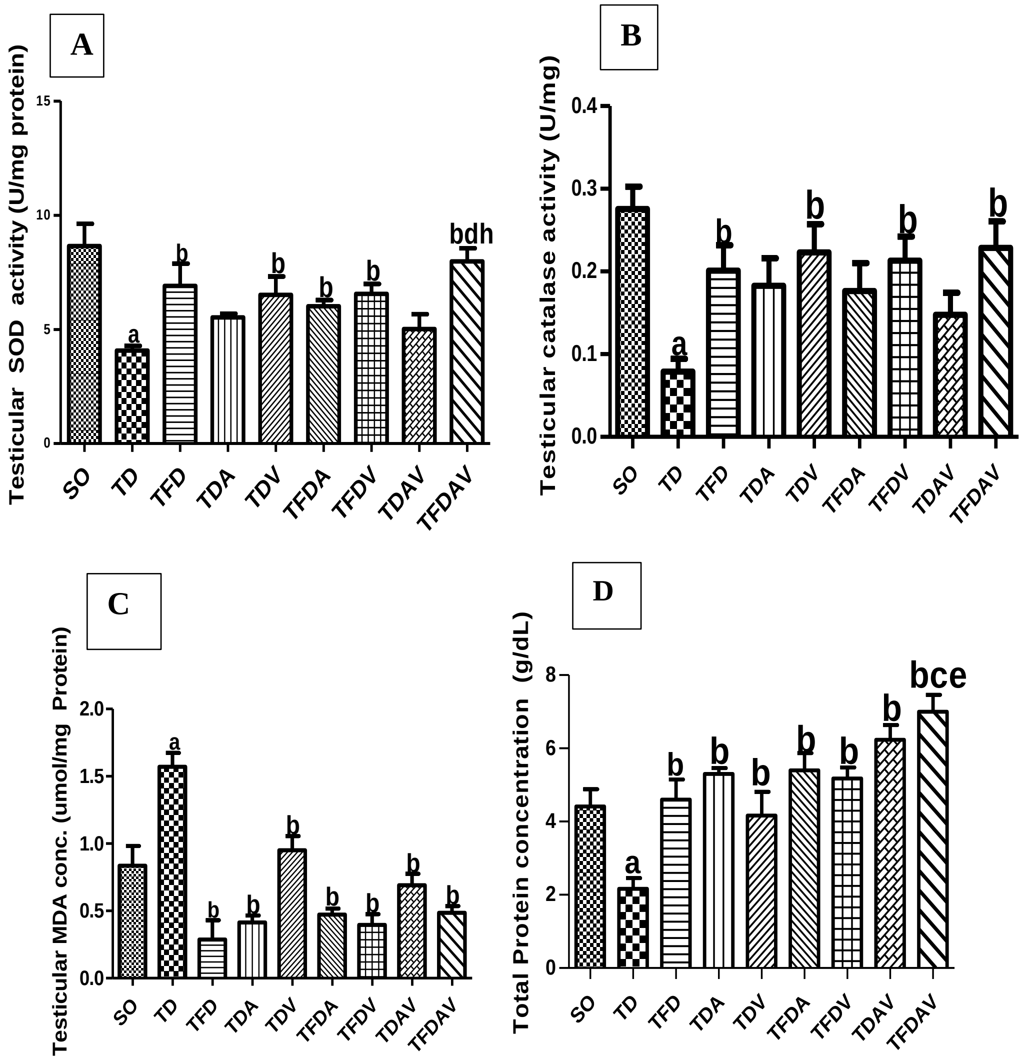

Fig. 1A-D: The effect of sequential administration of febuxostat, amlodipine and vitamin E on superoxide dismutase and catalase activity, malondialdehyde and total protein concentration in male Wistar rats 56 days after TIRI.

[a] represents significance at p<0.01 when compared to SO.
[b] represents significance at p<0.05 when compared to TD.
[c] represents significance at p<0.01 when compared to TFD
[d] represents significance at p<0.001 when compared to TDA.
[e] represents significance at p<0.01 when compared to TDV
[f] represents significance at p<0.001 when compared to TFDA.
[g] represents significance at p<0.01 when compared to TFDV.
[h] represents significance at p<0.001 when compared to TDAV.

Inflammatory markers

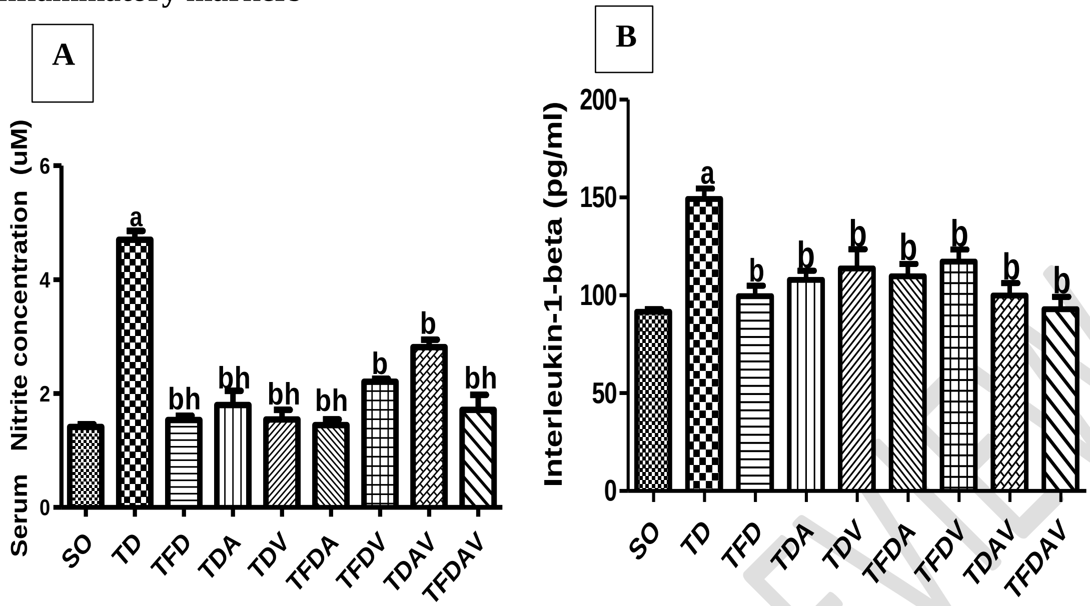

Fig. 2A-B: The effect of sequential administration of febuxostat, amlodipine and vitamin E on serum nitrite concentration and IL-1beta concentration in male Wistar rats 56 days after TIRI.

[a] represents significance at p<0.01; 0.001 when compared to SO.
[b] represents significance at p<0.05; 0.01;0.001 when compared to TD.
[h] represents significance at p<0.001 when compared to TDAV.

Testicular biopsy score and Leydig cell counts

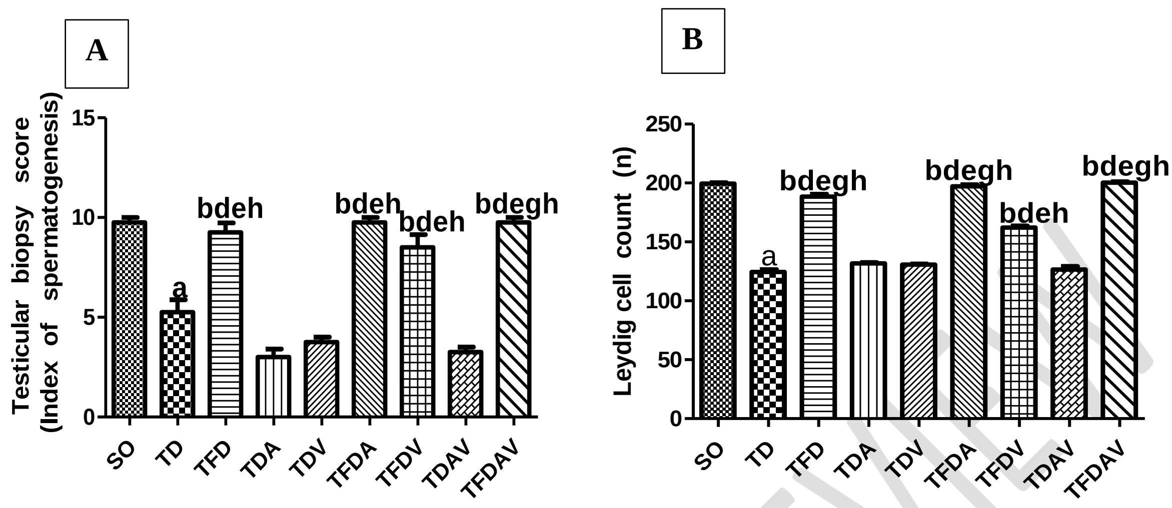

Fig. 3A-B: The effect of sequential administration of febuxostat, amlodipine and vitamin E on testicular biopsy score and Leydig cell count in male Wistar rats 56 days after TIRI.

[a] represents significance at $p<0.01$ when compared to SO.
[b]represents significance at $p<0.01$ when compared to TD.
[d] represents significance at $p<0.001$ when compared to TDA.
[e] represents significance at $p<0.001$ when compared to TDV.
[g] represents significance at $p<0.05$ when compared to TFDV.
[h] represents significance at $p<0.001$ when compared to TDAV.