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Ginger (*Zingiber officinale* Roscoe) Improves Ethanol-Induced Reproductive Dysfunction by Enhancing Steroidogenesis and Inhibiting Oxidative Stress and Inflammation

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HIGHLIGHTS

- Ethanol induces reproductive dysfunction.
- Ginger (*Zingiber officinale* Roscoe) improves steroidogenesis.
- Ginger inhibits oxidative stress and inflammation.
- Ginger improves ethanol-induced reproductive dysfunction.

Abstract: Ginger is traditionally used as a sexual enhancer in folk medicine. Despite extensive studies on the effect of ginger on reproduction, the molecular mechanism of ginger prevention effect on ethanol-induced reproductive disorder is not fully understood. Twenty-four adult male ratswereallocated into control, ethanol (4 g/kg of body weight (BW)/day), ginger (250 mg/kg of BW/day) and ginger-ethanol group. Ginger and ethanol were administrated by gavage for 28 days. Testicular concentration of testosterone, TNF-α, and antioxidant enzymes activity and serum concentration of gonadotropins hormone and testosterone were measured. The gene expression of Nrf2 and NF-κB which regulate oxidative damage and inflammation, respectively, and StAR, P450scc and 17βHSD which are involved in testosterone synthesis were detected. Ethanol significantly decreased gonadotropin hormones, oxidative markers, expression of genes involved in testosterone synthesis and Nrf2, and in reverse significantly increased TNF-α, MDA and gene expression of NF-κB compared to control (p<0.05). While ginger could significantly improve all of the above factors

compared to the ethanol group (p<0.05). These results were also supported by histological findings. It can be concluded that ginger prevents the ethanol-induced reproductive dysfunction by improving the gonadotropins, oxidative damage and inflammation and the genes involved in testosterone synthesis.

Keywords: Ginger; Ethanol; Steroidogenesis; Oxidative Stress; Inflammation.

INTRODUCTION

Infertility as "the failure to achieve a normal pregnancy after 12 months or more of regular unprotected sexual intercourse" is a major medicine and psychiatry problem [1]. 8% to 15% of couples are infertile in developed and developing countries [2] and half of these cases are related to male infertility [3]. Low sperm count (under 15 million/ml semen), low sperm motility and abnormal sperm are the major problems in male with infertility [3, 4]. Male infertility may cause by many factors such as cryptorchidism, cystic fibrosis, trauma, obstructive lesions, varicocele, tumors, infection, oxidative stress and inflammation [5, 6].In addition, cigarette smoking, anabolic steroids, medications, depression, physical inactivity, exposure to electromagnetic waves and ethanol abuse are closely related to our lives and are known as the main risk factors for male infertility [6-9]. Previous studies have shown that ethanol abuse causes reproductive disorders [10, 11] by changing the structure and function of the mitochondria [12], directing Sertoli cells, germ cells and interstitial cells toward apoptosis and necrosis pathways [13] and decreasing testosterone production. In addition, ethanol induced- inflammation and oxidative stress is one of the main mechanisms for lowering testosterone level and impairing reproductive function. Steroidogenic acute regulatory (StAR), Cholesterol side-chain cleavage enzyme (P450scc) and 17β-Hydroxysteroid dehydrogenases (17βHSD) along with other steroidogenesis enzymes involve in the synthesis of testosterone by Leydig cells. Oxidative stress and inflammation regulate by nuclear factor erythroid 2-related factor 2 (Nrf2) and Nuclear factor kappa B (NF-κB), respectively and are considered as the pathogenesis of alcohol-related testicular damage [11, 14, 15]. Oxidative testicular damage as a result of an overproduction of nitrogen and oxygen free radicals and antioxidant defense mechanism deficiency can be one of the factors reducing testosterone production and sperm quality in many diseases and disorders [8]. Many studies have shown that increased activity of proinflammatory cytokinesfactorssuch as interleukin-1 (IL-1) and tumor necrosis factor (TNF)-α can impair testosterone production and sperm quality as paracrine regulators[16, 17]. These cytokines physiologically regulate steroidogenesis function of Leydig cells; however, during inflammation, the high levels of TNF-α and IL-1 inhibit the production of testosterone. It is clear that oxidative stress and inflammatory status are closely related to lifestyle and can be controlled with a few simple strategies, including the use of herbal medicines and nutritional supplements, and increase the likelihood of fertility[18]. Moreover, herbal remedies and lifestyle modifications as a part of Complementary and Alternative Medicine can be acceptable therapeutic approaches that prevent the reproductive toxicity-induced by lifestyle-related factors including ethanol[7]. Ginger (*Zingiber officinale* Roscoe) as a flowering plant exhibits the pharmacological properties including antioxidant, anti-inflammatory, anti-diabetic and anti-cancer activities due to several biological active compounds such as gingerol, shogaols, gingerdiol and gingerdione[19, 20]. Ginger is traditionally used to enhance sexual potency around the world [5]. Ginger in the historical medicine of India, China and Persia traditionally used as a hot remedy to enhance male reproductive function [21, 22].Moreover ginger overcomes reproductive toxicity of cyclophosphamide [23], gentamicin [24], sodium arsenite[25] and ethanol[7] and increases the sperms counts, viability and motility and testosterones [26]. Despite the findings of these studies, the molecular pathway that ethanol disrupts testosterone production, and the role of ginger on steroidogenesis, inflammation, and oxidative stress in rats consuming ethanol, is not fully understood. Therefore, the aim of this study was to investigate the ginger prophylactic effect on ethanol-induced reproductive dysfunction in male rats.

MATERIALS AND METHODS

Preparation of Ginger Powder

Fresh ginger or rhizome of *Zingiber officinale* Roscoe was purchased from a local herbal shop. It was identified by a botanist and a voucher specimen was deposited in the Botanical Herbarium in Shandong University. The plant name was also checked on http://www.theplantlist.org. The ginger (3 kg) was shredded into small pieces and then dried at room temperature and subsequently mechanically milled to a fine powder.

Evaluation of the composition, the total phenolic content and the total antioxidant activity of ginger

The level of β-carotene, polyphenols and acid ascorbic in ginger powder was measured by a modified spectrophotometric method as previously described [27]. The amount of elements in ginger powder was measured as previously described [28]. To evaluate the total phenolic content (TPC) of ginger powder a modified Folin-Ciocalteu spectrophotometric method was used [29]. The scavenging activity of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical was also measured to evaluate the total antioxidant capacity of ginger powder. Briefly, it was measured via changing the intensity of the color in solutions containing the different amount of DPPH spectrophotometrically. First, 3mL of the DPPH (0.012 g/100 mL) solution was mixed well with a serial dilution (5-160 µg/mL) of ginger. The new solutions were incubated at room temperature in the dark. The absorbance after 15 min at 517 nm against methanol as blank was read.

Animals and Experimental Design

Twenty four male Sprague-Dawley rats (6-8 weeks, 190-230g) were purchased from the Laboratory Animal Research and Breeding Institute, Shandong University, Shandong, China. Rats were kept in polyethylene cages in animal room at 23±2 ºC and light cycle 12 hours lightness and 12 hours darkness and had free access to rodent pellet food and tap water. All research has been done in accordance with the Organizational Ethics Committee of Shandong University with reference number (586315/15). Animals were randomly assigned to four groups, including control group or vehicle group, which was assigned to receive 1mL/d of normal saline by oral administration, ginger group (250 mg/kg of BW/day [30]for four consecutive weeks by gavage), ethanol group (4g/kg of BW/day for four consecutive weeks by gavage) [31) and ginger-ethanol group, the animals were first given ginger and then ethanol. By adding a certain volume of normal saline as a solvent, the dose of ginger was prepared in the form of a suspension. The body weight of rats was measured at the beginning and the end of the study.

Sampling and Tissue and Blood Preparation

On the28th day of experiment, animals were weighted and sacrificed by deep anesthesia with ether. Blood samples were collected by heart puncture and were centrifuged (3000rpm for 15 min) to obtain sera and they were then separated and stored at -80 ºC for later biochemical assays. After blood collection, testes were weighted and testes index (%) was calculated by the equation testes weight (g)*100/body weight. Right testis was also removed and kept in formalin (10%) bottles. Another testis was manually homogenized and centrifuged at 3000rpm for 10 min in a cold phosphate buffer (pH 7.4, 0.1M). The obtained supernatant was used to evaluate the level of testosterone and TNF-α, and antioxidant enzymes activity.

Determination of Testosterone,Gonadotropin Hormones, Oxidative Stress and Inflammation Parameters

The testicular activity of antioxidant enzymes including total Superoxide Dismutase (SOD) and Catalase (CAT) and the level of Malondialdehyde (MDA) as a biomarker for lipid peroxidation were measured. The activity of SOD was evaluated according to the manufacturer's instruction from RANSOD kit (Randox Laboratories Company, UK). SOD is responsible for the dismutation of the superoxide to hydrogen peroxide and molecular oxygen. This method superoxide radicals react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyl tetrazolium chloride (INT) to hydrogen peroxide and molecular oxygen that form a red formazan dye. The activity of SOD is detected by the inhibition of this reaction. One unit of SOD is that which causes 50% inhibition of the rate of reduction of INT under the conditions of the assay. Catalase activity was also evaluated by a modified spectrophotometric method as described by Aebi (1984) based on the decomposition of H_2O_2. Briefly, 0.25 mL of H_2O_2 buffered solution (pH=7.0) and 0.5 ml of diluted tissue supernatant was mixed. This reaction was read at 240 nm for 2 min at 25ºC. Catalase activity was detected as the unit that is defined as µmol H_2O_2 consumed/min per mg tissue protein. The level of MDA in rat testis was also evaluated by a modified method as described by Lykkesfeldt (2001)[32). Briefly, 0.5ml of 30% trichloroacetic acid was mixed with0.4ml of the tissue serum, and 1.6ml of TrisKCl was added to it. 0.5ml of thiobarbituric acid was then added and the solution was incubated for 45min at room temperature and was read at wavelength of 532nm. The concentration of testosterone, LH, FSH and TNF-α in serum and testis were measured byenzyme-linked immunosorbent assay(ELISA) rat's specific kits (Shanghai Crystal day Biotech Co., Ltd.)**.**

Evaluation of gene expression involved in oxidative stress, inflammation and steroidogenesis

Total RNA extraction from testis tissue was performed by TRIzol™-based RNA extraction method (Thermo Fisher Scientific Co., USA). The extracted RNA was quantified by using the NanoDrop™ device (Thermo Fisher, NY, USA). Complementary DNA (cDNA) was synthesized by MMLV reverse transcriptase (Bioneer, Korea). Table 1 contains information on the primers used in this study. Real time PCR to evaluate the genes expression of StAR, P450scc, 17βHSD, Nrf2 and NF-κB was performed by Real Q Plus 2x Master Mix Green (Ampliqon, Denmark) in Rotor Gene 6000 (Corbett Research, Australia). The thermal cycling conditions were 95 °C for 2 min for preliminary PCR activation, denaturation at 95 °C for 20 s, annealing and extension at 58 °C for 30 s and melting at 72 °C for 30 s for 40 cycles. B-actin expression as a housekeeping gene was used to normalize the expression level of each target gene. The $2^{-\Delta\Delta CT}$ equation was used to calculate the fold of target genes.

Table 1. The sequences of primers used in this study

Gene	Sequence	PCR product (bp)
P450scc	Forward 5′- AACAACTACTTCCGCAGCCT-3′	170
	Reverse 5′- CGGTAGAACAATGAGCTGGA-3′	
StAR	Forward 5′- CTGAGGCAACAGGCTGTGAT-3′	114
	Reverse 5′-AGCCGAGAACCGAGTAGAGAG-3′	
17βHSD	Forward 5′-CGCCTCAGGAACCTCGTCT-3′ Reverse 5′- GCTGGCGCAATAAACGTCA-3′	62
Nrf2	Forward 5´-AAGACAAACATTCAAGCCGATTAG-3´	141
	Reverse 5´-TTGCTCCTTGGACATCATTTCAT-3´	
NF-κB	Forward 5′- GCACCAAGACCGAAGCAAT-3′	143
	Reverse 5′-CGTAACCGCGTAGTCGAAGA-3′	
β-actin	Forward 5′-TTGCTGACAGGATGCAGAAGG-3′	141
	Reverse 5´-CTGGAAGGTGGACAGTGAGGC-3′	

Histopathology assessment

The right testicle was removed and washed with normal saline and placed in a container containing 10% formalin. After at least 24 hours, the tissue samples were transferred to a container containing 4% formalin. It was then embedded in paraffin by a histologist and sections five microns thick were prepared. In the next step, it was stained with hematoxylin and eosin (H&E). Finally, these sections were examined to evaluate histopathological changes under a light microscope (BX-51, Olympus Corporation, Tokyo, Japan).

Statistical analysis

Data were analyzed by one-way analysis of variance (ANOVA) followed by post Hoc multiple comparisons Tukey test using the statistical package for social sciences (SPSS17.0) software for windows. Statistical significance was set at $p< 0.05$. The results were expressed as mean ±standard error of mean (Mean± SEM).

RESULTS

The results of the analysis of the composition of ginger powder

The data in Tables 2 and 3 show the total phenol content, total antioxidant capacity and ginger powder composition. The ginger powder total antioxidant activity (IC50%) and total phenolic content were 364.58± 2.54and 37.78± 2.21, respectively. The amount of β-carotene, polyphenols and acid ascorbic in ginger powder were 0.92±0.03, 23.47±1.463 and 5.47±1.463 (mg/100g dry sample), respectively. The results of the level of micro and macro-elements in ginger powder are presented in Table 2. The level of Mn and Cu as the essential microelements in this powder was 16.24 ± 10.24 and 13.24 ± 2.14, respectively.

Table 2. The analysis of the composition of ginger powder and its total antioxidants and total phenol

Test	Results
Description & Appearance	Clear, dark yellow, special odor
Specific Gravity (20 °C g/ml)	0.9104 - 0.9108
pH Value	5.12 – 6.5
Total phenolic (mg GAE. 100g-1)	37.78± 2.21
Total antioxidant activity (IC50%)	364.58± 2.54
Protein (g/100g dry sample)	18.2 ± 0.145
Fat (g/100g dry sample)	27.6 ± 0.208
Ash (g/100g dry sample)	11.8 ± 0.173
Crude fibers (g/100g dry sample)	37.8 ± 0.233
Carbohydrate (g/100g dry sample)	20.4 ± 0.463
β-carotene (mg/100g dry sample)	0.92± 0.03
Acid ascorbic (mg/100g dry sample)	5.47± 1.463
Polyphenol (mg/100g dry sample)	23.47± 1.463
Calcium (mg/100g dry sample)	143.24±23
Na (mg/100g drysample)	254.1 ± 36.8
K (mg/100g dry sample)	364.85 ±75.4
Mg (µg/100mg dry sample)	16.24 ± 10.24
Fe (µg/100mg dry sample)	1.14 ± 0.21
Cu (µg/100mg dry sample)	13.24 ± 2.14
Zn (µg/100mg dry sample)	1.04 ±0.07
Mn (µg/100mg dry sample)	4.16±0.3
Cr (µg/100mg dry sample)	0. 89±0.04

Table 3. DPPH radical scavenging activity of ginger powder

Sample	Concentration (µg/mL)	%Inhibition	IC50
1	5	7.79484	354.782
2	10	12.0601	
3	20	13.8622	
4	40	18.0529	
5	80	21.2625	
6	160	27.0847	

The effect of ginger and ethanol on body and testis weight

The results showed that the weights of body and testes in animals exposed to ethanol significantly were lower than those in other groups (Table 4, $p<0.05$). These parameters after pretreating with ginger significantly increased in the ginger-ethanol group. Our results showed that the highest weight of the body and testis was observed in the ginger group (Table 4, $p<0.05$).

Table 4. The mean± SEM of final body weight (g), testis weight (g) and tissue index (%) in studied groups*

Groups	Control	Ginger	Ethanol	Ginger-Ethanol
Body weight (g)	348.97±10.47 a	369.94± 12.36 b	271.26±10.34 c	310.14±15.69 d
Testis weight (g)	6.75±1.14 a	7.27±2.68 a	2.68±1.45 b	4.48±1.41 c
Tissue index (%)	1.93±0.11 a	1.97±0.21 a	0.988±0.14 b	1.44± 0.08 a

* Different small alphabetic letters show significant difference with other groups at $p < 0.05$.

The effect of ginger and ethanol on testicular and serum total testosterone, LH and FSH hormones

The concentration of total testosterone in serum and testis significantly decreased by ethanol (Table 5, $p<0.05$); and they significantly increased by ginger in alcoholic rats in comparison to ethanol group (Table 5, $p<0.05$). The level of LH and FSH (1.06±0.15 and 17.36±1.23, respectively) in the ethanol group were lower than those levels in control group (1.96±0.12 and 13.91±1.23) (Table 5, $p< 0.05$). Pretreatment with ginger in a dose of 250mg/kg for four consecutive weeks had no significant effect on the serum level of LH and FSH in alcoholic rats in comparison to other groups (Table 5, $p> 0.05$).

Table 5. The mean± SEM of testicular and serum total testosterone and *gonadotropin hormonesconcentration*in different groups*.

Groups	Testicular Testosterone (ng/mL)	Serum Testosterone (ng/mL)	LH (ng/mL)	FSH (ng/mL)
Control	1.25a±24.4	1.15a±3.456	1.964 ±0.12a	1.23a±17.36
Ethanol	1.27b±14.65	0.025b±0.974	1.06 ±0.15b	1.23b±13.91
Ginger	25.64 ±1.23a	0.717a±3.896	2.896 ±0.185c	1.75a±7.89
Ginger-Ethanol	1.15c±21.36	1.946 ±0.21c	1.746 ±0.125a	7.24 ±1.19a

*Different small alphabetic letters show significant difference with other groups at $p < 0.05$. LH: Luteinizing Hormone, FSH: Follicle-Stimulating Hormone

The effect of ginger and ethanol on the expression of genes involved in steroidogenesis, oxidative status and inflammation in testicular tissue

Ethanol ingestion for 4 weeks significantly decreased the expression of StAR and P450scc in testicular tissue compared to non-alcoholic rats, while pretreatment with ginger could significantly increase the expression of these genes (Figure 1, $p<0.05$). The expression of StAR and P450scc significantly were higher in the ginger group than those in other groups (Figure 1, $p<0.05$). The results of statistical analysis showed that Nrf2 expression significantly down regulated and expression NF-kB up regulated in testicular tissue by ethanol. However, pretreatment with ginger could reverse these effects in this tissue in alcoholic rats compared to alcoholic rats without any intervention. Moreover, the expression of Nrf2 significantly was higher in the ginger group than that in other groups (Figure 1, $p<0.05$).

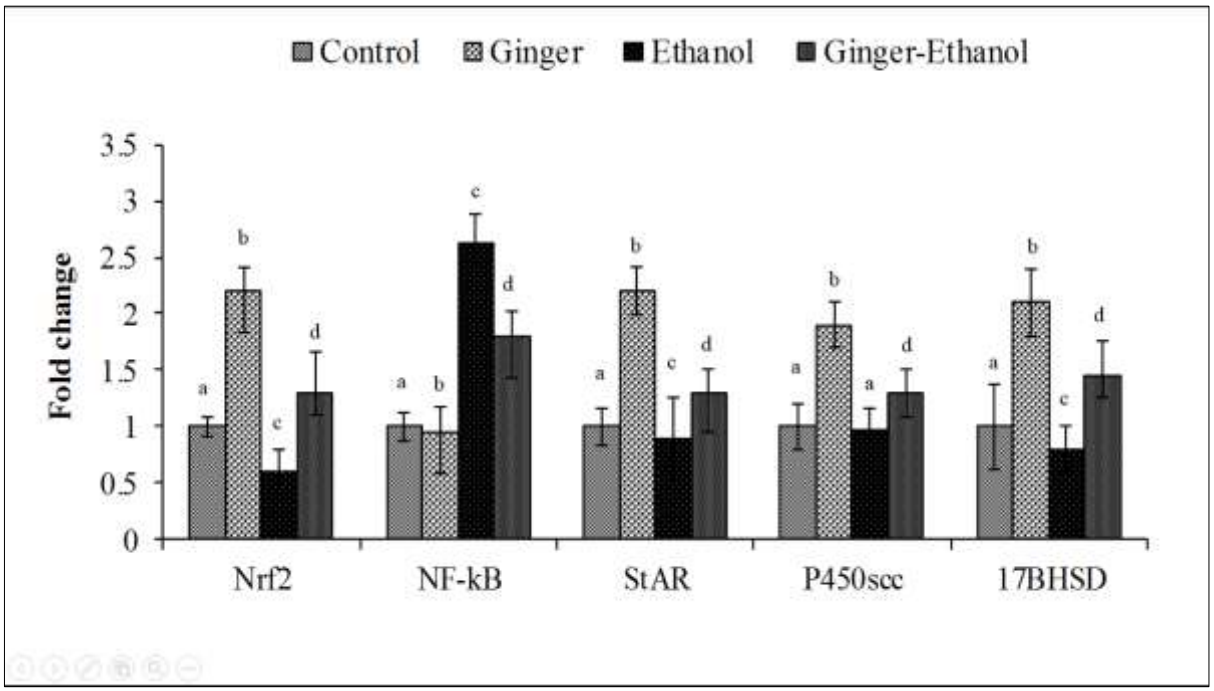

Figure 1. The mean ± SEM of the testicular expression of StAR, P450scc, 17βHSD, Nrf-2 and NF-κB in different groups. Different small alphabetic letters show significant difference with other groups at p<0.05.

The effect of ginger and ethanol on status of oxidative stress and inflammation in testicular tissue

The level of SOD and TAC was significantly lower in the ethanol group than that in other groups, while pretreatment with ginger could significantly increase the level of these bio factors compared to other groups (Figure 2, p< 0.05). The level of CAT was significantly lower in the ethanol group than that in other groups; while this level was significantly reverses by ginger in alcoholic rats. MDA and TNF-α levels were significantly higher in the ethanol group than those in other groups. These levels significantly decreased by ginger in the ginger-ethanol group in comparison to the ethanol group (Figure 2, p< 0.05).

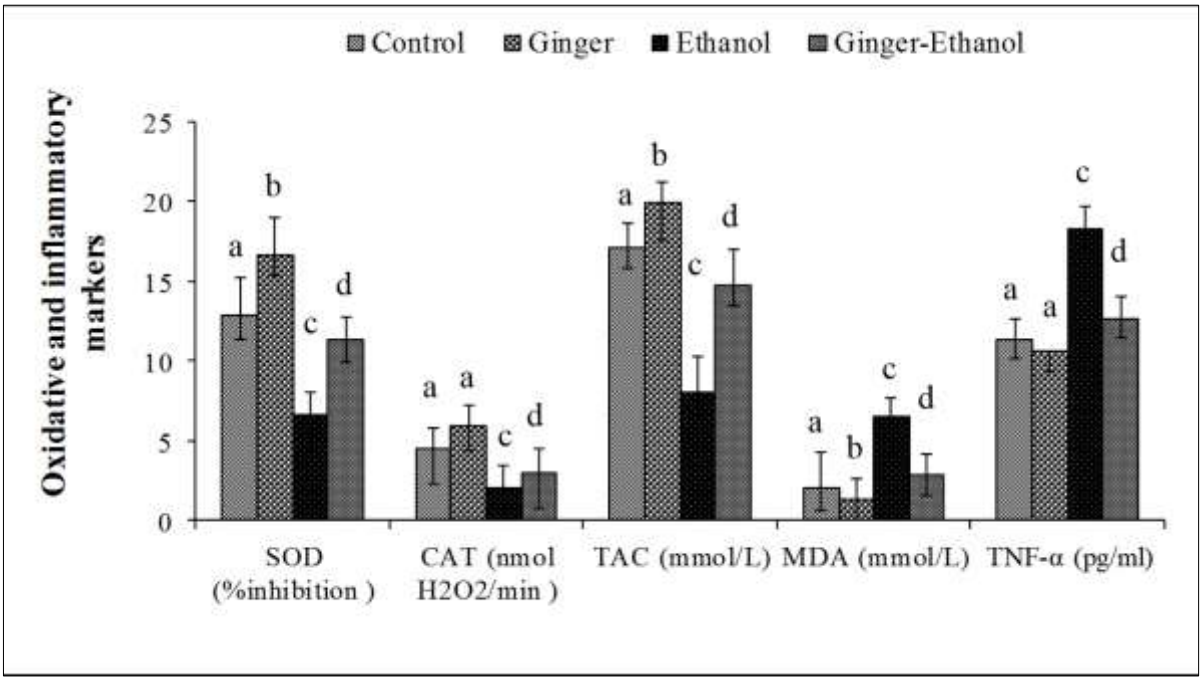

Figure 2. The mean± SEM of biomarkers of oxidative stress and inflammation in different groups. Different small alphabetic letters show significant difference with other groups at p<0.05.

Histopathological Results

Histopathological section studied groups are present in Figure 3 (Olympus, Japan; Magnification x40). The structure of seminiferous tubules was regular and normal in control groups (Figure 3 A and B). Moreover, the cycle of spermatogenesis was observed active and regular; the arrangement of spermatogenesis, spermatogonia, primary spermatocyte, and released sperm in lumen were regularly observed in these groups. While in the ethanol group (C) in the germinal epithelium, vacuolar-like structures and in some cases damaged germinal epithelium were observed in many parts of the seminiferous tube. Pretreatment with ginger before ethanol (D) could partially improve these damages on testicular tissue in alcoholic rats. In addition, the spermatogenic cell line shows better order than the ethanol group, and sperm cells can be seen in the lumen.

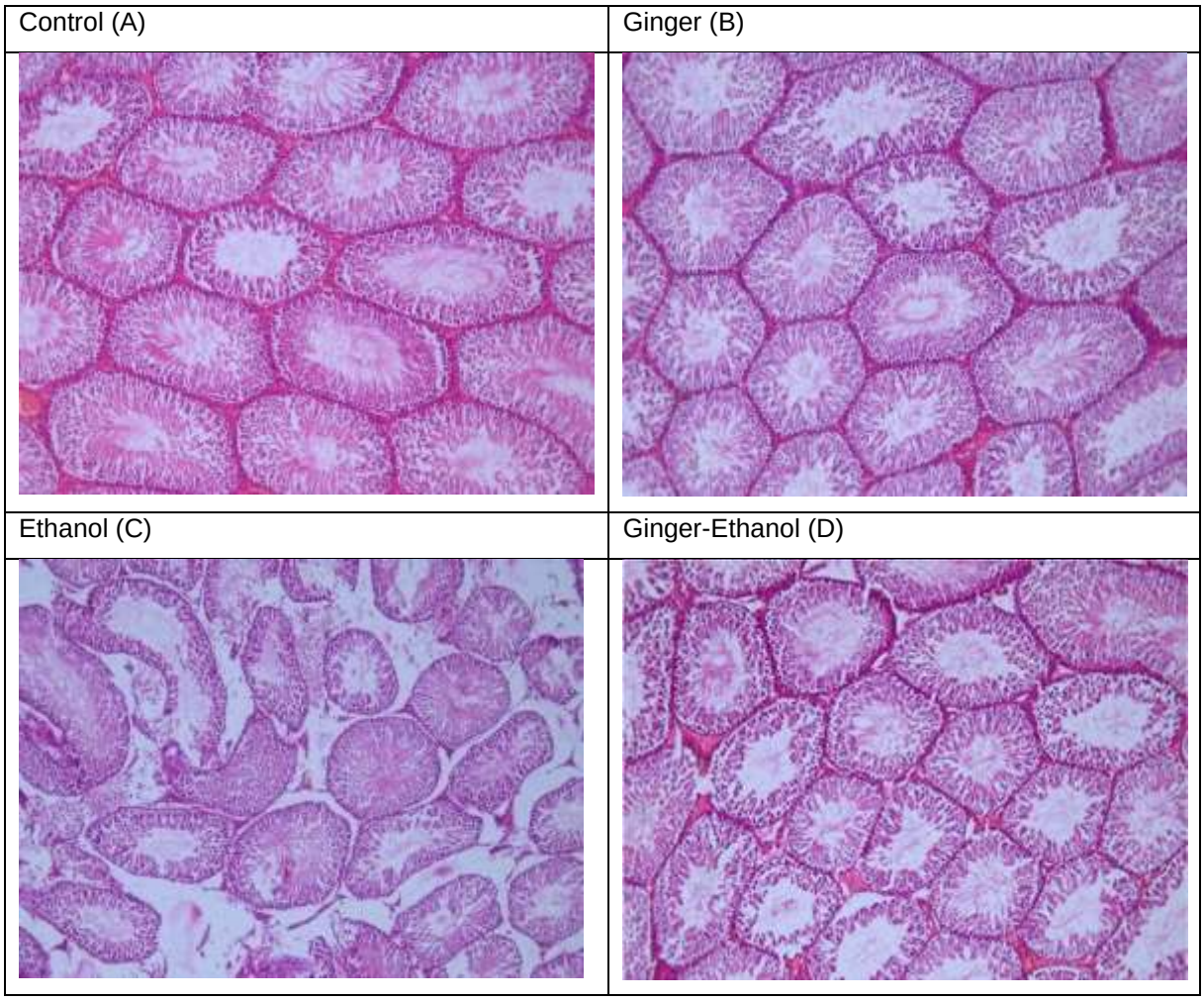

Figure 3. Histological section of seminiferous tubules in the testis of mature adult male rat in different groups (Hematoxylin and Eosin staining, Magnification ×40). Control (A) and Ginger (B) groups: Regular seminiferous tubule with normal germinal epithelium morphology; Ethanol group (C) seminiferous tubule with abnormal germinal epithelium morphology. Ginger-ethanol group (D) seminiferous tubule with improved germinal epithelium morphology.

DISCUSSION

In this study, the results indicated that ethanol could impair reproductive function through disruption of the pituitary-gonadal axis, steroidogenesis, oxidative stress and inflammation, which is consistent with previous studies [7,33,34]. Ethanol abuse can induce oxidative stress and inflammation through mitochondrial damage, production of homocysteine and acetaldehyde, disruption in homeostasis of essential elements and increased microsomal proliferation [7, 35-37]. In agreement with previous findings, our results indicated that ethanol could induce oxidative stress by reducing SOD and CAT activity and increasing MDA level in testis, which is consistent with previous results [14, 38]. It was reported that ethanol(4g/kg) for 28 days could induce oxidative damage and change in the serum level of total homocysteine (tHcy) and

testosterone and the homeostasis of some trace element [7]. Ethanol also can induce morphological abnormalities in sperm cells by inducing genotoxicity[33]and seminiferous tubules due to the accumulation of lipids in Sertoli cells and spermatogenic cells in alcohol-fed rats[39]. In agreement with these evidences, our histological results indicated that ethanol induces testicular atrophy and morphological abnormalities. Hence the testicular function may also be altered. In agreement with previous evidences, [7, 16, 40, 41] our results showed that serum and testicular testosterone concentration significantly reduced by ethanol. In addition, ethanol induced- inflammation and oxidative stress can be the main mechanisms for lowering testosterone concentration and impairing reproductive function [15, 42]. In this regard NF-κB and Nrf2 expression and TNF-α level along with StAR, P450scc and 17βHSD expression in the testicle significantly altered by ethanol. In agreement with our results, it was reported that inflammation decreases the production of testosterone by decreasing in P450scc and StAR expression [43]. Nrf2 and NF-κB are involved in oxidative stress, inflammation and apoptosis [44, 45]. Ahmed and coauthors indicated that Nrf2 regulates NLRP3 inflammasome activity which involves in NF-κB inflammatory pathways [44]. In addition, Zhao and coauthros showed that NF-κB /COX2 pathway is closely associated with inflammation and oxidative stress, and decrease production of testosterone[42]. High level of Cox2 expression in Leydig cells inhibits the expression of StAR[46, 47]. Therefore, ethanol by inflammation and oxidative stress could impair expression of StAR and P450scc and decreases in testosterone concentration in serum or testis. On the other hand, Loveland and coauthors reported that IL-1 and IL-6 are essential to protect sperm cells [16] by regulating the function and the development of Sertoli cells and germ cells [48]. Recent studies also showed that the production of testosterone decreased by ethanol [7] and radio frequency wave [49], which is associated with ROS overproduction. Moreover, another mechanism by which ginger or ethanol affects the testicles inflammatory status and its normal function is the concept of "immune privilege", regulation of local cell function to prevent pathogenic autoimmunity by inflammatory factors [16]. In addition, in agreement with our results the normal function of Leydig cells to produce testosterone is mainly influenced by the pituitary-gonadal axis, especially LH that this process can be disrupted by ethanol [50]. Our findings showed that ethanol not only destroys testicular structure and function, but also lowers the levels of LH and FSH, which is consistent with Noth and Walter Jr (1984) and Rachdaoui and Sarkar [51, 52]. Therefore, it can be well concluded that the ethanol-induced oxidative stress and inflammation along with pituitary-gonadal axis disruption reduces the expression of StAR, P450scc and 17βHSD. Our results also showed that ginger increase StAR, P450scc and 17βHSD expression in ginger group, this result clearly demonstrates that ginger has a direct effect on steroidogenesis and the production of testosterone. Our results in consistent with the finding of previous studies [7, 23, 24, 53] also showed that ginger improves ethanol-induced testicular damage and pituitary-gonadal axis disruption. Ginger or its derivatives also improves testosterone production by a wide range of cellular and molecular mechanisms [7, 23, 54-56]. Afolabi and coauthors showed that ginger rhizome inhibit oxidative damage in testis and improve epididymal sperm parameters in cryptorchid rats[54]. Mohammadi and coauthors also showed that ginger extract increases the testosterone concentration and the number of spermatogonial and Sertoli cells in seminiferous tubules [23]. Ghareib and coauthors also indicated that 6-gingerol stimulates cGMP pathway by enhancing the production of NO [57], it increases blood flow by dilating blood vessels [58]. Khaki and coauthors showed that ginger [50 and 100 mg/kg/day) improved sperm viability and motility, and serum total testosterones [41]. Improving homeostasis of the trace elements and tHcyby ginger is other mechanisms that help improve inflammation and oxidative stress induced by ethanol[7]. In addition, studies have shown that, improving blood sugar by ginger increases testosterone production and sperm parameters in diabetic patients [59]. Furthermore, Banihani reported that ginger improves production of testosterone by enhancing the entrance of cholesterol and recycling testosterone receptors, and inhibiting oxidative damage in the testes and the production of LH [56, 59]. In addition, the role of ginger in improving the expression of genes involved in oxidative stress and inflammation should not be forgotten. Oxidative stress and inflammation interact closely with each other and with cell apoptotic pathways [60]. Ginger could activate the Nrf2 signaling and inhibit NF-κB signaling, hence improves testosterone production in our study. Our results in agreement with previous studies [47, 53, 61, 62] showed that ginger improve the genes expression of Nrf2 and NF-κB and TNF-α level and thus can play an important role in reducing the damage caused by ethanol. [6]-gingerol also inhibits the phosphorylation of p38 mitogen-activated protein kinase and the expression of NF-κB and COX-2 [47].Our results showed that NF-kB and Nrf2 which are involved in regulating oxidative stress and inflammation could influence on steroidogenesis and sperm parameters. There is a negative correlation among the genes expression involved in steroidogenesis, oxidative stress and inflammation, it is clear from our results and the findings of other studies [43]. Therefore, it is safe to say that along with the direct effect of ginger on inflammation and oxidative stress improving the gene expression of

StAR and P4500scc involved in testosterone synthesizing along with gonadotropin hormones can be one of the main mechanisms of ginger to improve reproductive dysfunction induced by ethanol.

CONCLUSIONS

It can be concluded that ethanol could induce reproductive disorders by disrupting the testosterone synthesis pathway and reducing the levels of gonadotropin hormones and oxidative damage and inflammation, and ginger could improve ethanol-induced reproduction disorders by improving these pathways and increasing the expression of genes involved in testosterone synthesis.

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REFERENCES

1. WHO. https://www.who.int/reproductivehealth/topics/infertility/definitions/en/. ICMART glossary. 2009.
2. Agarwal A, Mulgund A, Hamada A, Chyatte MR. A unique view on male infertility around the globe. Reprod Biol Endocrinol. 2015;13(1):37.
3. Kumar N, Singh AK. Trends of male factor infertility, an important cause of infertility: A review of literature. J Hum Reprod Sci. 2015;8(4):191.
4. Gopalakrishnan K. Interpretation of semen analysis results: A practical guide. Indian J Med Res. 2001;113:114.
5. Saradha B, Mathur PP. Effect of environmental contaminants on male reproduction. Environ Toxicol Pharmacol. 2006;21(1):34-41.
6. Gaur DS, Talekar M, Pathak VP. Effect of cigarette smoking on semen quality of infertile men. Singapore Med J. 2007;48(2):119.
7. Akbari A, Nasiri K, Heydari M, Mosavat SH, Iraji A. The protective effect of hydroalcoholic extract of Zingiber officinale Roscoe (Ginger) on ethanol-induced reproductive toxicity in male rats. J Evid Based Complementary Altern Med. 2017;22(4):609-17.
8. Agarwal A, Prabakaran SA. Mechanism, measurement, and prevention of oxidative stress in male reproductive physiology2005.
9. Kıvrak EG, Yurt KK, Kaplan AA, Alkan I, Altun G. Effects of electromagnetic fields exposure on the antioxidant defense system. Journal of microscopy and ultrastructure. 2017;5(4):167-76.
10. La Vignera S, Condorelli RA, Balercia G, Vicari E, Calogero AE. Does alcohol have any effect on male reproductive function? A review of literature. Asian journal of andrology. 2013;15(2):221.
11. Emanuele MA, Emanuele N. Alcohol and the male reproductive system. Alcohol Research & Health. 2001;25(4):282.
12. Kiessling KH, Tobé U. Degeneration of liver mitochondria in rats after prolonged alcohol consumption. Exp Cell Res. 1964;33(1-2):350-4.
13. Kianifard D, Sadrkhanlou RA, Hasanzadeh S. The ultrastructural changes of the sertoli and leydig cells following streptozotocin induced diabetes. Iranian journal of basic medical sciences. 2012;15(1):623.
14. Dosumu OO, Akinola OB, Akang EN. Alcohol-induced testicular oxidative stress and cholesterol homeostasis in rats–The therapeutic potential of virgin coconut oil. Middle East Fertility Society Journal. 2012;17(2):122-8.
15. Akbari A, Jelodar G-A. The effect of oxidative stress and antioxidants on men fertility. Zahedan J Res Med Sci. 2013;15(7):1-7.
16. Loveland KL, Klein B, Pueschl D, Indumathy S, Bergmann M, Loveland BE, et al. Cytokines in male fertility and reproductive pathologies: immunoregulation and beyond. Front Endocrinol (Lausanne). 2017;8:307.
17. Lysiak JJ. The role of tumor necrosis factor-alpha and interleukin-1 in the mammalian testis and their involvement in testicular torsion and autoimmune orchitis. Reprod Biol Endocrinol. 2004;2(1):9.
18. Ruiz-Núñez B, Pruimboom L, Dijck-Brouwer DAJ, Muskiet FAJ. Lifestyle and nutritional imbalances associated with Western diseases: causes and consequences of chronic systemic low-grade inflammation in an evolutionary context. J Nutr Biochem. 2013;24(7):1183-201.
19. Ahmad I, Zahin M, Aqil F, Hasan S, Khan MSA, Owais M. Bioactive compounds from Punica granatum, Curcuma longa and Zingiber officinale and their therapeutic potential. Drugs of the Future. 2008;33(4):329.
20. Khan MMAA, Ansair MI, Maheshwari RK. Phytochemistry and Pharmacological Properties of Ginger (Zingiber officinale)2018.
21. Liu Y, Liu J, Zhang Y. Research Progress on Chemical Constituents of Zingiber officinale Roscoe. BioMed research international. 2019;2019.
22. R Vasanthi H, P Parameswari R. Indian spices for healthy heart-an overview. Curr Cardiol Rev. 2010;6(4):274-9.

23. Mohammadi F, Nikzad H, Taghizadeh M, Taherian A, Azami-Tameh A, Hosseini SM, et al. Protective effect of Zingiber officinale extract on rat testis after cyclophosphamide treatment. Andrologia. 2014;46(6):680-6.
24. Zahedi A, Khaki A, Ahmadi AH, Rastgar H, Rezazadeh SH. Zingiber officinale protective effects on gentamicin's toxicity on sperm in rats2010.
25. Morakinyo AO, Achema PU, Adegoke OA. Effect of Zingiber officinale (Ginger) on sodium arsenite-induced reproductive toxicity in male rats. Afr J Biomed Res. 2010;13(1):39-45.
26. Bordbar H, Esmaeilpour T, Dehghani F, Panjehshahin MR. Stereological study of the effect of ginger's alcoholic extract on the testis in busulfan-induced infertility in rats. Iranian journal of reproductive medicine. 2013;11(6):467.
27. Andarwulan N, Kurniasih D, Apriady RA, Rahmat H, Roto AV, Bolling BW. Polyphenols, carotenoids, and ascorbic acid in underutilized medicinal vegetables. J Funct Foods. 2012;4(1):339-47.
28. Basaki M, Saeb M, Nazifi S, Shamsaei HA. Zinc, copper, iron, and chromium concentrations in young patients with type 2 diabetes mellitus. Biol Trace Elem Res. 2012;148(2):161-4.
29. Kruawan K, Kangsadalampai K. Antioxidant activity, phenolic compound contents and antimutagenic activity of some water extract of herbs. Thai J Pharm Sci. 2006;30:28-35.
30. Atta AH, Elkoly TA, Mouneir SM, Kamel G, Alwabel NA, Zaher S. Hepatoprotective Effect of Methanol Extracts of Zingiber officinale and Cichorium intybus. Indian J Pharm Sci. 2010;72(5):564-70. Epub 2011/06/23. doi: 10.4103/0250-474x.78521. PubMed PMID: 21694986; PubMed Central PMCID: PMCPMC3116299.
31. Alirezaei M, Jelodar G, Niknam P, Ghayemi Z, Nazifi S. Betaine prevents ethanol-induced oxidative stress and reduces total homocysteine in the rat cerebellum. J Physiol Biochem. 2011;67(4):605-12.
32. Lykkesfeldt J. Determination of malondialdehyde as dithiobarbituric acid adduct in biological samples by HPLC with fluorescence detection: comparison with ultraviolet-visible spectrophotometry. Clin Chem. 2001;47(9):1725-7.
33. Cebral E, Abrevaya XC, Mudry MD. Male and female reproductive toxicity induced by sub-chronic ethanol exposure in CF-1 mice. Cell Biol Toxicol. 2011;27(4):237-48.
34. Dosumu OO, Osinubi AAA, Duru FIO. Alcohol induced testicular damage: Can abstinence equal recovery? Middle East Fertil Soc J. 2014;19(3):221-8.
35. Akbari A, Nasiri K, Heydari M. Ginger (Zingiber officinale Roscoe) extract can improve the levels of some trace elements and total homocysteine and prevent oxidative damage induced by ethanol in rat eye.Avicenna J Phytomed. 2019.
36. Manzo-Avalos S, Saavedra-Molina A. Cellular and mitochondrial effects of alcohol consumption. Int J Environ Res Public Health. 2010;7(12):4281-304.
37. Ji C. Mechanisms of alcohol-induced endoplasmic reticulum stress and organ injuries. Biochem Res Int. 2011;2012.
38. Abarikwu S, Duru Q, Chinonso O. Antioxidant enzymes activity, lipid peroxidation, and steroidogenesis in rats after co-exposure to atrazine and ethanol. Toxicol Lett. 2015;2(238):S303.
39. Weinberg J, Vogl AW. Effects of ethanol consumption on the morphology of the rat seminiferous epithelium. J Androl. 1988;9(4):261-9.
40. Hales DB, Diemer T, Hales KH. Role of cytokines in testicular function. Endocrine. 1999;10(3):201-17.
41. Khaki A, Fathi AF, Nouri M, Khaki AA, Ozanci CC, Ghafari NM, et al. The effects of Ginger on spermatogenesis and sperm parameters of rat2009.
42. Zhao Y, Liu X, Qu Y, Wang L, Geng D, Chen W, et al. The roles of p38 MAPK→ COX2 and NF-κB→ COX2 signal pathways in age-related testosterone reduction. Sci Rep. 2019;9(1):1-11.
43. Azimzadeh M, Jelodar G. Alteration of testicular regulatory and functional molecules following long-time exposure to 900 MHz RFW emitted from BTS. Andrologia. 2019;51(9):e13372.
44. Ahmed SMU, Luo L, Namani A, Wang XJ, Tang X. Nrf2 signaling pathway: Pivotal roles in inflammation. Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease. 2017;1863(2):585-97.
45. Tu W, Wang H, Li S, Liu Q, Sha H. The anti-inflammatory and anti-oxidant mechanisms of the Keap1/Nrf2/ARE signaling pathway in chronic diseases. Aging Dis. 2019;10(3):637.
46. Chen H, Luo L, Liu J, Zirkin BR. Cyclooxygenases in rat Leydig cells: effects of luteinizing hormone and aging. Endocrinology. 2007;148(2):735-42.
47. Kim SO, Chun KS, Kundu JK, Surh YJ. Inhibitory effects of [6]-gingerol on PMA-induced COX-2 expression and activation of NF-κB and p38 MAPK in mouse skin. Biofactors. 2004;21(1-4):27-31.
48. Hedger MP, Meinhardt A. Cytokines and the immune-testicular axis. J Reprod Immunol. 2003;58(1):1-26.
49. Jelodar G, Nazifi S, Akbari A. The prophylactic effect of vitamin C on induced oxidative stress in rat testis following exposure to 900 MHz radio frequency wave generated by a BTS antenna model. Electromagn Biol Med. 2013;32(3):409-16.
50. Emanuele MA, Emanuele NV. Alcohol's effects on male reproduction. Alcohol Health Res World. 1998;22(3):195.

51. Rachdaoui N, Sarkar DK. Effects of alcohol on the endocrine system. Endocrinology and Metabolism Clinics. 2013;42(3):593-615.
52. Noth RH, Walter Jr RM. The effects of alcohol on the endocrine system. Med Clin North Am. 1984;68(1):133-46.
53. Aktan F, Henness S, Tran VH, Duke CC, Roufogalis BD, Ammit AJ. Gingerol metabolite and a synthetic analogue Capsarol™ inhibit macrophage NF-κB-mediated iNOS gene expression and enzyme activity. Planta Med. 2006;72(08):727-34.
54. Afolabi AO, Alagbonsi IA, Oyebanji TA. Beneficial effects of ethanol extract of Zingiber officinale (ginger) rhizome on epididymal sperm and plasma oxidative stress parameters in experimentally cryptorchid rats. Annu Res Rev Biol. 2014:1448-60.
55. Shalaby MA, Hamowieh AR. Safety and efficacy of Zingiber officinale roots on fertility of male diabetic rats. Food Chem Toxicol. 2010;48(10):2920-4.
56. Banihani SA. Ginger and testosterone. Biomolecules. 2018;8(4):119.
57. Ghareib SA, El-Bassossy HM, Elberry AA, Azhar A, Watson ML, Banjar ZM. 6-Gingerol alleviates exaggerated vasoconstriction in diabetic rat aorta through direct vasodilation and nitric oxide generation. Drug Des Devel Ther. 2015;9:6019.
58. Banihani SA, Abu-Alhayjaa RF, Amarin ZO, Alzoubi KH. Pentoxifylline increases the level of nitric oxide produced by human spermatozoa. Andrologia. 2018;50(2):e12859.
59. Banihani SA. Effect of ginger (Zingiber officinale) on semen quality. Andrologia. 2019;51(6):e13296.
60. Li W, Khor TO, Xu C, Shen G, Jeong W-S, Yu S, et al. Activation of Nrf2-antioxidant signaling attenuates NFκB-inflammatory response and elicits apoptosis. Biochem Pharmacol. 2008;76(11):1485-9.
61. Fathi R, khadijeh n, Marjan C, Abolfazl A. Ginger (zingiber officinale roscoe) extract could upregulate the renal expression of NRF2 and TNFα and prevented toxicity-induced by ethanol in rat kidney. Avicenna Journal of Phytomedicine. 2020;AJP-1910-2477.
62. Pan MH, Hsieh MC, Hsu PC, Ho SY, Lai CS, Wu H, et al. 6-Shogaol suppressed lipopolysaccharide-induced up-expression of iNOS and COX-2 in murine macrophages. Mol Nutr Food Res. 2008;52(12):1467-77.