Research Article

# A potential protective mechanism of high-intensity interval training against tetracycline-induced hepatic steatosis and testicular apoptosis in male Wistar rat: A crosstalk between the liver and testis

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## Abstract

The presence of tetracycline in animal products has toxic and destructive effects on body tissues. In this study we investigate the potential protective mechanism of high-intensity interval training (HIIT) against tetracycline-induced hepatic steatosis (HS) and testicular apoptosis in male Wistar rat. In this study, forty-eight male Wistar rats (8-week, 220±10 gram) were randomly divaded into six groups of primary control (pre week one), primary HS (tetracycline-induced HS), secondary control (after week five), secondary HS, (5) HIIT, and HS+HIIT (after week five). Tetracycline was administered to rats 140 mg / kg for 7 days by gavage. HIIT was performed on rodent treadmill 5 days/week for 5 weeks. Oral exposure of tetracycline for 7 days caused severe testis damage as indicated by significant alterations in histomorphological, apoptosis, increase Bax, P53 and decease Bcl2 (gene and protein, p=0.001) compared to primary control. But the changes of PARP1 were not significant (p>0.05). However, HIIT and HS+HIIT groups significantly increased spermatogonium counts, spermatocyte cell counts & spermatid cell counts (p=0.001 for all) in line with Bcl-2 and PARP1 (gene and protein, p=0.001) and decreasing apoptotic cells, Bax and p53 compared with secondary HS group (p=0.001). This research provides the first evidence that the beneficial anti-apoptosis effects of HIIT on testis of rats poisoned with tetracycline. This beneficial effect of HIIT on hepatic steatosis and testicular damage and toxicity due to tetracycline might be mediated by inhibiting P53-induced BAX upregulation and preventing apoptosis-mediated degradation of PARP-1.

***Key Words:*** **Tetracycline, Testicular apoptosis, High-intensity interval training, Liver, Crosstalk**

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## Introduction

Non-alcoholic fatty liver disease (NAFLD), is one of the common metabolic diseases also known as metabolic syndrome (Bullón-Vela et al., 2020). Symptoms of this disease include increased concentration of fat or triglycerides in the hepatic parenchyma (Dongiovanni et al., 2018). NAFLD was discovered in 1980 and has been identified under various names, including steatosis (Ludwig et al., 1980). The prevalence of the NAFLD is increasing worldwide (Selvakumar et al., 2018). Increasing the prevalence of obesity is also one of the causes of the liver disease (Divella et al., 2019). With the rapid rise in obesity and type 2 diabetes (T2DM), the global prevalence of NAFLD continues to increase, as in the USA, NAFLD have a correlation with the obesity epidemic (Younossi et al., 1998). NAFLD can lead to the spread of other disorders, such as cardiovascular disorders, renal disease, and infertility disorders (Byrne & Targher, 2020; Hawksworth & Burnett, 2019; Henson et al., 2020). Recent studies provide substantial evidence for an association between NAFLD and atherosclerosis and cardiometabolic disorders (liver and heart crosstalk) (Lim et al., 2019). Obesity, NAFLD and metabolic syndrome are inversely correlated with semen volume, sperm concentration, sperm motility, and sperm morphology (Eisenberg et al., 2015). Metabolic syndrome associated with infertility (Martins et al., 2019). Since NAFLD is strongly associated with metabolic disorders, endocrine disorders, and CVD, it can also effect on men's semen and fertility. In animal research, tetracycline used to induce NAFLD. This antibiotic separately damages testicular tissue.

Nowadays antibiotics and the subsequent entry of these compounds into the environment has raised concerns worldwide. Among the various types of antibiotics, tetracycline is the second most common group of antibiotics in terms of production and consumption worldwide, which are widely used to treat a variety of infectious diseases (Kümmerer, 2009). Increase of this materials and drugs in the environment create antibiotic-resistant pathogens that increase risk of disease (Elmolla & Chaudhuri, 2010). Also, the presence of these risk

factor in the environment, including the aqueous environment, can cause reactions ranging from simple allergies to in some cases direct toxicity (induction of fatty liver with this drug) (Tanvir et al., 2019). Studies in human and animal sample show that long-term use of tetracycline is often associated with complications such as liver dysfunction and testicular damage (Wruble & Cummins, 1965; Yin et al., 2006). Previous studies on the effect of tetracycline on testicular tissue have shown that this drug can disrupt testicular tissue homeostasis by destroying the balance of oxidative stress and antioxidant capacity (Farombi et al., 2008). Also, testicular atrophy is another side effects of using this type of drugs, all of which can lead to male infertility (Azu et al., 2010). In current studies, tetracycline is used to induce hepatic steatosis (Yu et al., 2009). However, liver damage, including fatty liver, can also lead to testicular tissue damage (liver testis cross talk).

It has been demonstrated that increased lipid accumulation due to metabolic diseases increases the space of germ cells and it can have induced injuries in sertoli cells (Vidal & Whitney, 2014). Increased oxidative stress due to metabolic damage also induced apoptosis (Migliaccio et al., 2019). Although in physiological conditions apoptosis of testicular germ cells is essential, but in metabolic stresses, high apoptosis can have induced infertility. It has been confirmed P53 can increase apoptosis in testis therefor associated with male infertility. It is stated that, increased oxidative stress can be effective in releasing p53 responses and modulating subsequent pathways of apoptosis (Bax) (Gali-Muhtasib et al., 2015). Therefore, it seems that increased oxidative stress due to metabolic syndrome such as NAFLD is involved in activation of these pathways. Conversely, some factors are able to inhibit the damage caused by non-physiological apoptosis and inhibit DNA damage. Poly (ADP-ribose) polymerase-1 (PARP-1) is one of these factors.

PARP-1 mediate poly (ADP-ribosyl) ation (PARP polymerase ribosylation) of proteins, catalyses synthesis of over 90% of cellular poly (ADP-ribose) (PAR) following DNA damage (Meyer-Ficca et al., 2005). It has been stated PARP-1 play an important role in DNA repair and maintenance of genomic stability, transcriptional regulation and centromere function, centrosomal function, telomeric dynamics, apoptosis and necrosis (Bürkle, 2005). PARP1 gene expression occurred in the basal regions of the seminiferous tubules of mice. Therefore, this factor is increased in the early stage of spermatogenesis (Schreiber et al., 2002). However, studies regarding the effect of PARP-1 on apoptosis and structural damage of testicular tissue from hepatic steatosis metabolic syndrome are limited. It is stated exercise training is one of the strategies that can improve male infertility (Yi et al., 2020).

Based on previous research, physical activity that performs regularly improved metabolic disorder such as NAFLD and it can effect on sperm volume and count. Recently Maleki et al (2020) show that interval training attenuates inflammatory factors (IL-6 and TNF-α), oxidative stress (ROS and MDA), and antioxidants (SOD, CAT, and TAC). These changes can improve the semen parameters, sperm DNA integrity, and pregnancy rate (Maleki & Tartibian, 2020). It has been reported that a short-term aerobic exercise intervention program improves sperm count in obese adults (Rosety et al., 2017). Recently, it is stated that high-intensity interval training (HIIT) with anti-inflammatory and anti-oxidant effects, it can be a treatment strategy for male infertility. (Maleki & Tartibian, 2020). Hajizadeh Maleki and Tartibian (Maleki & Tartibian, 2017) examined the effects of high intensity interval training on improving the reproductive performance of infertile couples. Exercise program of the training group included HIIT on treadmill, 3 times a week, with intensity 70% to 85% of maximal oxygen consumption. In the exercise group, inflammatory biomarkers (interleukin-6 and tumor necrosis factor), oxidative stress (reactive oxygen species and malondialdehyde) significantly reduced, and antioxidants factors (superoxide dismutase, catalase) were increased. All of this change were consistent with favorable improvements in sperm DNA integrity, semen parameters and pregnancy rates (Zidi-Jrah et al., 2016).

Based on the previous research that mentioned at above, exercise training especially HIIT is able to control metabolic disorder such as NAFLD and reduce testis damage induced by toxin material. Fatty liver disease, whether caused by diet or toxins such as tetracycline, is a separate risk factor for testicular tissue homeostasis. Exercise training with reduce liver damage can have an indirect effect on improving testicular hemostasis. Therefore, our aim was to investigate protective effect of high-intensity interval training against tetracycline-induced hepatic steatosis and testicular apoptosis in male Wistar rat.

## Materials and Methods

### Animals

We purchased 48 males Wistar rats, 8-week-old that weighed 220 ± 10 g from posture Institute, Tehran, Iran. The rats were kept under standard conditions of a 12 h light/dark cycle at 65–75 F (18–23 C), the room humidity was 50–60%, and food and water were available ad libitum throughout the experiment. The animals were housed in collective cages (4 rats per cage, a polycarbonate cage 20 × 27 × 47 cm). This research was approved by the animal care and use committee at the Baqiyatallh University of Medical Sciences of Tehran, Iran (Approval reference number: IR.BMSU.REC.1396.632). After 2 weeks of acclimatization with laboratory environment, the rats were randomly divided into six groups of, (1) primary control (healthy rats that sacrifice at week 1), (2) primary HS (Hs rats that sacrifice at week 1), (3) secondary control (healthy rats that sacrifice at end of week 5), (4) seconda-

-ry HS (HS rats that sacrifice at end of week 5), (5) HIIT (healthy exercised rats that sacrifice at end of week 5), and (6) HS + HIIT (HS exercised rats that sacrifice at end of week 5) (n=8 in each groups).

### Induction of HS with Tetracycline

Tetracycline at a dose of 200 mg/kg of body weight (dissolved in 2 ml of water) was given to HS rats by gavage for 7 days (Tanvir et al., 2019). Confirmation of fatty liver (steatosis) associated by measuring liver enzymes and H&E staining after HS induction. Tetracycline was obtained from Amresco, Inc, USA.

### Exercise training protocol

HIIT was performed on a motorized treadmill at 0˚ inclination 5 days/week (Saturday to Wednesday) for 5 weeks. Exercise groups started with a warm-up at 4 m.min-1 for 5min. Rats ran 5 sets of 2min at 16 to 64 m.min-1 followed by 3min at 10 to 18 m.min-1 during five weeks (table 1). The protocols were originally designed to have the same total running distance for all groups, as proposed by Kalaki-Jouybari et al. 2018 (Kalaki-Jouybari et al., 2018). Control rats were placed in the training room during the sessions to expose them to the same environment and for the same time as the HIIT groups.

### Fatty liver confirmation (serum aminotransferase concentration & Histopathology of liver tissue)

At 24 h after last session of tetracycline gavage (primary control & primary HS groups) and at 48 h after last training session (secondary control, secondary HS, HIIT & HIIT+HS groups), blood samples given by harvesting eyeball and liver tissues of all animals were collected for further analysis. Serum concentrations of ALT&AST were determined by commercial assay kits according to the standard procedures, respectively.

In this study, a part of liver tissue was fixed in 9% formalin to examine histological changes. Fixed liver samples were embedded in paraffin to analyze liver tissue. Then, by making 5 Um thick incisions, histological examinations were performed by hematoxylin and eosin (H&E) staining.

### Real-Time PCR

Quantitative real-time PCR was performed for mRNA quantitation in the testis, as described previously, with specific modifications (Sakharkar et al., 2014). The total RNA was isolated using TRIZOL reagent (Invitrogen, Life Technologies, USA). The PCR conditions used for the reverse transcription process were 25°C for 10 minutes, 37°C for 120 minutes, and 85°C for 5 minutes. The duplicates of the cDNA were subjected to qRT-PCR on a StepOne™ RT- PCR System (Applied Biosystems) using SYBR green qPCR master mix (Thermo Fisher Scientific). The specific primers corresponding to the selected mRNAs (BCL2, P53, Bax and PARP-1) are listed in Table 2. The housekeeping gene GAPDH was measured in parallel as an internal control. The thermal profile used for the qRT-PCR had three stages: 95°C for 3 minutes (1 cycle); 95°C, 57°C, and 72°C for 30 seconds each (40 cycles); 95°C for 15 seconds and then 60°C for 1 hour (1 cycle). The fold change for each gene was determined after normalization to GAPDH using the $2^{-\Delta\Delta CT}$ method (Livak & Schmittgen, 2001).

$\Delta CT = CT_{target} - CT_{reference}$

$\Delta\Delta Ct = \Delta CT_{test\ sample} - \Delta CT_{control\ sample}$

Relative expression: $2^{-\Delta\Delta Ct}$

### Tissue preparation for staining

The investigated rats were anesthetized by using Ketamine/xylazine (100/10 mg/kg) and euthanized by CO2 gas. The testes of the rats were removed bilaterally and weighed. One of them was frozen at -20 temperature and the other one was fixed in 10% neutral buffered formaldehyde/NBF for 1 day at room temperature. Then, they were dehydrated in the ascending alcohol series, rinsed by xylene, and were blocked in paraffin following routine tissue follow-up procedures. Serial transverse sections (5 µm thickness) were collected at 50 µm intervals from the prepared paraffin blocks for each animal on a rotary microtome, and mounted on slides. All histology analysis was done without knowledge of treatment groups (blinded) by pathologist.

### Testicular histology analysis

Testis sections (5µm) were de-paraffinized in xylene and stained with hematoxyline-eosin (H&E) and observed under the light microscopy (Olympus BX51; Olympus, Waltham, MA), for evaluation of testicular cell density. Image Tools Software (ver.3, Microsoft, Texas, USA) was used to measure the number of spermatogonium cells, spermatocyte cells and spermatid cells (cells/mm2). For this purpose, five sections and four fields per section (an overall of 20 fields in each animal sample) were counted at magnification ×100 of light microscopy.

### TUNEL assay

The in situ cell death detection was used to assess the DNA fragmentation using POD Kit (Roche, Germany). the sections were de-paraffinized and rehydrated, incubated with proteinase K (15 µg/ml) for 30 min. then the sections were incubated in 3% hydrogen peroxide/methanol for 10 minutes in the dark at room temperature to block endogenous peroxidase activity. After washing by PBS buffer 3 times for 5 min, the sections were incubated with a TUNEL reaction mixture for 2 hours at 37°C. The sections were washed in PBS buffer 3 times for 5 min and then,

**Table 1.** Exercise training protocol in exercise groups.

| Weeks | Warm up (5 min) (m/m) | HIIT (m/m) | | Cool down (5 min) (m/m) |
|---|---|---|---|---|
| | | Exercise (5 set/2 min) | Active rest (5 set/2 min) | |
| 1 | 4 | 16-24 | 10 | 4 |
| 2 | 6 | 26-34 | 12 | 6 |
| 3 | 8 | 36-44 | 14 | 8 |
| 4 | 10 | 46-54 | 16 | 10 |
| 5 | 12 | 56-64 | 18 | 12 |

incubated with POD for 15 minutes at 37°C. After washing several time in PBS color development was performed in the dark room with DAB (3,3-Diaminobenzidine) for 15 minutes. Afterwards, hematoxylin solution was used as counter stain. The number of TUNEL positive cells was counted carefully in 3 sections of the seminiferous tubules of the testis per animal (Image J software v1.8, NIH, Wayne Rasband, USA).

### Western blot

For extraction of proteins, the testes of the rats were removed and homogenized in lysis buffer (RIPA, Beyotime Institute of Biotechnology) supplemented with protease inhibitors (PMSF, Aladdin). Proteins of sample was separated by 10% SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred onto a polyvinylidene difluoride (PVDF) membrane (Amersham TM Hybond, Merk, Germany). The membrane was blocked for one hour at room temperature, then incubated with mouse monoclonal antibodies against BAX, Bcl2, P53 and PARP1 (1:500, Santa Cruz Biotechnology, USA) and left at 4 °C overnight. The anti-GAPDH antibody (1:500, Santa Cruz biotech-nology, USA) was used as a loading control. The membrane was washed with TBST buffer and then incubated with goat anti-mouse IgG-HRP (1:5000, Santa Cruz Biotechnology, USA).

**Table 2.** Primer sequences used for real-time PCR amplification.

| Gene | Primer Sequence (5′-3′) | Accession Number |
|---|---|---|
| BCL-2 | F: CTTCAGGGATGGGGTGAACT | NM_016993.1 |
| | R: CACAGAGCGATGTTGTCCAC | |
| p53 | F: AGTGGGAATCTTCTGGGACG | NM_030989.3 |
| | R: TCTTTTGCTGGGGAGAGGAG | |
| Bax | F: GAGACACCTGAGCTGACCTT | NM_017059.2 |
| | R: CTGCAGCTCCATGTTGTTGT | |
| PARP1 | F: AAGGTGGAGATGCTGGACAA | NM_013063.2 |
| | R: GGGGTCCTTACTGCTGTCAT | |
| GAPDH | F:CAAGTTCAAGGGCACAGTCA | NM_017008.4 |
| | R: CCCCATTTGATGTTAGCGGG | |

F, forward; R, reverse; BCL-2, Rattus norvegicus repressor of programmed cell death BCL-2; Tp53, Rattus norvegicus tumor protein p53; Bax, Rattus norvegicus BCL2 associated X, apoptosis regulator; PARP1, R.norvegicus poly (ADP-ribose) polymerase 1; GAPDH, glyceraldehyde-3-phosphate dehydrogenase [ Rattus norvegicus (Norway rat) ].

### Statistical analysis

Means±SD and SEM were calculated using Graph Pad Prism 5 software (Graph Pad Software, San Diego, CA). Gene expression and protein analyzed by one-way ANOVA followed by Tukey post-hoc test using SPSS 24 (SPSS, Chicago, IL, USA).

## Results

### Liver enzyme and liver histopathology

In this study, to evaluate and confirm liver damage and induction of tetracycline-induced liver steatosis, ALT and AST enzymes as well as histopathological changes were evaluated. The results showed in the tetracycline consumer group. Serum ALT and AST concentrations increased significantly compared to the control group ($p = 0.001$). As shown in Figure 1 (a & b), HIIT reduced the increase in serum ALT and AST after tetracycline injection ($p = 0.001$).

Tissue changes induced by tetracycline and treatment are also shown in Figure 1C. According to the histological figure, induction of hepatic steatosis with tetracycline increase necrosis by lymphomacrophage infiltration, a violation of the structure of the liver sections. Also, the penetration of inflammatory cells hepatocyte in the tetracycline group showed a significant increase. However, all of these changes improved with HIIT (Figure 1c).

### Gene expression

Testicular apoptosis and decrease PARP-1 are other hallmark characteristics of testis disorder. Testis Bax and p53 mRNA levels were increased by tetracycline consumption ($p = 0.001$ compared to the control group), whereas Bcl-2 and PARP-1 mRNA levels have no significantly difference with consumption of tetracycline. However, HIIT exercise decreased Bax and p53 mRNA ($p = 0.001$) and increased Bcl-2 and PARP1 mRNA ($p = 0.001$), with or without tetracycline (Fig 2).

### Histomorphometric evaluation of testicular tissues

Markers of testicular morphology were assessed because of their importance in the regulation of spermatogenesis. There are significant increases in spermatogonia counts in primary C, secondary C, HIIT and HS+HIIT groups compared to Primary HS and Secondary HS groups, which was the highest increase in HIIT group ($P < 0.001$). Meanwhile, compare to HIIT group, all Control and HS+HIIT groups had a significant decrease in Spermatogonia counts, which was the lowest decrease in the Primary C group ($P < 0.1$) (Fig 3, a&b).

Spermatocyte cell count in the HIIT and HS+HIIT groups have a significant increase compared to the both control groups ($P < 0.001$), while primary HS and Secondary HS groups significantly decreased compare to Control groups ($P < 0.01$) (Fig 3, a&c).

In spermatid count, HIIT group have an increase compared to other groups ($P < 0.001$), whereas Primary HS and Secondary HS groups have a decrease compared primary control group, which was the lowest decrease in the Primary HS group ($P < 0.01$). (Fig 3, a&d).

## Tunnel staining

TUNEL-positive cells (apoptotic cells) (Fig. 4 a) were measured in different groups 72 h after the reperfusion. Based on quantitative analysis (Fig.4 b) the HIIT group had the lowest the number of TUNEL-positive cells compares to other group ($P < 0.001$). the number of these cells in the primary HS and secondary HS were increased compared to HIIT group ($P < 0.001$). The rate of TUNEL-positive cells in the Primary C and Secondary C groups were not significantly different from the HIIT group ($P > 0.05$).

## Western blot of Bax, Bcl2, P53 and PARP1 proteins

We compared apoptosis process by measuring the expression of Bax, Bcl2, P53 and PARP1 proteins from the testes of rats that were harvested from Primary C, Primary Control, Primary HS, Secondary C, Secondary Control, Secondary HS, HIIT and HS+HIIT group rats by western blotting (Fig.5). The Primary HS, Secondary HS and HS+HIIT groups significantly elevated the expression of Bax in the testes compared with that in HIIT group

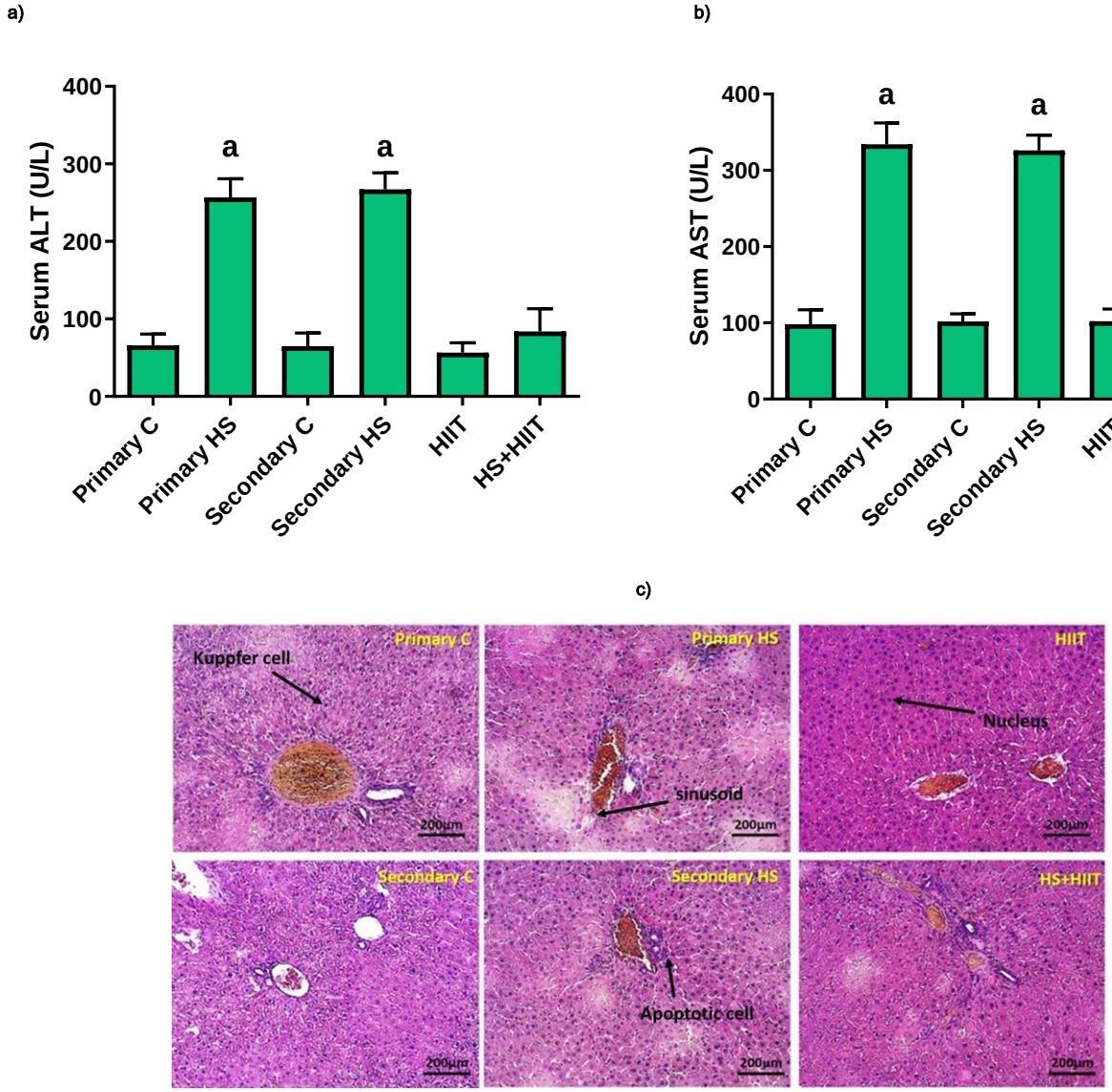


**Figure 1.** Effects of tetracycline gavage in serum ALT and AST concentration (a&b mean±SD) (a and b), and induced fatty liver in rats with H&E images (c). Liver specimens were collected at 24 h after sacrifice and liver sections were stained with hematoxylin–eosin. Original magnification, × 200um. a, serum ALT; b, serum AST. A: sign of significant compare to primary control group, $P < 0.001$

rats (Fig.5a). Additionally, the expression of Bax in the Primary C and Secondary C groups were not significantly different from the HIIT group (P > 0.05). The HIIT group had the highest of Bcl2 compared with other groups (P<0.001). The expression of Bcl2 was increased significantly in Primary C, (P<0.1), Secondary C (P<0.001), and HS+HIIT (P<0.001) groups rats compared with that in Primary HS group rats (Fig.5b). Meanwhile, P53 expression was significantly increased in Primary HS, Secondary HS and HS+HIIT group rats compared with that in HIIT group rats (Fig.5c). Additionally, the expression of P53 in the Primary C and Secondary C groups were not significantly different from the HIIT group (P > 0.05) (Fig.5c). The PARP1 concentration in HIIT and HS+HIIT group has a significant increase compared to other groups (Fig.5d). The rate of PARP1 expression in the Primary C Primary HS and Secondary C groups were not significantly different from the Secondary HS group (P > 0.05).

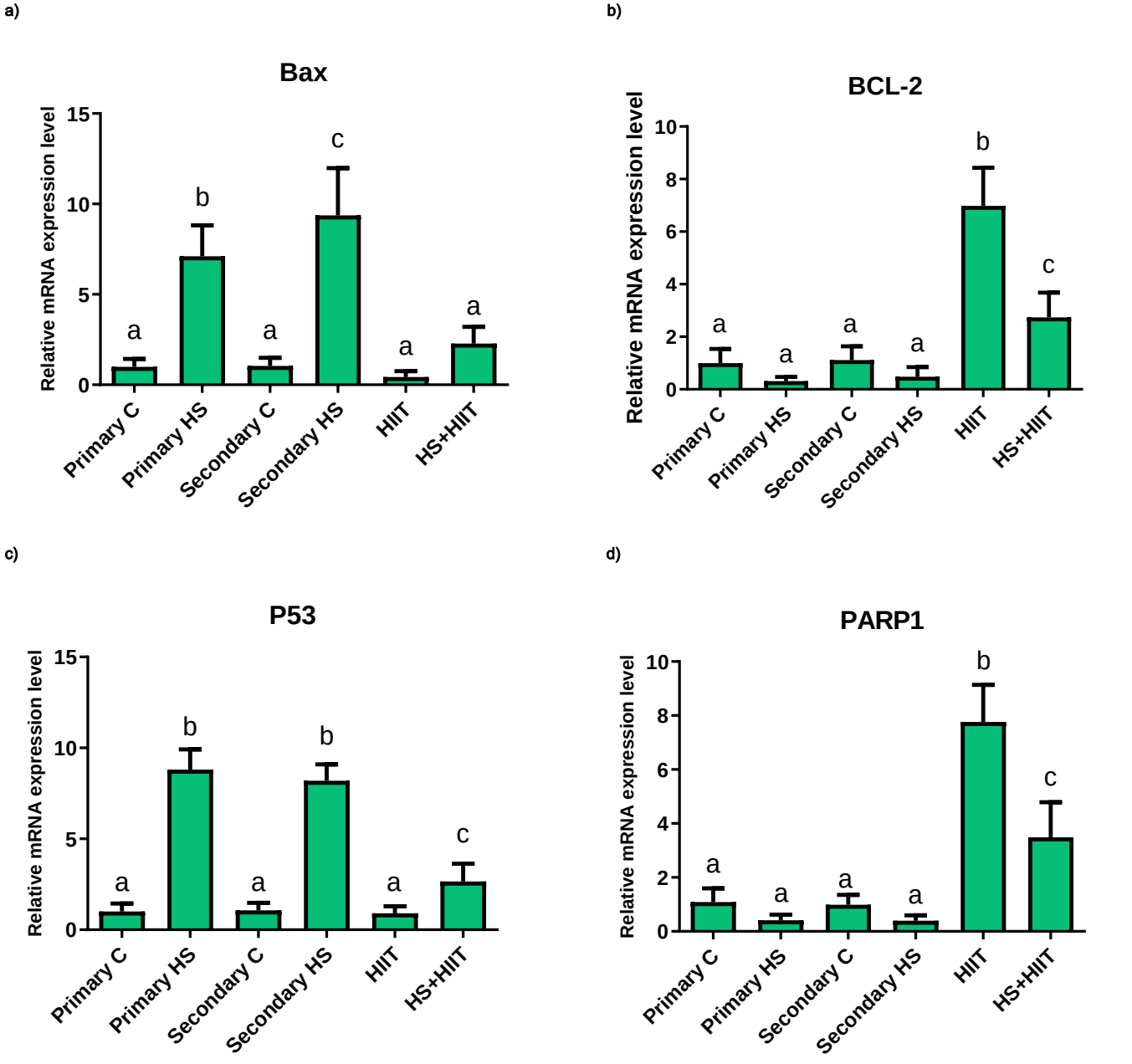


**Figure 2.** Effect exercise on pro and anti-apoptosis Bax (a), Bcl2 (b), P53 (c) and PARP1 (d) (Poly(ADP-Ribose) Polymerase 1) mRNA in testicular tissue. Data are expressed as the mean ± SEM; n = 8, P ≤ 0.05; The difference signs represent statistically significant differences between groups (P<0.05) and the same sign are not significant (P>0.05). Abbreviation: Primary C, Primary Control (healthy control group pre week 1); Primary HS, Primary Hepatic Steatosis (induced-hepatic steatosis group pre week 1); Secondary C, Secondary Control (healthy control group after 5 weeks); Secondary HS, Secondary Hepatic Steatosis (induced-hepatic steatosis group after 5 weeks. HIIT, High Intensity Interval Training (healthy exercise group after 5 weeks). HS+HIIT, Hepatic Steatosis + High Intensity Interval Training (induced-hepatic steatosis with exercise group after 5 week).

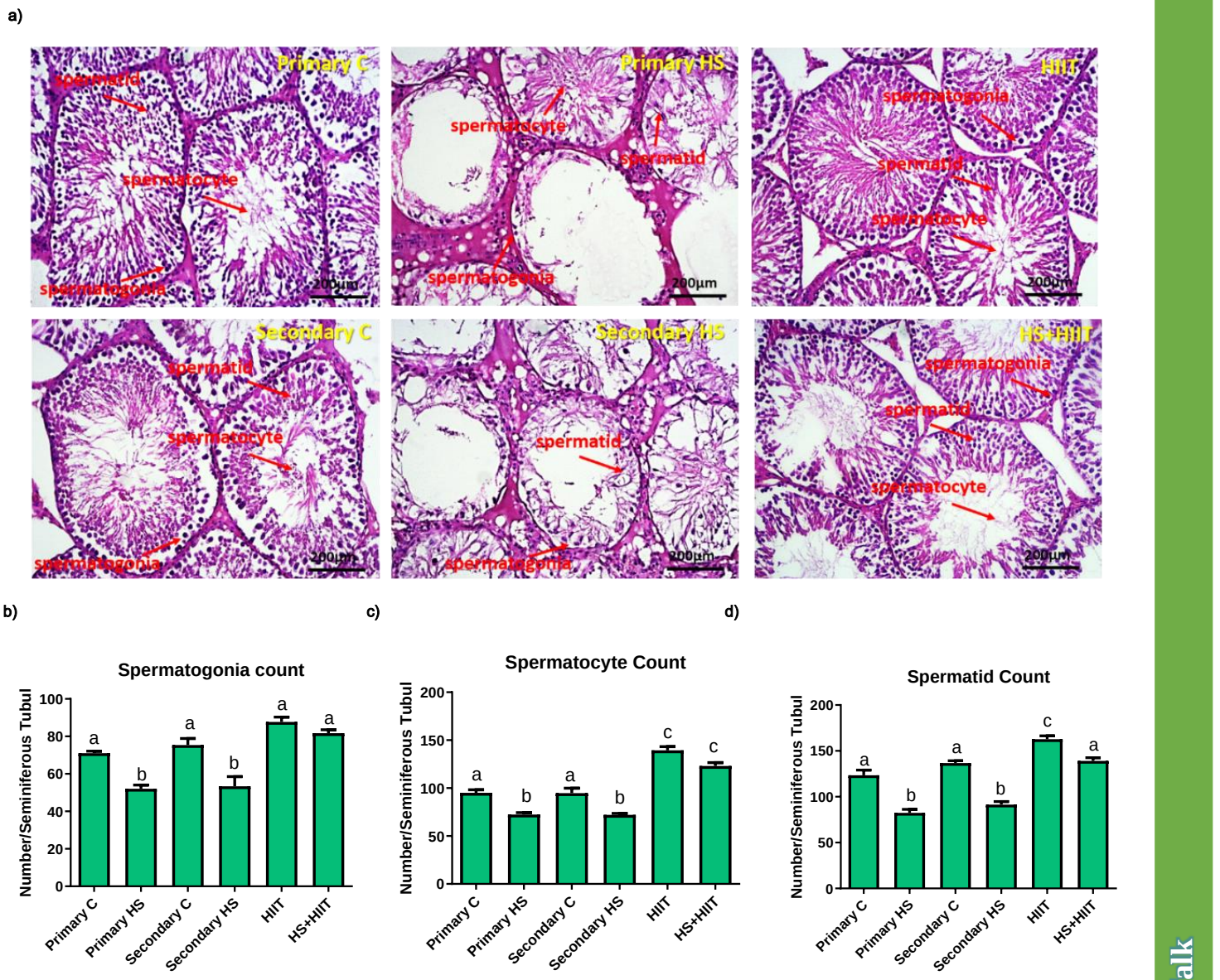


**Figure 3.** Histomorphological assessment in testicular tissue. H&E staining; Magnification is 100X (a). also the graph show different change in spermatogonium counts (b), spermatocyte cell counts (c) & spermatid cell counts (d) of groups study; Data are expressed as the mean ± SEM; n = 8, P ≤ 0.05; The difference signs represent statistically significant differences between groups (P<0.05) and the same sign are not significant (P>0.05). Abbreviation: Primary C, Primary Control (healthy control group pre week 1); Primary HS, Primary Hepatic Steatosis (induced-hepatic steatosis group pre week 1); Secondary C, Secondary Control (healthy control group after 5 weeks); Secondary HS, Secondary Hepatic Steatosis (induced-hepatic steatosis group after 5 weeks. HIIT, High Intensity Interval Training (healthy exercise group after 5 weeks). HS+HIIT, Hepatic Steatosis + High Intensity Interval Training (induced-hepatic steatosis with exercise group after 5 week).

## Discussion

The results showed that tetracycline causes hepatotoxicity and metabolic degradation, including hepatic steatosis. On the other hand, hepatic steatosis and tetracycline toxicity can lead to structural and functional damage to testicular tissue, which can lead to infertility. Exercise can be effective in controlling this destruction by inducing new mechanisms in apoptosis down regulation. Because, after inducing hepatic steatosis with tetracycline, increase of PARP1 with HIIT seems to be a critical factor to decrease p53 and reduce apoptosis in testicular tissue and potentially increase sperm count with improvement of testicular histopathology.

Apoptotic cells and BAX and p53 gene and protein (western blotting) in tetracycline receiving groups significantly increased compared to healthy control and HIIT group. However, HS + HIIT group inhibited the increase in p53 and testicular tissue apoptosis indices. BCL-2 and PARP1 gene and protein were less affected

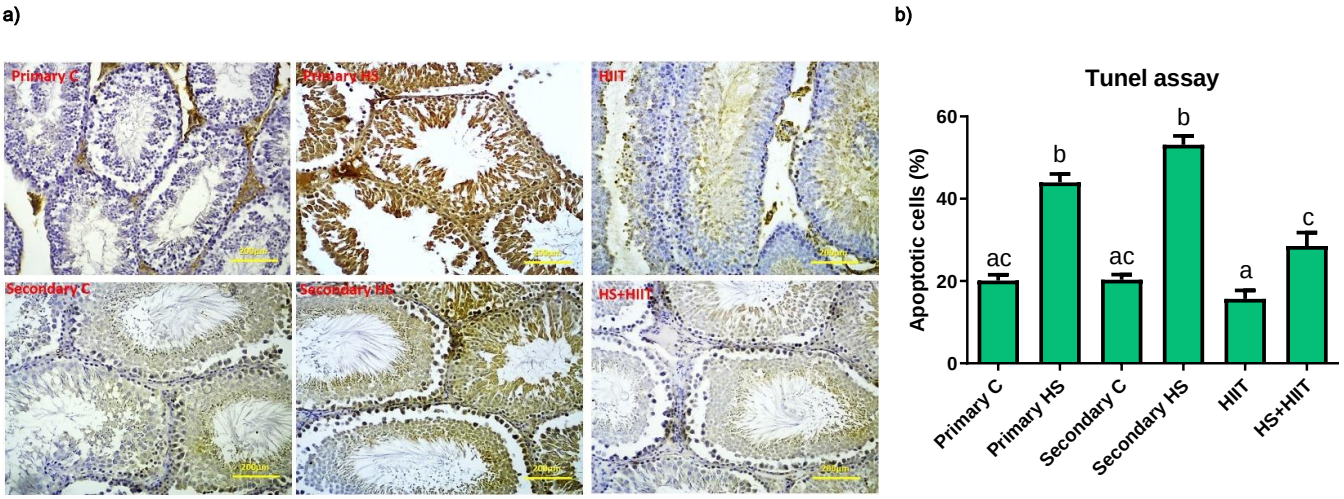


**Figure 4.** Photomicrographs of TUNEL-positive cells in the testes of rats, Date shows at means±SD. The difference signs represent statistically significant differences between the mean values (P<0.05) and the same sign are not significant (P>0.05). Abbreviation: Primary C, Primary Control (healthy control group pre week 1); Primary HS, Primary Hepatic Steatosis (induced-hepatic steatosis group pre week 1); Secondary C, Secondary Control (healthy control group after 5 weeks); Secondary HS, Secondary Hepatic Steatosis (induced-hepatic steatosis group after 5 weeks HIIT, High Intensity Interval Training (healthy exercise group after 5 weeks). HS+HIIT, Hepatic Steatosis + High Intensity Interval Training (induced-hepatic steatosis with exercise group after 5 week).

by tetracycline. But HIIT significantly increased these two agent in comparison to the other groups. Also, incremental changes of BCL-2 and PARP1 gene and protein in HS + HIIT group were significant. Consists with this results, testicular BAX and Bad (pre-apoptotic factors) increased after metabolic diseases such as diabetes (Koh, 2007a, 2007b). Since exercise training, especially intermittent exercise, reduces and improves blood sugar levels (by increasing muscle GLUT4) as well as lowering blood lipids, it can be effective in controlling apoptosis in different tissue especially in testis (Ghaderpour et al., 2021; Parastesh et al., 2020). In the present study, the induction of HS increases p53 in testicular tissue. PARP1 inhibition, enhances p53-dependent and -independent DNA damage with different agent (Nguyen et al., 2011). Therefore, PARP1 and p53 are negatively correlated with each other which were confirmed in testis tissue in the present study. Although the correlation between the factors was not evaluated in the present study, it was observed that with the increase of PARP1 in the exercise groups with and without HS the p53 values also decreased significantly. Limited study considers the effects of PARP1 on P53 in testis. PARP1 has a variety of genetic effects within the cell. PARP1 appears to inhibit P53 degradation by controlling the cell nucleus (Pleschke et al., 2000). Thus, increased changes of PARP1 and decrease change of P53 in testicular tissue can benefit DNA repair and inhibit cell death. Also, increased p53 regulate cell death signals and increase apoptosis (Zhao et al., 2017). In this study, the increase of p53 was in line with the increase of Bax (as a marker of pre-apoptosis) and apoptotic cell images (Tunnel assay). However, Bcl2 was negatively correlated with these variables. All of these indicated increased apoptosis or programmed cell death in the t-estis tissue of the HS model. The apoptosis of testicular cells leads to DNA damage and ultimately to cell destruction. The use of antioxidants is one of the strategies to counteract these degradations and inhibit cell apoptosis. In oxidative damage, oxidants target DNA strands and terminate apoptosis by DNA damage. Various factors are capable of repairing DNA resulting from this damage, including PARP1. PARP1 activate in response to single- or double-strand break DNA in cells (Desai et al., 2009). Thus, increased changes in this factor could compensate for DNA damage induced by apoptosis, which in the present study there was a significant increase in PARP1 concentration in testicular tissue in HIIT training groups. Few studies have investigated this factor in testicular tissue associated with exercise training. As previously stated, PARP1 involved in gene injury and recruitment of X-ray repair cross-complementing protein 1 (XRCC1), DNA polymerase B and DNA ligase III to damage sites on DNA strands (Atorino et al., 2001). Agarwal et al. (2009) showed increased PARP1 expression in response to DNA damage in testicular tissue and explained its role in DNA strand repair (Agarwal et al., 2009). Therefore, the increased changes of PARP1 in HS + HIIT group indicate DNA repair after HS. In this group, apoptotic cells (using TUNEL assay) and apoptosis inducers such as P53 and BAX, unlike PARP1, have a significant decrease compared to HS groups. Therefore, it can be concluded that HIIT exercise was effective in DNA repair by increasing PARP1, that PARP1 overexpression is involved in repairing different types of DNA damage in different cell types (Iyama & Wilson III, 2013). It is stated that, before apoptosis, caspases and the pre-apoptotic factors degraded PARP1. But increased expression of PARP1 inhibits this action. Conversely, if PARP1 overexpression is exce-

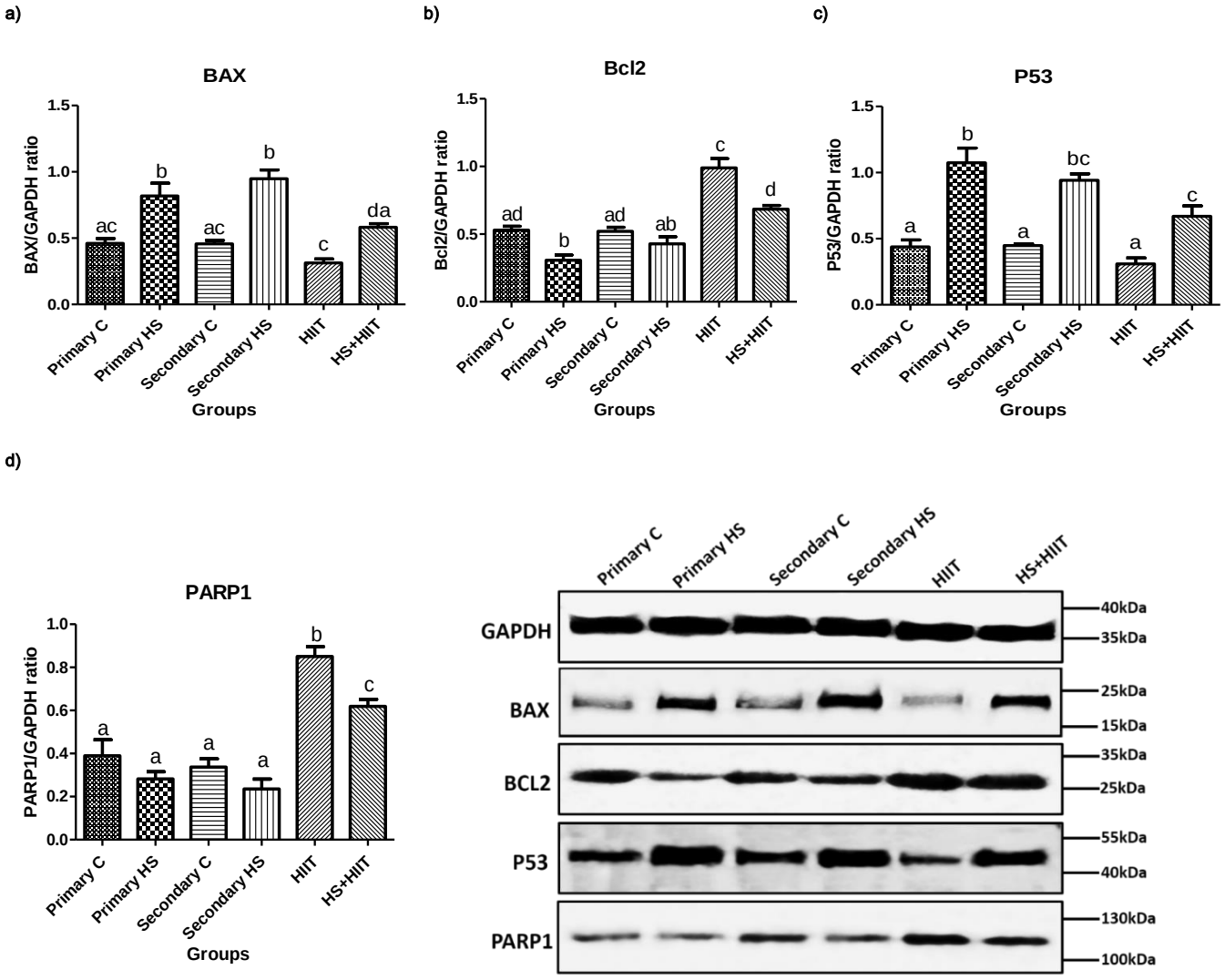


**Figure 5.** The Western blot analysis of BAX (a), BCL2 (b), P53 (c) and PARP1 (d) proteins. Date shows at means±SD. The difference signs represent statistically significant differences between the mean values (P<0.05) and the same sign are not significant (P>0.05). Abbreviation: Primary C, Primary Control (healthy control group pre week 1); Primary HS, Primary Hepatic Steatosis (induced-hepatic steatosis group pre week 1); Secondary C, Secondary Control (healthy control group after 5 weeks); Secondary HS, Secondary Hepatic Steatosis (induced-hepatic steatosis group after 5 weeks HIIT, High Intensity Interval Training (healthy exercise group after 5 weeks). HS+HIIT, Hepatic Steatosis + High Intensity Interval Training (induced-hepatic steatosis with exercise group after 5 week).

-ssive, it leads to increased poly (ADP) ribosylation and eventual depletion of NAD +/ ATP, then apoptosis ensues (Agarwal et al., 2009; Heeres & Hergenrother, 2007). Therefore, overexpression of PARP1 in the HIIT group could be risky in some ways, but apoptosis changes were not evaluated.

In this study examination of morphological changes of testicular tissue revealed that spermatogonium counts and spermatid cell counts have a significant decrease only in the tetracycline-treated groups, whereas the exercise group alone did not make a significant difference. Only the HS + HIIT group have a significant increase compared to the primary and secondary HS groups. While spermatocyte cell counts in the HS groups have a significa-

-nt decrease compared to the healthy groups, also the exercise groups with and without HS have a significant increase in spermatocyte cell counts compare to HS groups. All of these changes affect the quantity and quality of sperm. Since fatty liver and other liver damage cause changes in the concentrations of liver enzymes and blood lipids, these changes lead to thedevelopment of oxidative stress in testicular tissue that affects the anatomical structure of spermatozoa (Dallak, 2018; Li et al., 2015). It has been suggested that regular exercise training, especially interval exercise, significantly reduces fat mass, lipid profile and body weight (Coll-Risco et al., 2016; Khammassi et al., 2018). The correlation between degradation of quantitative and qualitative of sperm with abdominal obesity has been confir-

-med in different studies (Rosety et al., 2017). Therefore, a weight decrease induced by exercise can affect testicular anatomy and sperm content. Endurance exercise decrease blood flow to the testes and decrease testosterone concentration, thereby affecting on spermatogenesis (Hackney, 2001). Most studies have identified professional endurance training as a destructive factor in testicular homeostasis (Vaamonde et al., 2018). However, the present study used HIIT exercise for 8 weeks. Maleki and Tartibian (2017) examined the effects of HIIT on improving the reproductive performance of infertile couples. In this study, the exercise program consisted of interval running protocols on treadmill, three times a week, with intensity of 70% to 85% of maximal oxygen consumption. There was a significant reduction in inflammatory biomarkers (interleukin-6 and tumor necrosis factor), oxidative stress (reactive oxygen species and malondialdehyde) and a significant increase in antioxidants (superoxide dismutase, catalase and antioxidant capacity) in the exercise group. These changes were associated with favorable improvement in semen parameters, sperm DNA integrity and semen concentration. This researcher reported that their exercise program (HIIT) was sufficient to improve reproductive performance markers in infertile couple (Maleki & Tartibian, 2017). In our study, only testicular morphology (spermatogonium counts, spermatid cell counts and spermatocyte cell counts) was investigated. The training groups with and without HS have a significant increase in these indices. This change demonstrates the development and improvement of testicular morphology that can improve sperm quantitatively and qualitatively.

## Conclusion

Tetracycline-induced hepatic steatosis led to a significant increase of P53 and BAX expression in rat testicular cells (liver and testis crosstalk) that was accompanied by decreased PARP1, and HIIT significantly relieved apoptosis cell and inhibited P53 expression. These findings suggested that the beneficial anti-apoptosis effects of HIIT on testicular cells of tetracycline-induced hepatic steatosis rat might be mediated by inhibiting P53-induced BAX upregulation and preventing apoptosis-mediated degradation of PARP-1. Considering low number of studies and their different limitations such as low measurement factors (especially in liver tissue), small volume of sample, and single-center study, further studies with high methodological quality and adequate sample size specially in human sample are necessary to attain more conclusive findings.

## What is already known on this subject?

Recent studies provide substantial evidence for an association between NAFLD and atherosclerosis and cardiometabolic disorders (liver and heart crosstalk). Obesity, NAFLD and metabolic syndrome are inversely correlated with semen volume, sperm concentration, sperm motility, and sperm morphology.

## What this study adds?

It seems that high-intensity interval training can control apoptosis in testicular tissue by controlling tetracycline-induced liver damage, and this cross talk between liver and testis can be a therapeutic target in future studies.

**Organ Cross-Talk Tips:**

- High intensity exercise training can also control testicular tissue damage by controlling fibrosis and liver tissue damage.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical approval** This research was approved by the animal care and use committee at the Baqiyatallh University of Medical Sciences of Tehran, Iran (Approval reference number: IR.BMSU.REC.1396.632).

**Informed consent** Animal study.

## Author contributions

Conceptualization: M.E.Z.; Methodology: F.R., M.E.Z.; Software: F.R., M.E.Z.; Validation: F.R., M.E.Z.; Formal analysis: F.R., M.E.Z.; Investigation: F.R., M.E.Z.; Resources: F.R., M.E.Z.; Data curation: F.R., M.E.Z.; Writing - original draft: F.R., M.E.Z.; Writing - review & editing: F.R., M.E.Z.; Visualization: F.R., M.E.Z.; Supervision: F.R..; Project administration: F.R., M.E.Z.; Funding acquisition: F.R.

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