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Atorvastatin Mediates Down Regulation of IFN-λ / Il-1β /NF-kB on Testicular Inflammation in Experimental Male Rats

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ABSTRACT: Testicular inflammation (TIN) provokes reproductive impairments via endocrine disruption. Atorvastatin (AT) is one of the statins that have a crucial role in attenuating metabolic disorders. In this study, the prospective impacts of AT on TIN generated by a high-fat fructose diet (HFFD) in the testicular tissue were investigated through the oxidative stress-inflammatory pathway. Male albino rats were employed in the research and split into three groups control (CR), induction (TIN), and treated (TIN-AT). Animals in TIN administrated 3 ml/kg Bwt ghee and coconut oil (3:1) (v/v) along with 25% fructose in daily drinking water, for 3 months, while AT used against TIN in AT-TIN was given for one month at 10 mg/Kg Bwt after TIN induction period. Indicators of liver and kidney functions in serum, oxidative stress, and inflammation in the testicular tissue were assessed using biochemical and molecular techniques. In addition, a histopathological examination was conducted. The acquired results evoked that TIN induced by HFFD leads to the reduction of antioxidant enzymes and an increase in the reactive oxygen species and inflammatory parameters. On the other hand, AT exhibited antioxidant influence by improving minimized glutathione levels, and glutathione-S-transferase and glutathione peroxidase enzyme actions, in testicular tissue. AT also attenuated lipid peroxidation and nitric oxide levels. Moreover, gene expressions of interferon-gamma, interleukin-1 beta, and nuclear factor-kappa B were downregulated in TIN-AT rats. AT could counter the pathomorphological alterations of TIN. Further studies are recommended to address other molecular roles of AT versus the TIN.

KEYWORDS: Testicular inflammation; atorvastatin; prooxidants; antioxidant enzymes; inflammatory markers.

1. Introduction

Inflammatory disorders are a cluster of diseases in which the immune system is attacked and altered inflammatory mediators are released [1]. The testicles are a key organ in the male reproductive system. It is comprised of seminiferous tubules and interstitial tissue. The seminiferous tubules contain spermatogonia and Sertoli cells, which are the site of the formation and generation of sperm. However, Leydig cells, which produce androgens that are necessary for male reproductive function, are found in the interstitial tissue. [2]. Likewise, the testicles implies an immunological response to defend its elements against hostile immune reactions [3].Testicular inflamation (TIN) is a pathological condition that affects the spermatogenesis and steroidogenesis process [4]. It involves two types, local TIN due to acute infection and systemic TIN [5]. TIN is triggered by diverse contributors including physiological as weight and age, pathological as metabolic disorders [6], and psychological factors such as stress and anxiety [7]. Also, elevated temperature [8], environmental pollution and sustained exposure to toxins and heavy metals are other causes of TIN [9]. Other lifestyles like smoking and alcohol sipping increase the prevalence of TIN [10]. Statins (STs) are categorized according to their origin into two candidates, i: natural STs which are obtained from fermentation products such as simvastatin, and ii: synthetic STs such as atorvastatin (AT), and rosuvastatin [11]. AT is a penta-substituted pyrrole that consists of two opposing fractions with an asymmetric heterocyclic core unit and a 3,5-dihydroxypentanoyl side chain similar to its parent molecule [12]. AT is a second-generation lipophilic statin characterized by its rapid absorption by passive diffusion into the non-hepatic organs and its level reaches the peak in the serum within two to three hours

[13]. AT has a longer action, and it is metabolized by cytochrome P3A4 in the liver to ortho and para-hydroxylated active metabolites [14]. AT has an extensive range of pharmaceutical applications. It ameliorates hypertension and restores vascular redox homeostasis [15]. Moreover, AT upgrades the cardiac hypertrophy incidence and atherosclerosis [16]. Besides, AT has neuroprotective [17] and antiproliferative impacts [18].In addition, the role of AT in respiratory disorders has been recorded in the case of acute pulmonary embolism [19]. As well, AT diminishes arteriosclerosis and atheromatosis [20]. In addition, it has immunomodulatory [21] and anticancer actions [22]. The main purpose of this research is to use molecular and histopathological approaches to ascertain how AT affects TIN caused by a high-fat fructose diet.

2. Materials and methods

2.1. Procurement of chemicals and drugs

Reduced glutathione (GSH), thiobarbituric acid (TBA), 5,5´-dithio-bis-2-nitrobenzoic acid (DTNB), p-nitro benzyl chloride, cumene hydrogen peroxide, tris hydrochloride, and agarose were gathered from Sigma-Aldrich Chemical Co (USA). Sodium nitrite and sodium nitroprusside were gotten from Merck company. Sulfosalicylic acid and sulfanilamide were acquired from Win Lab (UK). Additionally, N-1-naphthyl-ethylenediamine-dihydrochloride and trichloroacetic acid (TCA) were obtained from Coinbrook Bucks (England) and LOBA-Chemie, respectively. Further, phosphoric acid, ethyl alcohol, xylene, disodium hydrogen phosphate, sodium dihydrogen phosphate, sodium chloride, formalin, and normal saline were bought from El-Nasr Pharmaceutical Chemical Company, Egypt. Hematoxylin and eosin (H&E) stain was obtained from Ricca Chemical Company (Arlington). Isoflurane® and AT® were purchased from a pharmacy. Kits of alanine aminotransferase (ALT), aspartate aminotransferase (AST), urea, uric acid, creatinine, and total protein were purchaced from spectrum, Egypt. Easy-RedTM Total RNA Extraction Kit (Cat. No. 17063) and Maxime RT-PCR Premix Kit (Cat. No. 25131) were picked up from iNtRON Biotechnology, Inc. Additionally, 100bp DNA ladder RTU (Cat. No. DM001-R500) was got from genedirex. Interferon-gamma (IFN-λ), interleukin-1 beta (Il-1ß), and nuclear factor-kappa B (NF-kB) primers were procured from Willowfort (UK).

2.2. Animal grouping and study design

Twenty-five male albino rats (100-130 g) were obtained from animal residences, Faculty of Science for Boys Al-Azhar University, Assiut branch. Rats were housed in typical circumstances of a 12-hours light/dark cycle, humidity (45 ± 5 %), and temperature (22 ± 2°C). Animals were kept for two weeks for appropriate familiarization with the animal house circumstances in the Faculty of Veterinary Medicine, New Valley University. Rats had free access to food and water. Animals in each batch were kept in polypropylene cages. The death rate was four during the induction time. All the experimental procedures were granted permission by ethical regulation of the Institutional Review Board, Faculty of Medicine, Assiut University, 04-2023-10066. Animal model of TIN was implemented according to the adapted way [23, 24] where rats received a high-fat fructose diet (HFFD) by oral gavage of 3 ml/kg Bwt ghee and coconut oil (3:1) (v/v) along with 25% fructose in daily drinking water, for 3 months. The rats were arbitrarily dispensed into three sets (n=7) as follows, control (CR) received distilled water (dH_2O) orally all over the experimental period, induction (TIN), and treated (TIN-AT) received AT (10 mg/Kg Bwt) dissolved in dH_2O [25] orally for one month after induction time. Following the end of the experiment, rats were fasted for six hours before killing, weighed, and anatheized by 1% isoflurane, and all animal welfare was applied to minimize any stress. The Bwt changes were calculated by subtracting the final Bwt from the initial Bwt [26]. Blood samples were gathered in plain tubes from eye-canthus, centrifuged at 4000 rpm

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for 20 min., and sera were collected and retained at -20°C. Testicles from each rat were gathered, cleaned from peripheral structures carefully, washed with ice-cold normal saline, and weighed and their relative weight was recorded by dividing both testicles' weight on final rat Bwt and multiplying by 100 [27]. The right testicle was stored in liquid nitrogen and put at -80°C for biochemical and molecular inspection. The left one was anchored in 10% formalin for histopathological rating.

2.3. Biochemical analysis

2.3.1. Serum assays

In all studied groups, liver activity was estimated by analyzing both ALT (EC 2.6.1.2) and AST (EC 2.6.1.1) activities by commercial kits following the instructions of manufacture. Also, the kidney operation was ascertained by quantifying the concentration of urea, uric acid, and creatinine. Urea was determined using colorimetric kits based on its hydrolysis in serum samples in the presence of H_2O by urease enzyme at alkaline media and yields ammonia and carbon dioxide [28]. Additionally, the estimation of uric acid depended on the oxidation of uric acid in the sample by the uricase enzyme into allantion and hydrogen peroxide (H_2O_2). After that, the created H_2O_2 reacted with 4-amino antipyrine and 3,5-dichloro-2-hydroxybenzenesulfonic acid in the existence of peroxidase enzyme and produced quinonimine dye and H_2O. Finally, the concentration was measured at 546 nm by spectrophotometer [29]. Nevertheless, creatinine was analyzed by the kinetic fixed rate method where the creatinine in the specimen interacted with picric acid in an alkaline media [30]. First, to create the workable solution, equal parts of 38 mmol/l picric acid, and 0.4 mol/l sodium hydroxide were added in the tube. Forward that in quartz cuvette 100 µL of standard and every sample was introduced to 1 ml of working solution individually and the absorbance of each one was read at 490 nm exactly following 30 seconds, and represented as A_1, then after another 2 minutes precisely the absorbance was re-read, and it denoted as A_2. The concentration of creatinine in each specimen was computed by executing the formula [(ΔA of the sample / ΔA of the standard)*standard concentration] and the results were expressed as mg/dl.

2.3.2. Preparation of testicular tissue homogenate (TTH) and estimation of protein

The right testicle was decapsulated, and 2/3 of the parenchyma was weighed and blended in frigid cold phosphate buffer saline (PBS), 0.1 M, pH 7.4. The ratio of the PBS to the tissue was 9:1 (v/w). The homogenized tissue was centrifuged at 4000 rpm for 10 min. using cooling centrifuge (LE30000161, EU) and the supernatant was handled at -80°C for further assays. Determination of protein content in homogenate was accomplished in compliance with the rules of photometric kits and the concentration was expressed as g/dl.

2.3.3. Assessment of testicular pro-oxidative stress indicators

Lipid peroxidation (LPO) assay was performed in TTH by measurement of malonaldehyde (MDA). The methodology relies on the reaction of the MDA, a byproduct liberated when free radicals assault lipid membranes, and TBA [31]. The experiment entailed, adding 500 µL of dH_2O (in blank tube) and samples (in sample tubes) to 1 ml of 15% TCA, thoroughly mixed, and centrifuged at 3000 rpm for 10 min. After that, 1 ml of each supernatant was added to 500 µL of TBA, boiling the tubes for 10 min. in boiling H_2O bath (S/N.Ø4ØØ7Ø2149HØ14, Korea) until the purple color was formed. The MDA level was estimated following the cooling of the tubes at 532 nm versus the blank. Nitric oxide (NO) level in TTH was assessed using a modified method of *Griess* reagent assay. It depends on measuring the nitrite accumulation as a marker of NO creation [32]. In this adaptation, 100 µL of sodium nitrite standard, dH_2O, and each sample were added to 150 µL of 10 mM sodium nitroprusside in standard, blank, and examine tubes, respectively. The tubes were kept at 37°C for 2 hours in the dark. Following that, 250 µL of *Griess*

reagent (10 mM sulfanilamide and 1 mM N-1-naphthyl-ethylenediamine-dihydrochloride in 5% phosphoric acid) was put in all tubes. The produced purple color was measured by an ELISA reader at 540 nm and stated as nm/mg protein.

2.3.4. Assessment of testicular antioxidant stress markers

Glutathione is a tripeptide encompassing three amino acids, glutamic acid, cysteine, and glycine. It is responsible for preserving the redox status inside the cell and is involved in antioxidant defense [33]. Two forms of glutathione were recognized, oxidized (GSSG) and reduced (GSH) ones. The non-enzymatic thiol as GSH in TTH was quantified by *Ellman's* technique, where DTNB is reacted with GSH in the sample producing thiol with 2-nitro-5-thiobenzoic acid (2N5TA) [34]. In blank, reference, and specimen tubes, 100 µL of dH_2O, GSH, and TTH, respectively, were added to 100 µL of 4% sulfosalicylic acid. All tubes were incubated at 4°C for one hour. Then, the tubes were centrifuged for 10 min. at 3000 rpm using a cooling centrifuge. Finally, 100 µL of supernatant was integrated into 2.7 ml of phosphate buffer (0.1 M, pH 7.4) and 200 µL of DTNB. The developed yellow color in tubes was measured at 412 nm against the blank and the GSH content was affirmed as µmol/mg protein. Additionally, glutathione-S-transferase (GST) (EC 2.5.1.18) activity in TTH was evaluated depending on the catalyzed reaction of the substrates, p-nitrobenzyl chloride with glutathione to yield glutathione nitrobenzyl [35]. The GST-specific activity was referred to µmol/min/mg protein. Moreover, the activity of glutathione peroxidase (GPx) (EC 1.11.1.9) is another indicator of protection of the cell membranes against damage caused by LPO. It also prevents the accumulation of hazardous intracellular hydroperoxides. The GPx activity was calculated by deducting the overabundance of GSH after enzymatic reaction from the total GSH when the enzyme was absent. GSH interacts with DTNB to create yellow-colored 2N5TA [36].

2.4. Determination of testicular contents of IFN- λ, Il-1β, and NF-κB by polymerase chain reaxtion (PCR)

The entire RNA was isolated from testicular tissue via an easy-RED^{TM} Total RNA Extraction kit (Cat. No. 17063) in line with the manufacturer's directions. After that, PCR was employed utilizing a one-step Maxime RT-PCR PreMix kit (Cat. No. 25131) in a PCR machine (Prime, Code 3 PRIME G/02, Bibby Scientific Ltd, UK). The primers utilized in the reaction are demonstrated in Table 1. GAPDH served as an internal control. The final PCR product was run on 1.5% agarose gel in the electrophoresis horizontal gel unit (SCIE-PLAS, UK). The length of each gene was identified according to the DNA ladder (1.5 Kbp) and the bands were visualized by an ultraviolet radiator (Analytic Jena, USA). Finally, the quantification of the bands was performed via GelQuant.NET software provided by biochemlabsolutions.com, and relative gene expression was calculated.

2.5. Histopathological study

The left testicles acquired from all rats were preserved in 10% formalin and dehydrated using progressively higher grades of ethyl alcohol. Then, the dehydrated specimens were cleansed in xylene and passed through three paraffin wax modifications for 1-2 hours. Lastly, segments (3-5 µm thick) were stained with Ehrlich's hematoxylin and counterstained with eosin (H&E) for histopathological appraisal. The samples underwent light microscope inquiry [37].

2.6. Statistical analysis

he data's normalcy was conducted using a measure of central tendency, skewness, and kurtosis tests. Kolmogorov-Smirnova and Shapiro-Wilk tests were also accomplished. Statistical data assessment was undertaken using SPSS Version-25 software. For non-parametric results, the Kruskal-Wallis test was used and post hoc analysis was undertaken using the Mann-Whitney U test to compare the means between each experimental group. All data

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are presented as means ± standard error (SE). Statistically significant disparities were concurred as (P ≤ 0.05) [38].

3. Results

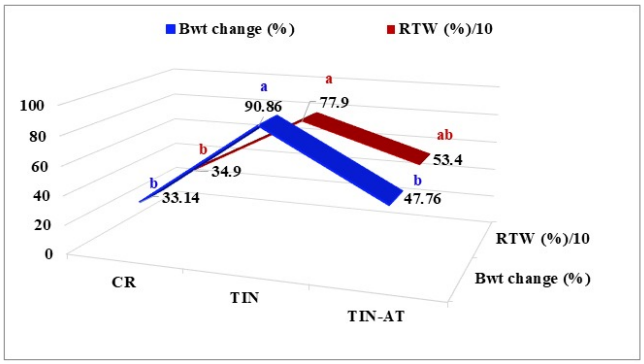

Figure 1: Effect of AT on Bwt change and RTW in TIN. Values are stated as means ± SE (n=7). Kruskal-Wallis test was used and post hoc analysis was done using the Mann-Whitney U test. Means with an uppercase letter (a) are significantly different at p ≤ 0.05 when compared to the CR group and means with an uppercase letter (b) are significantly different at p ≤ 0.05 when compared to the TIN group.
Abbreviations: CR, control group; TIN, induction group; TIN-AT, treated group with atorvastatin; Bwt, body weight; RTW, relative testicle weight.

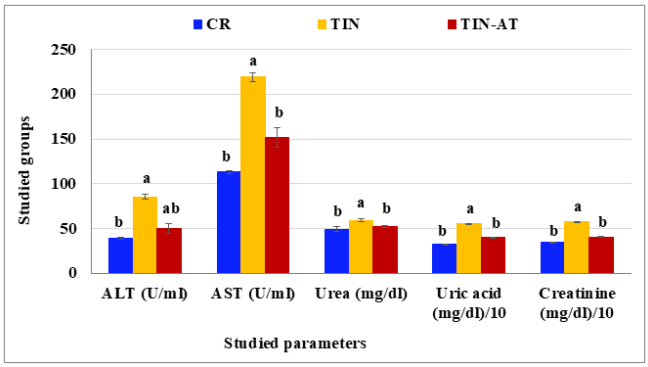

Figure 2: Effect of AT on liver and kidney function issues in TIN. Values are stated as means ± SE (n=7). Kruskal-Wallis test was used and post hoc analysis was done using the Mann-Whitney U test. Means with an uppercase letter (a) are significantly different at p ≤ 0.05 when compared to the CR group and means with an uppercase letter (b) are significantly different at p ≤ 0.05 when compared to the TIN group.
Abbreviations: CR, control group; TIN, induction group; TIN-AT, treated group with atorvastatin; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

3.1. *Effect of AT on Bwt change and relative testicle weight (RTW) in TIN*

The changes in the Bwt and RTW are demonstrated in Figure. 1 The Bwt and RTW were increased by 1.7 and

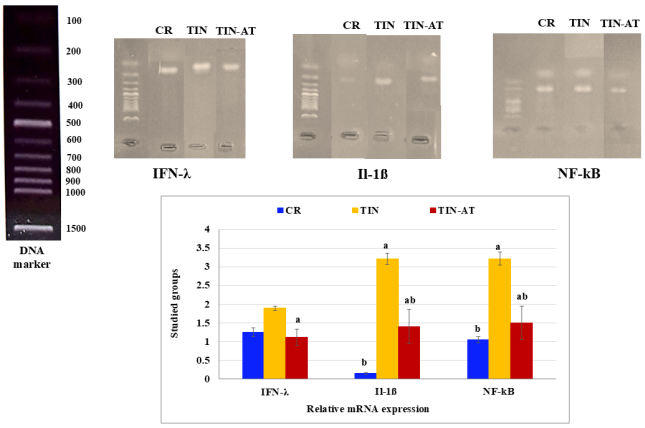

Figure 3: Effect of AT on IFN-λ, Il-1β, and NF-kB expression in TIN. Values are stated as means ± SE (n=3). Kruskal-Wallis test was used and post hoc analysis was done using the Mann-Whitney U test. Means with an uppercase letter (a) are significantly different at p≤ 0.05 when compared to the CR group and means with an uppercase letter (b) are significantly different at p≤0.05 when compared to the TIN group.
Abbreviations: CR, control group; TIN, induction group; TIN-AT, treated group with atorvastatin; IFN-λ, Interferon-gamma; Il-1β, interleukin-1 beta; NF-kB, nuclear factor-kappa B.

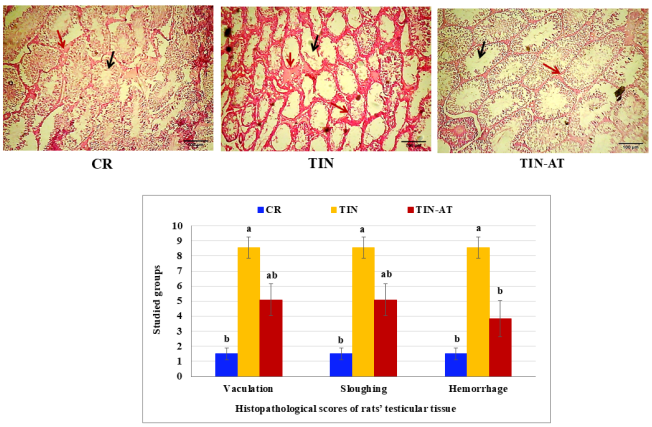

Figure 4: Effect of AT on testicular histopathological alterations in TIN (X= 40). Values are stated as means ± SE (n=3). Kruskal-Wallis test was used and post hoc analysis was done using the Mann-Whitney U test. Means with an uppercase letter (a) are significantly different at p ≤ 0.05 when compared to the CR group and means with an uppercase letter (b) are significantly different at p ≤ 0.05 when compared to the TIN group.
Abbreviations: CR, control group; TIN, induction group; TIN-AT, treated group with atorvastatin.

1.2-folds, respectively in TIN as opposed to CR. Using AT reversed the Bwt and RTW nearly by 0.47 and 0.31-folds, correspondingly compared to TIN.

Table 1: PCR primers used to investigate testicular inflammation

Gene	Primer sequence		Gene bank accession No.	Product size (bp)	Annealing temperature (ºC)
GAPDH	F	5´-GAGACAGCCGCATCTTCTTG-3´	NM_017008.4	200	53.8
	R	5´-TGACTGTGCCGTTGAACTTG-3´			
IFN-λ	F	5´-GAGGTGAACAACCCACAGATCCA-3´	NM_138880.3	100	53.8
	R	5´-CGACTCCTTTTCCGCTTCCTTAG-3´			
Il-1ß	F	5´-TCTGAAGCAGCTATGGCAAC-3´	NM_031512.2	129	55.8
	R	5´-TCAGCCTCAAAGAACAGGTCA-3´			
NF-kB inhibitor alpha	F	5´-GTGACTTTGGGTGCTGATGT-3´	NM_001105720.2	200	57.4
	R	5´-ACACTTCAACAGGAGCGAGA-3´			

Table 2: Effect of AT on oxidative stress parameters on testicular inflammation

Groups	LPO (nm/mg protein)	NO (nm/mg protein)	GSH (µmol/ mg protein)	GST (µmol/min/mg protein)/10^2	GPx (U/mg protein)
CR	0.37 ± 0.11^{b}	0.18 ± 0.00^{b}	0.83 ± 0.06^{b}	0.3 ± 0.2^{b}	0.38 ± 0.06^{b}
TIN	3.75 ± 0.90^{a}	0.38 ± 0.08^{a}	0.18 ± 0.01^{a}	0.01 ± 0.00^{a}	0.07 ± 0.01^{a}
TIN-AT	1.46 ± 0.46^{b}	0.14 ± 0.05^{b}	0.43 ± 0.07^{ab}	0.1 ± 0.06^{a}	0.20 ± 0.04^{ab}

Values are stated as means ± SE (n=7). Kruskal-Wallis test was used and post hoc analysis was done using the Mann-Whitney U test. Means with an uppercase letter (a) are significantly different at $p \leq 0.05$ when compared to the CR group and means with an uppercase letter (b) are significantly different at $p \leq 0.05$ when compared to the TIN group. **Abbreviations:** CR, control group; TIN, induction group; TIN-AT, treated group with atorvastatin; LPO, lipid peroxidation; NO, nitric oxide; GSH, reduced glutathione; GST, glutathione-S-transferase; GPx; glutathione peroxidase

3.2. Effect of AT on liver and kidney functions issues in TIN

The activity of both ALT and AST was significantly raised by 1.2 and 0.93-folds in TIN in comparison to CP. This elevation dropped in TIN-AT to 0.41 and 0.03-folds, respectively. Moreover, in the TIN group the concentration of urea, uric acid, and creatinine exhibited significant elevation ($P \leq 0.05$) (59.19 ± 1.41, 5.52 ± 0.45, and 5.76 ± 0.24, respectively) in comparison to CR. However, in TIN-AT their level reduced to (52.40 ± 1.51, 4.04 ± 0.29, and 4.07 ± 0.29, correspondingly) (Figure. 2).

3.3. Effect of AT on oxidative stress and antioxidant status in TIN

Data retrieved in Table 2 announced the altitude of LPO and NO by 9.1 and 1.1-folds in TIN more than CR. After administration of AT, both LPO and NO declined nearly 0.6-fold. The GSH content in TIN decreased by 78.3% than CR, and it increased by 63.2% in TIN-AT when compared to TIN. Additionally, in the TIN group, the activity of both GST and GPx deteriorated by 96.7% and 81.6% in comparison to CR, and they were well backed to their normal after AT administration.

3.4. Effect of AT on IFN-λ, Il-1β, and NF-kB expression in TIN

The changes in the expression of IFN-λ, Il-1β, and NF-kB are presented in Figure. 3. The upregulation of IFN-λ, Il-1β, and NF-kB by 0.5, 20.4, and 2.1-folds, respectively was discovered in TIN relative to CR. However, IFN-λ, Il-1β, and NF-kB downregulated in TIN-AT by 0.41, 0.56, and 0.53-folds, respectively, in comparison to TIN.

3.5. Effect of AT on testicular histopathological alterations in TIN

Results obtained from histopathological examination indicated that testicles of CR rats disclosed well-developed seminiferous tubules with active spermatogenesis and apparent interstitial cellularity (red arrows), lumens are entire of grow-up spermatozoa (black arrows). Nevertheless, in TIN, the testicles revealed a complete loss of

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architecture with a dilated lumen due to a limited amount of germ cells. The lumen is empty and all seminiferous tubules appear degenerated (black arrows). Also, there was extreme sloughing of spermatogenic cells, thickening, hemorrhage, and fibrosis of the lumen (red arrows). The testicles in the group treated by AT exposed degeneration and vacuolation of some seminiferous tubules (black arrows) with mild sloughing of spermatogenic cells and thinning of the lumen (red arrows) (Figure. 4).

4. Discussion

The TIN is among the valuable issues in male infertility [39]. The present study reveals that HFFD leads to TIN which hallmarks a serious public health problem [40]. Also, HFFD causes oxidative stress (OS) and influences thyroid function via modification of hormones and cytokines changes [41]. In addition, OS has destructive consequences on the male reproductive system by interrupting the hypothalamic/pituitary/testicular (HPT) axis, steroidogenesis, and spermatogenesis [42]. Spermatozoa are characterized by the presence of an extreme amount of polyunsaturated fatty acids, which makes them vulnerable to reactive oxygen species (ROS)[43]. It is known that the testicles' weight is reliant upon the mass of undifferentiated spermatogenic cells[44]. In our study, the Bwt and RTW of rats significantly elevated. In contrast, other studies have been informed that TIN causes a reduction of both Bwt and RTW [45, 46] Further, as opposed to the investigations of [47], our outcomes revealed TIN increased the rats' RTW. These variations may be attributed to altered animal patterns of TIN induction and alterations in the seminiferous tubules [48]. The information in this research indicated hyperactivity of both ALT and AST in TIN due to the degenerative impact of ROS on the hepatic cell membrane causing cellular leakage and loss of its functional integrity [49]. Further, TIN leads to the rise of renal markers because of the destruction of renal tubules and glomerulus due to the alteration of renal lipogenesis and lipolysis [50]. TIN causes extreme manufacture of ROS, which deteriorates the scavenging aptitudes of the antioxidant guarding enzymes [51]. These modifications lead to a boost of LPO and NO, while GSH, GST, and GPx are slowed down. The increased LPO levels in TIN disrupt DNA methylation, and synthesis and repair and damage the testicular cells through activation of the transforming growth factor-ß [52]. Elevation of testicular NO level also inhibits the activity of glyceraldehyde-3-phosphate dehydrogenase, that required for the glycolytic cycle via intersection of NO to the thiol group (SH) of the enzyme and retards its action [53]. The current data is in harmony with Barakat et al.,[54] where testicular NO rose due to the inflammatory effect of cadmium injection as a single dose. Further, the adverse implications of TIN upon antioxidant enzymes may be attributed to the joining of reactive oxygen molecules by the SH group of GST and GPx and restraining their activities [55]. The decline in those enzyme activities makes the testicles more susceptible to OS and damage. Further, it is known that GSH is catalyzed by GPx [56] so the diminished GSH content and GPx activity promote the LPO due to the inability of H_2O_2 detoxification to water and oxygen, hence H_2O_2 accumulated and broke the lipid membrane of the testicular cell with a subsequent loss of testicular architecture [57]. Our discoveries were matched with prior studies [58]. Following the appearance of OS in tissues, inflammatory mediators are probable to develop. The accumulated NO initiates the cell damage and promotes an inflammatory signaling cascade, which yields several inflammatory cytokines from macrophages, and Sertoli and Leydig cells [59]. It was determined that TIN intensified IFN-λ, Il-1β, and NF-kB levels in this study. NF-kB is a member of the transcription element family, which has a beneficial role in numerous procedures, primarily inflammation, apoptosis, and cell expansion [60]. It is very sensitive to OS due to redox inside the cell [61]. In TIN, NF-kB superfluous the inducible NO synthase enzyme and triggers a boost

in NO fabrication, which in turn-initiated NF-kB over-exaggeration [62]. It was reported that Il-1ß has been involved in the alteration of the HPT axis, through its direct impact on gonadotropin-producing hormones, FSH, and LH [63]. A high level of Il-1ß has been observed during TIN, which influences testicular steroidogenesis and spermatogenesis through openings of the connections between epithelial cells and Sertoli, producing disruptions in the position of the seminiferous epithelium which need for appropriate spermatogenesis [64]. IFN-λ belongs to the interferon type II family and it is secreted from T helper and natural killer cells and involved in TIN [65]. It was noted that OS enhanced IFN-λ over secretion leading to endothelial dysfunction [66]. The observed upregulation of IFN-λ in TIN may be related to the chronic stress from ROS that activates both apoptosis signal-regulating kinase 1 and Jun N-terminal kinase by phosphorylation process, hence higher IFN-λ is released [55]. An elevation of inflammatory markers in TIN observed in this study corroborates previous reports [67]. Statins are a group of pharmaceutical drugs that have a key role in some metabolic disorders and include AT, fluvastatin, lovastatin, simvastatin, rosuvastatin, pravastatin, cerivastatin, and pitavastatin [68] In this experiment, AT significantly ameliorated many of the observed parameters of TIN. One month of supplementation of AT attenuates Bwt and RTW according to the study of [69]. Although some literature pointed out the hepatotoxic effect of AT [70] our data evoked that administration of AT to TIN-AT restored both ALT and AST, this variation related to using of different dosages and the administration routes. Our results are in harmony with [71]. Further, our results disclosed AT restored the urea, uric acid, and creatinine levels nearly to normal, and this is consistent with [72] who attributed that to the hypolipidemic action of AT. Improving the antioxidant defense system in TIN should be considered. AT reduced the LPO and NO levels but increased testicular activities of GST and GPx and GSH content which could be a compensatory scheme to avoid the TIN that arises from ROS [73, 74]. In a similar study Lee et al. [75], AT normalized the prooxidant and antioxidant enzymes created from kidney inflammation resulting from diabetes mellitus. The antioxidant mechanisms of AT are attributed to various concepts. Firstly, AT upsurges the NO biosynthesis in blood vessels and binds it to the phospholipids surface of lipoproteins [72]. Further, AT decreases the redox balance of low-density lipoprotein particles [76] and impedes the vascular nicotinamide adenine dinucleotide phosphate oxidase [9]. AT protects the testicular vascular endothelial cells and preserves endothelial stability [77]. Moreover, AT intensifies the heme oxygenase-1 [78], isoenzyme of heme oxygenase that breaks the heme into biliverdin, carbon monoxide (CO), and ferrous iron [79]. The produced biliverdin and CO activate the p38 mitogen-activated protein kinase (p38 MAPK) signaling network which blocks ROS [80]. Moreover, AT inhibits the mevalonic acid pathway, so cholesterol biosynthesis is decreased which plays a role in ROS initiation [81]. Also, AT is a competitive suppressor of 3-hydroxy-3-methylglutaryl coenzyme-A reductase that has a role in dipping cholesterol concentration [82]. Additionally, it was reported that AT protects antioxidant enzyme levels in the liver by hindering endogenous antioxidant enzyme reduction[83]. The current results demonstrated the decline of the IFN-λ, Il-1β, and NF-kB in the TIN-AT group that ascribed to AT mitigates numerous testicular inflammatory signaling cascades through triggering anti-apoptotic kinase, MAPK [55], and nuclear factor erythroid 2-related factor 2 [56]. Similar to our findings, Zhao and his colleagues displayed downregulation of serum Il-1β following AT administration in hypertensive rats through activation of peroxisome proliferators-activated receptor λ [46]. The present biochemical and molecular data is confirmed by the insights from the histopathological executions of TIN and TIN-AT. The alterations caused by TIN were efficiently restored by AT in the TIN-AT rats. These findings agreed with

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the histological characteristics of the testicular tissue of experimental TIN [84].

Conclusion

Ultimately, this research exposed that testicular inflammation (TIN) hinders the function of the testicles by advertising oxidative stress (OS) and creating inflammatory mediators. Nevertheless, atorvastatin (AT) mitigated testicular malfunction through means tied to minimizing the regulation of OS, and inflammatory responses as well as recuperation of testicular histoarchitecture. It is advisable to perform further studies to elucidate the precise molecular mechanisms of AT in testicular inflammatory disorders.

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Conflict of interest statement

There are no disclosed conflicts of interest for the authors.

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