Alqalam Journal of Medical and Applied Sciences. 2026;9(5):1231-1235
https://doi.org/10.54361/ajmas.269514

Original article

Aspirin-Induced Alterations in Male Reproductive Function: Evidence from A Murine Model

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Abstract
Aspirin (ASA) is one of the non-steroidal anti-inflammatory drugs; it is used for treating pain, cold, and fever. This study aimed to evaluate the effect of ASA on sperm parameters, testicular tissues, and the embryos in adult male mice. Sixteen male mice were divided into two groups: the control group received distilled water, while the second group was given ASA at a dose of 75 mg/kg BW for 15 days. At the end of the dosing, measurements were taken for body and testicular weights, testes were removed, and sperm parameters were evaluated, as well as a histopathological assessment of the testes was carried out. Three treated males from each group were put with untreated females (1: 2) for mating. The results showed a significant decline in testicular weight, sperm count, motility, and histopathological criteria and Johnsen's score, while the percentage of abnormal sperm increased. Histopathological analysis indicated pronounced testicular alterations in the ASA-treated group compared to the control group. Furthermore, a reduction was observed in both the mean body weight of embryos and the average number of live embryos. Aspirin at the dose of 75 mg/kg exerted adversely influences on sperm quality, testicular tissues, and the embryos. Therefore, ASA should be used with caution.
Keywords. Aspirin, Mice, Testes, Sperm quality, Embryo.

Introduction

Aspirin or acetylsalicylic acid (ASA) is a non-steroidal anti-inflammatory drug; it is used for treating cold, fever, pains [1], and as a cardioprotective agent [2; 3], in terms of preventing coronary artery disease, stroke, and myocardial infarction [3]. Moreover, it has anti-pyretic [4; 5], antiplatelet [6], and analgesic benefits [4]. long-term therapeutic use of aspirin leads to gastrointestinal ulcerations, hepatotoxicity, nephrotoxicity, and renal cell cancers [7]. In addition, ASA causes an increased risk of cerebral micro-bleeding and Reye's syndrome in children [8], alters progesterone and estrogen biosynthesis [9], and decreases the activity of sorbitol dehydrogenase and hyaluronidase [10].

Aspirin has also been reported to increase the size of spermatocytes nuclei [11], as well as it reduces the hemoglobin values [12], and the amount of oxygen delivered to tissues [13]. Several studies have shown that aspirin inhibits prostaglandin and alters cholesterol metabolism, leading to changes in androgen biosynthesis [10,12]. Other researchers have demonstrated that ASA led to a reduction in the number and movement of sperm, an increase in abnormal sperm [14; 15]. Vyas *et al* [12] elucidated that treatment of male rats with aspirin at the dose of 12 mg/kg for 30 and 60 days caused a decrease in sperm count and motility, as well as induced histopathological changes in testes. Additionally, Banihani [16] stated that aspirin affected Leydig cell function and sperm count. Due to widespread use of aspirin without a medical prescription, and a lack of data about its effects on male reproductive function in Libya. Therefore, this study was designed to evaluate its influences on sperm quality, testicular histology, and the embryos in adult mice.

Methods

Aspirin

Aspirin (acetylsalicylic acid) tablets were purchased from a pharmacy in Tripoli; its molecular structure ($C_9H_8O_4$) and its molecular weight is 180.159 g/mol.

Experimental animals

This study was carried out on 16 male Swiss albino mice weighing 25 to 30 g and aged 8-12 weeks. They were bred in the animal house at the Zoology department/ Faculty of Science/ University of Tripoli under a natural photoperiod and at room temperature 22-25 °C. Mice were given the food and drinking water ad libitum.

Treatment

Mice were divided into two groups, consisting of 5 animals each. The control received distilled water, while the treated group was given 75 mg/kg BW of aspirin for a duration of 15 days. At the end of the treatment

Received*: 07-03-2026 -* ***Accepted****: 04-05-2026 -* ***Published****: 11-05-2026*

period, five male mice from each group were weighed, then killed, while the remaining male mice were used for mating with untreated females.

Sample collection
The sperm were isolated from the vas deferens for the evaluation of sperm biological characteristics. The testes were removed, weighed, and kept in 10 % formalin for histological procedures.

Sperm function assessment
Sperm were collected by squeezing each vas deferens in a petri dish containing 1 ml of physiological saline solution (0.9 % NaCl). The sperm suspension was incubated for 10 minutes at 37 $^{\circ}$C, then thoroughly mixed using a small pipette. A Neubauer hemocytometer counting chamber groove was filled with the suspension, and then, the motile and immotile sperm were counted under a light microscope according to the method of Soheir and Haya [17]. The examination of sperm morphology was carried out by dropping a drop of sperm suspension on a slide. After that, air-dried, stained with 1 % eosin, the smears on the slides were examined under a light microscope, and the percentage of abnormal sperm was recorded [18]. Sperm count was determined and expressed as sperm count X 10^6 /ml.

Testicular histology
The testicular tissues were passed through ascending gradients of ethanol, then cleared with xylene, embedded in paraffin, and sectioned to a 5- µm thick using a rotatory microtome. The sections were stained with hematoxylin and eosin, examined under a microscope, and photographed. One hundred seminiferous tubule cross sections were examined at 100x magnification per animal to evaluate histological changes, including disorganization and vacuolization in germinal epithelium. The average percentage was calculated for each specimen according to the method described by D' Cruz and Uckun [19]. Moreover, the level of sperm production (spermatogenesis) was evaluated using Johnsen's scoring method [20].

Effect of aspirin on the embryo
Treated males were put with untreated females at a ratio of 1:2 for mating. When the vaginal plug was observed, that day was considered the zero day of gestation. On the 18th day of pregnancy, pregnant females were killed. The embryos were extracted from the uterus, and then the number of live embryos and their weight were recorded.

Statistical analysis
The results were presented as the mean ± standard deviation (SD). Statistical analysis of data was performed using SPSS software version 26. A t-test was used to determine the level of significance between the control and treated groups. P. value < 0.05 was considered statistically significant.

Ethical approval
The study received ethical approval from the ethics committee of the University of Tripoli (SREC- UOT 20-2024).

Results

Body and testes weights in mice exposed to aspirin
Our results showed no significant changes (P > 0.05) in the body weight of mice treated with aspirin compared to those of the control group (Table 1). A significant decline (P < 0.05) in testes weight was observed in male mice treated with aspirin as compared to the control (Table 1).

Table 1. Body and testes weights in mice treated with aspirin.

Variables	Body weight (g)	Testes weight (g)
Control group	30.72 ± 0.56	0.25 ± 0.04
Aspirin-treated group	30.04 ± 0.82	0.22 ±0.02 *

*Data represent the mean ± SD of 5 mice in each group. * P < 0.05 is significantly different from the control.*

Sperm parameters
According to the outcomes, exposure to aspirin led to a significant decrease (P < 0.05) in sperm count and motility, and significantly elevation (P < 0.05) in the percentage of abnormal sperm (Table 2).

Table 2. Sperm count, motility, and morphology in mice exposed to aspirin

Variables	Sperm count (10^6)	Sperm motility (%)	Abnormal sperm (%)
Control group	42.2 ± 8.35	0.88 ± 0.03	0.31 ± 0.09
Aspirin treated group	35.2 ± 0.57*	0.61 ± 0.36*	0.72 ± 0.10*

*Data represent the mean ± SD of 5 mice in each group. * P < 0.05 is significantly different from the control*

Received*: 07-03-2026 -* ***Accepted****: 04-05-2026 -* ***Published****: 11-05-2026*

https://doi.org/10.54361/ajmas.269514

Testicular tissues examination

The results of histological evaluation showed that testes from the control have normal histological structure and an organized cellular arrangement of seminiferous tubules. Furthermore, closely packed seminiferous tubules are separated from each other by narrow interstitial spaces containing Leydig cells. These seminiferous tubules were lined by germinal epithelium. This epithelium is composed of concentric layers of spermatogonia, primary and secondary spermatocytes, spermatids, and spermatozoa in the lumen (Figure 1A). While administration of aspirin led to marked damage in the histological structure of testes, which caused severe vacuolization of the seminiferous tubules' cells, disorganization of germinal epithelium, thickening of the basement membrane, as well as little amounts of sperm in lumens (Figure 1B). Also, there was a significant decrease in normal histology criteria of the ASA-treated group in comparison to the control ($P < 0.05$). As well as a significant decline in the mean Johnsen's score ($P < 0.05$) was observed in the ASA-treated group when compared to the control group (Table 3).

Table 3. Histological assessment of the testis and Johnsen's score of mice exposed to aspirin

Variables	Percentage of the seminiferous tubule			Johnsen's score
	Normal %	Vacuolization %	Disorganized %	
Control group	86.60 ± 1.52	12.33 ± 1.51	1.31 ± 0.55	10.00 ± 0.00
ASA treated group	1.65 ± 0.50*	53.3 ± 3.40*	45.1 ± 4.22*	4.70 ± 0.51*

*Data represent the mean ± SD in each group. * P < 0.05 is significantly different from the control.*

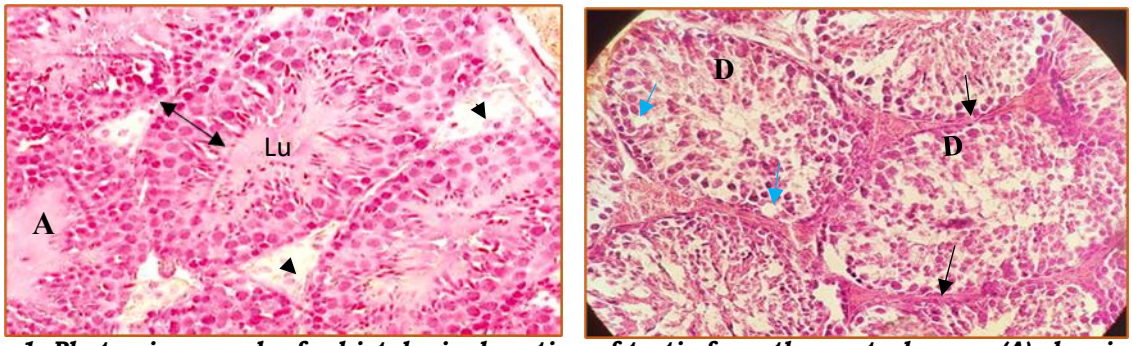

Figure 1. Photomicrograph of a histological section of testis from the control group (A) showing normal histological structure of seminiferous tubules, these tubules are lined by germinal epithelium (double-headed arrow), with sperm in the lumen (Lu), and interstitial spaces contain Leydig cells (arrow head). (B) A histological section of the testis of the aspirin-treated group showing severe vacuolization of the seminiferous tubules' cells (blue arrow), disorganization of germinal epithelium (D), thickening of the basement membrane (black arrow) (H & E 40 x)

Effect aspirin on the embryos

The results revealed a significant decrease ($P < 0.05$) in the average number of live embryos, and also in the mean body weight of embryos sired by treated males Table 3.

Table 3. Effect of aspirin on the embryos.

Treatments	No. of pregnant females	No. of live embryos	Body weight (g)
Control group	6	9.2 ± 0.5	1.60 ± 0.03
Aspirin-treated group	6	5.1 ± 0.2*	1.33 ± 0.02*

*Data represent the mean ± SD of 6 mice in each group. * P < 0.05 is significantly different from the control.*

Discussion

The mice treated with aspirin did not show significant changes in body weight. This result was similar to the result documented by Vyas *et al* [12], who reported that rats that received aspirin did not record significant alterations in body weight. Testicular weight significantly declined in mice treated with aspirin. This outcome was consistent with the results of Scott and Persaud [21] and Vyas *et al* [12], who revealed a reduction in testicular weight of treated rats with aspirin. Previous research demonstrated a decrease in the weight of testes in mice treated with aspirin [22]. This reduction can be attributed to inhibition of androgen biosynthesis [23] or to degeneration of spermatogenic elements [24].

Testis weight is an important indicator of reproductive health; reduced testis weight means a decrease in length for seminiferous tubules that are the primary site for spermatogenesis, subsequently leading to low activity of reproduction. We consider that the observed alterations in the testis tissue by H & E analysis are responsible for the decrease in testicular weight. Our findings indicate that aspirin has a detrimental effect on sperm parameters. The administration of aspirin led to a significant decline in sperm count and motility, and an increase in sperm abnormalities. These results were supported by the studies of Al-Taei [10] and

Received: *07-03-2026* - ***Accepted***: *04-05-2026* - ***Published***: *11-05-2026*

Vyas *et al* [12]. Other studies explain that the impact of aspirin on sperm parameters is due to its effect on spermatogenesis process [25], or to hypercholesteremia in testis which is a marker of low production of androgen that affect physiological maturation of spermatozoa and indicator of impairment in spermatogenesis [26], or its influence on the flow of calcium ions which is necessary for movement and activation of sperm [27].

Mohammed *et al* [28] reported that administration of aspirin to adult mice resulted in a reduction in the activity of hyaluronidase, which plays an important role in the penetration of sperm to the ovum during the process of fertilization. Morphological abnormalities of sperm may be attributed to alterations in testicular DNA [29, 1], or a result of mistakes in the sperm differentiation process during spermatogenesis [30]. Histopathological examination showed structural alterations in the testes, including disorganization of germinal epithelium, thickness in the basement membrane, and oligospermia. These consequences were in accordance with other studies that documented similar histopathological changes in testicular tissues of rats exposed to aspirin [12, 31]. Similar results were recorded by Mahmoudi-Lafout and Mohammadghasemi [32], who revealed histopathological changes in testes according to Johnson's score, and by Chaloob et al [22], who showed that administering aspirin to male mice caused structural alterations in testicular tissues. These detrimental effects of aspirin on testicular tissues may be the result of low levels of testosterone or its effect on the blood tissue [33] by inhibiting prostaglandin production, which affects constriction of arterioles leading to epithelial cell death [34], or low antioxidant levels [35]. The current study also demonstrated that aspirin at the dose of 75 mg/kg for 15 days affected the embryos. A similar outcome was obtained by Ibrheem and Al-Janaby [36] who found a significant reduction in litters number sired by treated males with aspirin at the doses 50 and 150 mg/kg. This impact on the embryos may be due to alterations in sperm DNA [32] as a result of reducing antioxidants [35].

Conclusion

It can be concluded that administering aspirin to male mice for 15 days at a dose of 75 mg/kg BW adversely affected reproductive effectiveness and the embryos. Therefore, ASA should be used with caution.

Acknowledgments

The authors are grateful to the University of Tripoli for providing the laboratory facilities necessary to achieve this research.

Conflict of Interest

The authors declare that there is no conflict of interest.

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Received*: 07-03-2026 -* ***Accepted****: 04-05-2026 -* ***Published****: 11-05-2026*

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Received*: 07-03-2026 -* ***Accepted****: 04-05-2026 -* ***Published****: 11-05-2026*