

Asian Pacific Journal of Reproduction

Journal homepage: www.apjr.net



doi: 10.4103/2305-0500.241179

©2018 by the Asian Pacific Journal of Reproduction. All rights reserved.

Effect of graded doses of acetylsalicylic acid on sperm chromatin integrity and maturity of germinal epithelium in adult male mouse

Farzaneh Mahmoudi-Lafout¹, Fahimeh Mohammadghasemi²✉

¹Department of Anatomy, School of Medicine, Guilan University of Medical Sciences, Rasht-Iran

²Cellular & Molecular Research Center, School of Medicine, Guilan University of Medical Sciences, Rasht-Iran

ARTICLE INFO

Article history:

Received 28 June 2018

Revision 10 July 2018

Accepted 29 July 2018

Available online 18 September 2018

Keywords:

Acetylsalicylic acid

Chromatin

DNA fragmentation

Sex hormones

ABSTRACT

Objective: To evaluate the effect of graded doses of acetylsalicylic acid (ASA) on sperm chromatin integrity and sex hormones in the adult male mouse. **Methods:** Forty-nine male adult mice were divided into seven groups. Control group received no drug, sham group received ASA solvent (dimethylsulfoxide 0.1%), groups 3 to 7 received different doses of 0.05, 0.10, 0.50, 1.00 and 5.00 mg ASA, daily for 14 d, respectively. On day 15, evaluations were made by sperm chromatin dispersion test for the study of DNA sperm integrity, radioimmunoassay for the study of testosterone and luteinizing hormone (LH) level and histopathology of testis for Jhonson's scoring. **Results:** ASA in groups 0.50, 1.00 and 5.00 mg reduced big halo sperms. DNA fragmentation significantly increased just in 5 mg group. Serum level of testosterone in doses of 0.50, 1.00 and 5.00 mg groups reduced significantly ($P < 0.01$) while LH level was not affected. Johnson's score reduced in all ASA treated groups. **Conclusions:** Administration of ASA over the 14 days in dose of 5.00 mg increases sperm DNA fragmentation index and therefore reduces DNA integrity and in doses of 0.50, 1.00, 5.00 mg reduces serum testosterone level with no effect on LH. Generally, ASA has deleterious effects on the male reproductive indices even in low doses.

1. Introduction

Infertility is now a globally growing problem in the whole world. About 10%-20% of couples are prone to infertility. Male factors appear to be involved in 20%-50% of cases[1]. Although male fertility assessment is based on the analysis of sperm parameters, 15%-25% of infertile men have normal sperm analysis[2], as a result, the typical sperm analysis cannot be a powerful assessment to determine male infertility[3].

Drugs such as paracetamol and non-steroidal anti-inflammatory drugs (NSAIDs), which are considered as mild analgesia, are pharmacologically active effective drugs in use throughout the world for selling[4]. These medications are both abundantly available in the environment and are widely used as self-therapy and for prevention of the disorder pain[5]. Some athletes also use them to prevent pain[4]. The risks of misuse of mild analgesia include liver toxicity, cardiovascular complications and asthma complications[4].

First author: Farzaneh Mahmoudi-Lafout, Department of Anatomy, School of Medicine, Guilan University of Medical Sciences, Rasht-Iran.

✉ Corresponding author: Fahimeh Mohammadghasemi, Cellular & Molecular Research Center, Faculty of Medicine, Tehran-Rasht Road, Iran.

Tel: +98 9125153436

Fax: +98-31- 33128843

E-mail: parsahistolab@gmail.com

Foundation project: This work was supported financially by Research & Technology Vice-Chancellor Guilan University of Medical Sciences.

This is an open access article distributed under the terms of the Creative Commons Attribution-Non Commercial-Share Alike 4.0 License, which allows others to remix, tweak and build upon the work non-commercially, as long as the author is credited and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

©2018 Asian Pacific Journal of Reproduction Produced by Wolters Kluwer- Medknow

How to cite this article: Mahmoudi-Lafout F, Mohammadghasemi F. Effect of graded doses of acetylsalicylic acid on sperm chromatin integrity and maturity of germinal epithelium in adult male mouse. *Asian Pac J Reprod* 2018; 7(5): 214-219.

Aspirin or acetylsalicylic acid (ASA) is an anti-inflammatory and anti-pyretic drug that is commonly used in heart patients for the prevention of coronary artery disease, myocardial infarction and stroke[6]. It also is used for the treatment of fever, cold, and pains[7]. Long-term use consumption of aspirin may have detrimental effects on some of the body's systems, such as leading to the gastrointestinal bleedings, subarachnoid or intra cerebral hemorrhage, nephrotoxicity and hepatotoxicity[8]. In addition, some of its side effects have been shown on male reproduction potentials, such as decreased hormones and changes in human and animal sperm parameters[9].

Human studies have shown that mild analgesia in pharmacological doses causes endocrinology endocrinological disorders in the testicles during adulthood, especially in the interstitial tissues[4]. In addition, the use of aspirin administration during in pregnancy has been shown to be associated with an anti-androgenic effect as well as increased risk of cryptorchidism in newborn[10]. The use of aspirin or indomethacin in adult rats disrupts spermatogenesis[11,12]. Aspirin also reduces plasma testosterone and changes luteinizing hormone (LH) level. *In vitro* studies on human testicle culture cells have shown that mild steroidogenesis analgesia is reduced by about 10%-43% compared to control by aspirin[4].

NSAIDs drugs such as aspirin directly or indirectly target cyclooxygenase[12] induce apoptosis in various animal and human cell lines[13].

Recently, molecular and genetic factors have been identified as important signs of male fertility and its outcomes[14]. The integrity of the sperm DNA is essential for the accurate transmission of genetic content[15]. According to some studies, sperm DNA damage occurs in 5%-8% of infertile men with normal spermatogram[16,17]. Genetic damage can occur in several stages of spermatogenesis, all of which can lead to male infertility. Although low levels of DNA damage can be repaired by oocytes[18], fertilization with sperm with a fragmented DNA can disrupt fetal development, induces fetal mutation, and increases the risk of cancer in the offspring[18].

Some male factors such as apoptosis, DNA fractures without restoration, oxidative stress, drug use, cigarette smoking and illness are known to be major sources of DNA damage[14,19].

Few studies are available about different doses of aspirin (low, pharmacological and high) on men's fertility indices. The aim of this study was to evaluate the effects of different doses of ASA on the integrity of sperm chromatin, sperm parameters, and testicular function in adult male mouse.

2. Materials and methods

2.1. Animals

This project was done on 49 adult male Balb-c mice with 6-8 weeks age and 30 to 35 g weight. Mice were obtained from Institute of Pasteur, Karaj-Iran. Animals were placed in 12-hour light and 12-

hour darkness cycle for 2 weeks in room temperature (23±2) °C. Animals in all groups had full access to food and drinking water. Ethical considerations were made based on the guidelines for working with animals in Department of Research and Technology of Guilan University of Medical Sciences (No.: Ir.GUMS.REC.1394.644; Dated: 8 May 2016).

Animals were randomly divided into 7 groups. Control group received no drug. Sham group received ASA solvent (dimethylsulfoxide 0.1%). Groups 3 to 7 received different doses of 0.05, 0.10, 0.50, 1.00, and 5.00 mg ASA (Sigma, USA) for 2 weeks, respectively. All treatments were done orally through gavage.

On the day 15, animals were killed by cervical dislocation. Left testis and epididymis were removed from the mouse body and blood samples were collected from inferior vena cava for histologic studies and assay of hormones.

2.2. Hormone assay

After collecting the blood sample from inferior vena cava, the samples were stored at room temperature for half an hour and then centrifuged at 3 000 rpm for 10 min. After serum separation from the blood cells, the serum sample was kept at -20 °C. To assess sex hormones (LH and testosterone), a radioimmunoassay kit (Monobind, USA) was used. Assay was performed on the bases of the kit's instructions. Measurements were repeated twice.

2.3. Sperm parameters analysis

For analysis of sperm parameters such as count, motility and morphology, we used epididymis's tail that was put in a petri dish which contained 1 mL of Ham'sF10 and was previously incubated for half an hour. Then 50 µL of solution was evaluated for sperm motility which was expressed as percentage. Five optical microscopic fields with 400 × magnification were used for study of motility. In order to count the cells, 10 µL sperm's suspension was placed on a Neubauer slide. The number of sperms were counted in 5 cells and expressed as 10⁶ per mL. For study of morphology, we prepared smear, dried and then fixed them in 96% ethanol and then stained them with hematoxiline and eosine. With an optical microscope and 1 000 × magnification, we observed cells and reported abnormal heads and tails in 200 cells in each animal.

2.4. Sperm chromatin dispersion test

In order to evaluate the sperm DNA fragmentation and the quality of chromium, the sperm chromatin dispersion test was used. For the preparation of sperm samples, the tail of epididymis was used. At first, the epididymis was stored in a dish containing Ham'sF10 media, and then transferred to a 37 °C incubator for 45 min. In order to remove more sperm, the epididymis was cut into smaller pieces with the aid of a bifurcated razor before placing the dish into the incubator. Then

1 mL of solution was transferred into the centrifugal tube. After that, the instant solution was discarded and used for the test.

First, 30 μ L of a sperm sample with 70 μ L agarose with a low melt point were put at 37 °C. The mixed sample was then placed on a lamina previously coated with 65% agarose and then placed in a refrigerator horizontally for 4 min by placing a lamella on it for 4 min. Then, lamella was carefully removed from the surface of the slide, each slide then was put in HCl 0.12 N for 7 min in darkness. Slides were immersed in the acid denaturation solution (acidic tris 0.4 m, 2-mercaptoethanol 0.8 m, sodium dodecyl sulfate 1%, ethylene-diamine-tetra-acetic acid 50 mM, and sodium chloride 2 m) at room temperature for 25 min.

After being washed in distilled water twice, slides were dehydrated in ethanol 70%, 90% and 100% (each for 2 min), and dried out in the air. Samples were stained with phosphate buffer saline - Wright's stain 1:1 for 10 min. Then, the slides were examined by optical microscope. The cells were evaluated and the rate of DNA fragmentation of the sperm nucleus was evaluated based on the size of the halo. The larger the halos, the healthier the cell. We divided cells on 3 types: large halo, small halo and without halo or fragmented. In each animal, 200 cells were studied. The sperm DNA fragmentation index (SDFI) was calculated from the ratio of the number of fragmented cells to the total number of healthy and fragmental cells, and the overall response was multiplied by 100 and expressed as percentages[20].

2.5. Histological study and maturity of germinal epithelium

Testis samples were fixed in Buins's solution for 72 h. Then they were processed for histologic study. For dehydration ethanol with graded doses, infiltration xylene and melted paraffin were used. Using a rotary microtome (Leitz, Germany), tissues were sectioned in 5 μ m thickness. Then they were stained with hamatoxylin-eosin. They were observed using an Olympus light microscope with 400 \times magnification. In each animal, three slides were observed. For study of maturity of germinal epithelium, they were scored using Jhonson's score. One hundred seminiferous tubule in transverse section were scored in each animal and graded from 1 to 10[21].

Table 1

Effect of ASA on sperm parameters and maturity of seminiferous tubules.

Group	Sperm ($\times 10^6$ /mL)	Count motility (%)	Abnormal head morphology (%)	Abnormal tail morphology (%)	Maturity (Jhonson's score)
Control	14.70 \pm 3.83	57.14 \pm 5.28	6.29 \pm 2.43	1.29 \pm 1.11	9.41 \pm 0.56
Sham	14.75 \pm 6.00	58.97 \pm 5.08	7.14 \pm 2.34	1.00 \pm 1.82	9.32 \pm 0.64
0.05 mg	13.78 \pm 2.67*	49.72 \pm 5.47	10.14 \pm 2.61	11.71 \pm 0.95	8.88 \pm 0.89*
0.10 mg	12.60 \pm 3.11*	41.72 \pm 5.07*	10.29 \pm 2.81	12.00 \pm 1.91	8.40 \pm 1.01*
0.50 mg	11.08 \pm 9.39*	40.05 \pm 5.60*	12.00 \pm 0.81*	13.29 \pm 1.11	8.16 \pm 0.90*
1.00 mg	8.98 \pm 6.41*	34.05 \pm 2.83*	12.71 \pm 3.03*	12.57 \pm 1.90	7.92 \pm 0.93*
5.00 mg	5.57 \pm 3.40*	31.84 \pm 3.12*	14.71 \pm 3.35*	14.43 \pm 2.57*	7.88 \pm 0.75*

Data are shown as mean \pm SD; *Compared to control group $P < 0.05$.

2.6. Statistical analysis

For analysis of data, SPSS one way analysis of variance test were used. Also Tuckey test was used to evaluate the difference among groups. For assay of relationship, correlation test was used. Data were expressed as mean and standard deviation (mean \pm SD) and $P < 0.05$ were considered significant.

3. Results

The results of sexual hormones evaluation showed that serum LH level had no significant difference in any of the groups (Figure 1). However, serum testosterone level in groups of 0.50, 1.00 and 5.00 mg was significantly lower than control ($P < 0.01$). There was no significant difference in serum testosterone in sham, 0.05 and 0.10 mg group compared to control (Figure 1).

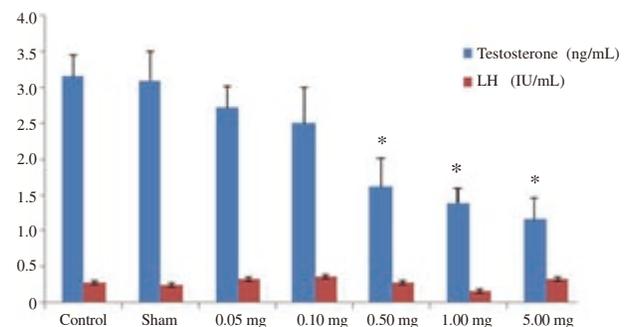


Figure 1. Effect of ASA on sex hormones.

Data are shown as mean \pm SD; *Compared to control group $P < 0.001$.

Histologic observation showed active spermatogenesis in seminiferous tubules in control group. There were different types of germ cells and Sertoli cells in tubules. Lumen was circular, regular and clear. Acidophilic Leydig cells in small groups were observed around small vessels in interstitial tissue. Jhonson's score of seminiferous tubules were 9.41 \pm 0.56 and almost all tubules had mature cells. All of these items were the same in sham group and also Jhonson's score of seminiferous tubules was 9.32 \pm 0.60 (Table 1) and (Figure 2).

In the other groups, Jhonson's score of seminiferous tubules was decreased in comparison with control ($P < 0.01$). Vacuole was

observed in some area, especially in the last three groups (0.50 mg, 1.00 mg and 5.00 mg). The regular margin of the tubules was torn and the number of elongated spermatids was reduced (Figure 2).

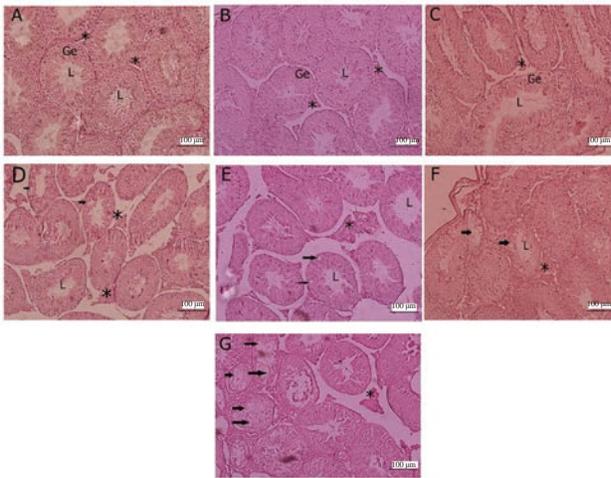


Figure 2. Photomicrograph of mouse seminiferous tubules (hematoxylin-eosin, 200 ×).

A: control, B: sham, C: 0.05 mg ASA, D: 0.10 mg ASA, E: 0.50 mg ASA, F: 1.00 mg ASA, G: 5.00 mg ASA. L: lumen of seminiferous tubules, Ge: germinal epithelium. *Shows Leydig cells in interstitial tissue; arrows show vacuoles inside the germinal epithelium.

In this experimental trial, different doses of aspirin altered some parameters of sperm. The number of total sperm was decreased in all of treated groups ($P < 0.001$) in compare with control. Motility of sperms was decreased in the last 4 groups ($P < 0.01$). Also, the sperms with head and tail abnormality significantly were increased in the last 3 groups and 5.00 mg group respectively ($P < 0.01$) compared with control (Table 1).

The percentage of big halo, small halo and SDFI (or sperm without halo) was shown in Table 2. Based on the findings of this study, the number of big halo was not significantly altered in sham, 0.05 and 0.10 mg group, but a significant reduction was found in 0.50, 1.00 and 5.00 mg group when compared with control ($P < 0.001$) (Table 2).

Table 2

Effect of aspirin on DNA fragmentation in adult mouse.

Group	Big halo (%)	Small halo (%)	Without halo (%)
Control	39.71 ± 5.11	46.00 ± 8.62	14.28 ± 6.47
Sham	38.50 ± 5.55	45.78 ± 5.12	15.71 ± 5.81
0.05 mg	38.85 ± 5.55	42.07 ± 2.43	19.07 ± 5.60
0.10 mg	36.00 ± 5.18	46.50 ± 7.48	17.50 ± 4.13
0.50 mg	27.29 ± 4.23*	52.71 ± 3.34	20.00 ± 3.52
1.00 mg	22.64 ± 8.49*	57.28 ± 4.13*	22.21 ± 4.81
5.00 mg	14.21 ± 4.68*	61.78 ± 6.61*	24.00 ± 7.09*

Data are shown as mean ± SD; *Compared to control group $P < 0.001$.

Number of small halo was significantly increased in 1.00 and 5.00 mg group in comparison to control group ($P < 0.01$) but in other groups there was not significant alteration. Also, SDFI was significantly increased in 5.00 mg group ($P < 0.01$), but in other groups no significant difference was shown when compared with

control (Table 2). There was no significant relationship between LH hormone and small and big halo and SDFI. There was a positive significant correlation between testosterone and big halo ($r = 0.61$, $P < 0.001$). Small halo ($r = -0.50$, $P < 0.001$) and SDFI ($r = -0.34$, $P = 0.02$) showed a negative significant correlations with serum level of testosterone.

4. Discussion

This study showed ASA both in low and high doses has adverse effects on sperm parameters, quality and maturation of spermatogenesis, sex hormone levels and DNA fragmentation, all of which are important factors for fertility of men. Aspirin is one of the most important NSAID drugs known for cardiovascular benefits[13]. Changes in fertility indices in this study are likely to occur through indirect inhibition of androgen. Like to other studies, administration of ASA for 14 days reduces the number and movement of sperm and increases the abnormal morphology of head and tail[11,22-24]. Vane and Botting reported that aspirin administration in rats significantly reduced the overall parameters of sperm[24]. Vyas *et al* also showed that ASA consumption for 30 and 60 days in rats reduced sperm motility parameters, total number and sperm density in the tail and testicles[13]. Aspirin inhibits prostaglandin and thus can alter the metabolism of cholesterol and thereby affect androgen synthesis[13,25]. It has been shown that aspirin by inhibition of prostaglandins can cause ischemia in tubules and epithelial cells of arteriole that cause cell death and decrease in sperm count[26]. Low motility of sperm may occur by alternation in calcium transmissions that is essential for cell formation and movement, uncoupled oxidative phosphorylation and cytochrome oxidase inhibiting activity pathway[27].

ASA reduced sperm count and movement maybe due to a decrease in androgen level. In this study, serum testosterone was decreased. Epididymis is the main site of sperm maturation and is an organ that is completely androgen dependent. Obviously, in episodes of decreased testosterone, the function of epithelium of epididymis is altered, which may also be due to the toxic effects of aspirin on epithelial cells.

The main reason for the increase in head abnormality following aspirin is not exactly known. In general, damage to the sperm head can be due to one or a combination of genetic, physiological and cytotoxic agents. Head abnormality may be due to changes such as deletion and point mutation in the testicular DNA, which by itself leads to impairment of sperm differentiation[7]. Morphology and morphometry of sperms occur at the later stages of spermatogenesis[28]. Abnormal morphology can be induced by abnormalities in androgens, followed by impaired spermatogenesis or DNA damage[7,29]. Reduction of testosterone leads to an increase in stem cell apoptosis[30] and a decrease in spermatogenesis[9]. Moreover, impaired spermatogenesis can decrease the maturity

and increases low quality of seminiferous tubules[9]. Vacuoles in the germinal epithelium in the last three groups can indicate a poor tissue linkage to reduce some adhesion molecules, such as cadherin, or a sign of the pre apoptotic process[30]. In this regard, it has been shown that nonsteroidal anti-inflammatory drugs induces apoptosis in various human cell lines, such as fibroblasts, colon cancer, and colon adenomatous cells. The apoptotic activity of NSAIDs may be induced by the mechanism of cytochrome oxidase[12,13].

During the last decade, the integrity of sperm DNA as a functional parameter of sperm was considered to play an important role in human assisted reproduction technology[31]. Halosperm test is a sperm chromatin dispersion test based on the ability of a healthy DNA to form a loop around the nucleus. The deproteinated nucleous creates a scattered halo that resembles loose DNA loops attached to the remaining nuclear axis. The nucleus does not produce halo with fragmented DNA, or creates very small halos. The sperm nucleus creates a large halo without DNA fragmentation. In fact, halosperm testing mostly measures sperms that do not damage until it measures the sperm DNA fragmentation[31].

Although low levels of DNA damage are restored through compensatory oocyte mechanisms[18], this process can be followed by some pathological and environmental conditions such as testicular damage, drug toxicity, rays, hormone inactivation, exposure to oxidative stress and cigarette smoking[19,31,32]. In addition, it has been shown that DNA fragmentation of more than 25%-30% can impair male fertility[19,33]. As Zhang *et al* have shown infertile men with fragmentation less than 27% are more hopeful to fertility output[32]. The reasons for DNA damage include impairment in regeneration of chromatin with topoisomerase, oxidative stress, and cell death[34]. DNA fragmentation can be created as a result of one or a combination of different mechanisms[34]. Increased DNA damage in this study may be due to reduction in antioxidant levels. In this regard, it has been shown that administration of ASA for 14 days reduces total antioxidant serum level in mouse[35]. It also may be due to androgen deficiency that our study confirmed it.

It has been shown that the sperm DNA fragmentation affects both natural fertility and fertility assisted reproductive cycles. Studies have shown that 18% of men with normal semen parameters have a degree of DNA fragmentation and an increased risk of poor blastocyst evolution[31].

In this study, although no significant relationship was found between LH and fragmentation, an inverse relationship was observed between testosterone and fragmentation, which could be due to decreased testosterone and subsequent disruption of the spermatogenesis process[9,29]. Some studies have reported a positive relationship between abnormal morphology and abnormal chromosomal structure[33,34]. Veles dela *et al* showed a significant relationship between DNA damage and sperm parameters[36]. The exact mechanisms about the effect of ASA on male reproduction is unknown and needs further molecular researches.

In conclusion, results of the present study showed that administration

of ASA over the 14 days in dose of 5.00 mg increases sperm DNA fragmentation index and therefore reduces DNA integrity and in doses of 0.50, 1.00, 5.00 mg reduces serum testosterone level without having effect on LH. Generally ASA has deleterious effects on the male reproductive indices even in low doses.

Conflict of interest statement

The authors declare that there is no conflict of interest.

References

- [1] Ricci E, Vigano P, Cipriani S, Somigliana E, Chiaffarino F, Bulfoni A, et al. Coffee and caffeine intake and male infertility: A systematic review. *Nutr J* 2017; **16**(1): 37.
- [2] Sadeghi S, Garcia-Molina A, Celma F, Valverde A, Fereidounfar S, Soler C. Morphometric comparison by the ISASR CASA-DNA system of two techniques for the evaluation of DNA fragmentation in human spermatozoa. *Asian J Androl* 2016; **18**(6): 835-839.
- [3] Colacurci N, Monti MG, Fornaro F, Izzo G, Izzo P, Trotta C, et al. Recombinant human FSH reduces sperm DNA fragmentation in men with idiopathic oligoasthenoteratozoospermia. *J Androl* 2012; **33**(4): 588-593.
- [4] Albert O, Desdoits-Lethimonier C, Lesne L, Legrand A, Guille F, Bensalah K, et al. Paracetamol, aspirin and indomethacin display endocrine disrupting properties in the adult human testis *in vitro*. *Hum Reprod* 2013; **28**(7): 1890-1898.
- [5] Verlicchi P, Al Aukidy M, Zambello E. Occurrence of pharmaceutical compounds in urban wastewater: Removal, mass load and environmental risk after a secondary treatment—A review. *Sci Total Environ* 2012; **429**: 123-155.
- [6] Wurtz M. Aspirin in coronary artery disease: An appraisal of functions and limitations. *Dan Med J* 2015; **62**(4): B5011.
- [7] UB Ekaluo, EV Ikpeme, AE Udokpoh. Sperm head abnormality and mutagenic effects of aspirin, paracetamol and caffeine containing analgesics in rats. *Int J Toxic* 2009; **7**(1): 1-9.
- [8] Ma N, Liu XW, Yang YJ, Li JY, Mohamed I, Liu GR, et al. Preventive effect of aspirin eugenol ester on thrombosis incarrageenan-induced rat tail thrombosis model. *PLoS One* 2015; **10**(7): e0133125.
- [9] Ibtisham F, Chen J, Niu Y, Wang Z, Wu J, Xiao M, et al. Effect of aspirin on reproductive profile of male tar—An overview. *Int J Res Dev Pharm L Sci* 2016; **5**(4): 5-12.
- [10] Kristensen DM, Lesne L, Le Fol V, Desdoits-Lethimonier C, Dejuq-Rainsford N, Leffers H, et al. Paracetamol (acetaminophen), aspirin (acetylsalicylic acid) and indomethacin are anti-androgenic in the rat fetal testis. *Int J Androl* 2012; **35**(3): 377-384.
- [11] Didolkar AK, Gurjar A, Joshi UM, Sheth AR, Roychowdhury D. Effects of aspirin on blood plasma levels of testosterone, LH and FSH in maturing male rats. *Int J Androl* 1980; **3**(3): 312-318.

- [12]Elwood PC, Gallagher AM, Duthie GG, Mur LA, Morgan G. Aspirin, salicylates, and cancer. *Lancet* 2009; **373**(9671): 1301-1309.
- [13]Vyas A, Ram H, Purohit A, Jatwa R. Adverse Effects of subchronic dose of aspirin on reproductive profile of male rats. *J Pharm (Cairo)* 2016; **2016**: 1-9.
- [14]Ruvolo G, Roccheri MC, Brucculeri AM, Longobardi S, Cittadini E, Bosco L. Lower sperm DNA fragmentation after r-FSH administration in functional hypogonadotropic hypogonadism. *J Assist Reprod Genet* 2013; **30**(4): 497-503.
- [15]Roodbari F, Abedi N, Talebi AR. Early and late effects of Ibuprofen on mouse sperm parameters, chromatin condensation, and DNA integrity in mice. *Iran J Reprod Med* 2015; **13**(11): 703-710.
- [16]Shefi S, Turek PJ. Definition and current evaluation of subfertile men. *Int Braz J Urol* 2006; **32**(4): 385-397.
- [17]Aitken RJ. Sperm function tests and fertility. *Int J Androl* 2006; **29**(1): 69-75.
- [18]Agarwal A, Said TM. Oxidative stress, DNA damage and apoptosis in male infertility: A clinical approach. *BJU Int* 2005; **95**(4): 503-507.
- [19]Evenson DP. The sperm chromatin structure assay (SCSA) and other sperm DNA fragmentation tests for evaluation of sperm nuclear DNA integrity as related to fertility. *Anim Reprod Sci* 2016; **169**: 56-75.
- [20]Mohammadghasemi F, Khajeh Jahromi S. Melatonin ameliorates testicular damages induced by nicotine in mice. *Iran J Basic Med Sci* 2018; **21**(6): 639-644.
- [21]Ghasemnezhad R, Mohammadghasemi F, Faghani M, Bahadori MH. Oxytocin can decrease germ cells apoptotic index in testis under acute ischemia reperfusion in a rat model. *Iran J Reprod Med* 2015; **13**(5): 283-290.
- [22]Sostres C, Lanas A. Gastrointestinal effects of aspirin. *Nat Rev Gastroenterol Hepatol* 2011; **8**(7): 385.
- [23]Emre MH, Polat A, E refolu M, Karabulut AB, Gül M. Effects of melatonin and acetylsalicylic acid against hepatic oxidative stress after bile duct ligation in rat. *Acta Physiol Hung* 2008; **95**(4): 349-363.
- [24]Vane JR, Botting RM. The mechanism of action of aspirin. *Thromb Res* 2003; **110**(5-6): 255-258.
- [25]Al-Taei BSD. Effect of aspirin on sperm specification and some hematological parameters in male albino white rat. *J Biotechnol Res Cent* 2014; **8**(4): 1-4.
- [26]Yasmeen T, Yasmin F, Qureshi GS. To evaluate the role of diclofenac sodium on renal parenchyma of young albino rats. *Pak J Pharm Sci* 2008; **21**(2): 98-102.
- [27]Macias-Garcia B, Gonzalez-Fernandez L, Loux SC, Rocha AM, Guimaraes T, Pena FJ, et al. Effect of calcium, bicarbonate, and albumin on capacitation-related events in equine sperm. *Reproduction* 2015; **149**(1): 87-99.
- [28]Pal PC, Manocha M, Kapur MM, Rao DN, Sharma RS, Rajalakshmi M. Obstructive infertility: Changes in the histology of different regions of the epididymis and morphology of spermatozoa. *Andrologia* 2006; **38**(4): 128-136.
- [29]Sun F, Ko E, Martin RH. Is there a relationship between sperm chromosome abnormalities and sperm morphology? *Reprod Biol Endocrinol* 2006; **4**: 1.
- [30]El-Sharaky AS, Newairy AA, Elguindy NM, Elwafa AA. Spermatotoxicity, biochemical changes and histological alteration induced by gossypol in testicular and hepatic tissues of male rats. *Food Chem Toxicol* 2010; **48**(12): 3354-3361.
- [31]Tandara M, Bajic A, Tandara L, Bilic-Zulle L, Šunjić M, Kozina V, et al. Sperm DNA integrity testing: Big halo is a good predictor of embryo quality and pregnancy after conventional IVF. *Andrology* 2014; **2**(5): 678-686.
- [32]Manente L, Pecoraro S, Picillo E, Gargiulo U, Gargiulo P, De Luca A, Politano L. Molecular evidence of apoptotic pathway activation in semen samples with high DNA fragmentation. *Vivo* 2015; **29**(2): 289-294.
- [33]Zhang LH, Qiu Y, Wang KH, Wang Q, Tao G, Wang LG. Measurement of sperm DNA fragmentation using bright-field microscopy: Comparison between sperm chromatin dispersion test and terminal uridine nick-end labeling assay. *Fertil Steril* 2010; **94**(3): 1027-1032.
- [34]Aitken RJ, De Iulius GN. On the possible origins of DNA damage in human spermatozoa. *MHR: Basic Sci Reprod Med* 2009; **16**(1): 3-13.
- [35]Emami NH, Lafout FM, Mohammadghasemi F. Administration of melatonin protects against acetylsalicylic acid-induced impairment of male reproductive function in mice. *Iran J Basic Med Sci* 2018; **21**(2): 124-129.
- [36]Velez dela Calle JF, Muller A, Walschaerts M, Clavere JL, Jimenez C, Wittemer C, et al. Sperm deoxyribonucleic acid fragmentation as assessed by the sperm chromatin dispersion test in assisted reproductive technology programs: Results of a large prospective multicenter study. *Fertil Steril* 2008; **90**(5): 1792-1799.