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Effect of Perinatal and Postnatal Exposure of Aloe Vera Gel on Male Mice Epididymis at Puberty

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Abstract

Background: In medicine, plants considered the main resource for drugs. In fact, it is estimated that 25% of prescription drugs have a plant origin. Aloe vera which is a pharmaceutical plant is useful for improving the body's physiology and could be used for curing many diseases. However, there are a few studies related to the effects of Aloe vera on the reproductive system.

Aim of study: The main objective of this study is to investigatethe effect of perinatal and postnatal exposure to Aloe vera gel extract on the histological status of male mice epididymis at puberty.

Materials and Method: Forty mature female Swiss Webster mice were divided intoexperimental and control groups. These female mice were mating with fertile males.Following conception,thefemales given orally 10 µl of fresh Aloe vera gel extract started from the onset of gestationand continued throughout the gestation period (20 days). After parturition and through weaning time these male births were given orally 10 µl of fresh Aloe vera gel extract and continuous till six weeks. The female mice in the control group were given normal saline only by the same dose and route. Around puberty (aged six weeks) the male births were sacrificed then an incision was made in the pelvic region to get their testes. The epididymis was grasping gently, fixed, and then histological sections with a thickness of 5 microns were prepared.

Results: Histological observation of male mice epididymis prenatal exposure to a low dose of Aloe vera gel extract and continuous through weaning time till puberty showed a normal structural pattern with a great number of sperms in caput (head), corpus (body) and caudal (tail) epididymis. The lumen of the cauda epididymis contains a larger density of sperm cells.

Conclusions: Using a low dose of Aloe vera gel extract revealed a normal structural pattern of the epididymis, and it leads to enhancing the sperm amounts inside the epididymal lumen.

Keywords: Aloe vera, Male infertility, Natural therapeutic remedies, Male reproductive system, Epididymis

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Introduction

Aloe Barbadensis Miller is a perennial succulent herb, mostly referred to as Aloe vera. It is one of the over 400 species of Aloe, belongto family *Asphodelaceae*. Aloe vera has been used medicinally for centuries and is frequently used in herbal medicine [1]. In fact, because they are generally safer than synthetic drugs, many users of chemical drugs prefer to use herbal drugs [2]. However, chemistry of Aloe vera revealedmore than 200 different materials which are biologically active substances such as: anthraquinones or phenolic compound, saponins, lignin and salicylic acid, in addition to vitamins, sugars and minerals [3]. It also contains amino acids, lipids, sterols tannin and enzyme [4], polysaccharides, steroids, organic acids, antibiotic agents [5].

Aloe vera considered as an important pharmaceutical plant because of its many medicinal properties [6]. In fact, the presence of the antioxidant polyphenols, indoles, and alkaloids, in the Aloe vera leaf gel shows antioxidant capacity [7]. However, Researches has been confirmed the linked of antioxidants present in the Aloe vera extractwith lower blood lipids in hyperlipidaemic patients [8]. Aloe vera also possesses hypoglycaemic, hypotensive, hepatoprotective, blood purifying properties [9]. It alsoused in patients complain from ulcerative colitis to reduce inflammation [10]. However, the bioactive compounds from Aloe vera are very effective in treatments of various diseases, such as allergic reactions, rheumatoid arthritis, diabetes, skin diseases, dysentery, diarrhoea, piles. Aloe vera could be effective in treatments in other condition such as inflammatory of the digestive system, a blood purifier, diuretic, uterine tonic and fever reliever [11]. Most of the health benefits associated with Aloe vera can be becausethe presence of polysaccharidesin the gel of the leaf [12]. In fact, 40% of infertility cases are male factor infertility [13]. Low fertility of male has been attributed toinability to produce sperm and ejaculation, premature ejaculation and decreased libido [14]. Therefore, men

have a significant proportionin relation with infertility.Meanwhile, damage in the spermatogenesis ismain causes of infertility in men [15]. However, medicinal plants extracts arealso curing infertility and low fertility. Previous studiesimproved that Aloe veracould enhance spermatogenesis because of its antioxidative effects. In fact, this miracle plant could improve the testosterone hormone level and has a positive effect on histological features of the testis [16]. Many medicinal plants have been used to treat infertility including male infertility problems [17]. The epididymisare a pair comma-shaped structure (Figure 1), covered by the visceral tunica vaginalis [18,19]. These irregular, long tubules (4- 5 meters), lied on the dorsal surface of testes, are lined by a pseudostratified columnar epithelium with many cell typesincluding: tallcells (principal cells) with stereocilia and small cells (basal cells), in addition to cells are thought to be intraepithelial lymphocytes called small halo cells with dark rounded nuclei and pale cytoplasm [18]. Another population ofcells found in the head region named apical cells, while clear cells predominantly found in the tail region. Epididymal cells form ablood-epididymis barrier,protects the antigenic sperm from the (host immune system) [20]. However, epididymis, a highly coiled tube, which mature sperm move from the testis to the vas deferensis surrounded by smooth muscle and embedded within a loose, vascular stroma. Each epididymis divided into a caput (head region), corpus (body) and cauda (tail region) (Figure 2). The primary storage site for mature sperm is cauda (tail region of the epididymis), which continuous with a highly muscular duct, the ductus deferens were sperm undergo final maturation in which it acquires motility andability to fertilize an egg [20]. Epididymis tubules secrete an important substance thatmention the sperm survive and maturation [17]. Mean while, vascularisation of epididymis is enriched by the testicular artery branches.

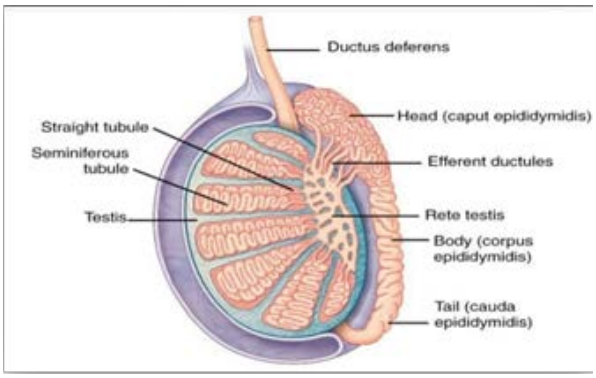

Figure 1: Comma-shaped structure epididymis attached to the dorsal surface of the testis.

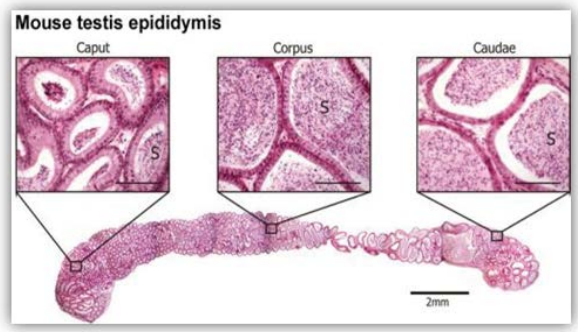

Figure 2: Panoramic view of a longitudinal section of the mice epididymis illustrating thecaput, corpus and cauda epididymis H&E [21].

Materials and Method

Forty mature female Swiss Webster micewere randomly selected from animal house of the High Institute for Infertility Diagnosis and Assisted Reproductive Techniques/Al-Nahrain University. The female mice were about 27-30 g weight and aged 8 weeks old. Thesefemales were kept at room temperature (27℃-30℃) and exposed to photo periodicity 12:12. The female mice were divided into two groups (experimental and control groups).

Aloe vera gel extracts preparation

Fresh leaves of Aloe verawere washed with fresh water.Juicefrom this plant leaves were extracted mechanically. Then the terminal tip and lateralhorns of Aloe Vera were eliminated. Leaves were cut transversely into pieces. The gelatinous material inside the leaves were separated from the thick epidermis, the protective external envelop. The solid gel in the centre of the leaf was homogenized. The resulting mucilaginous, thick and straw-coloured homogenate were filtered through cloth and the filtrate centrifuged at 20000 rpmfor 30 min, at 2°C in a refrigerated centrifuge. The filtrate of Aloe vera extract was divided into 2 ml volume tubes. The clear filtrate was stored in dry sterilized small containers for 3 days at 20°C before being used [22]. The pregnant females were given orally 10 µl of Aloe vera gel extract started from the first day of gestation and continuous throughout the gestation period (20 days). After parturition and through weaning time, male births separated, given orally 10 µl of Aloe Vera gel extract and continuous till puberty around aged six weeks. The control groups were givennormal saline only by the same dose and rout. The dosing schedule used was once per day. After six weeks (around puberty) and at the end of the treatment period, the pentobarbital sodium was administered for anaesthesia. The male births, sacrificed. Then an incision was made in pelvic region to get their testes. Epididymis was grasping gently, fixed with 10% formalin.The routine histological technique was done and histological sections with thickness of 5 microns were prepared [23].

Results

The histological section of control mice revealed that the epididymis is lined by pseudostratified columnar epithelium. The predominant cell type is the tall principal cell with a moderately dense cytoplasm and basally located nucleus. These cells extended from the basement membrane to the lumen of epididymis. In the caput (head region) these cells have long stereociliathat project into the lumen.The other basal cells are small cells which located between the bases of the principal cells and characterized by a littleamount ofcytoplasm.The sperms are obviously seen inside the lumen of epididymis tubules (Figure 3).

While histological observation of male mice epididymis prenatal exposed to low dose of Aloe vera extract and continuous through weaning time till puberty showed normal structural pattern and the epididymis consists of caput (head), corpus (body) and caudal (tail) epididymis. Epithelium height in different segments is found to be highest in the caput and lowest in cauda with great quantity of sperms (Figure 4). The epididymal caput observed with large number of sperms in its lumen (Figure 4, 5 and 6), and lined by epithelium with numerous tall principal cells with deeply stained nucleus. These tall cells extended from the basement membrane to the lumen, while the pyramid-shaped cells, basal cells has nucleus showing a densely stained chromatin (Figure 6), and the lumen of the cauda epididymis contains larger density of sperm cells, the stereocilia is much shorter in the cauda (tail) segment (Figure 7).

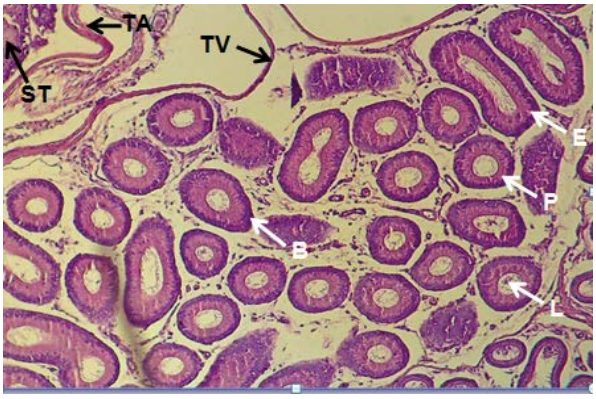

Figure 3: Normal caput epididymis structure from control group. The epididymis tubules are lined by pseudostratified epithelium (E). The predominant cell types are ciliated tall columnar dark-staining cells, the principlecells (P) and basal cells (B). Section also revealed epididymis lumen (L) with sperms (white arrows). Seminiferous tubules of tests (ST), tunica albuginea (TA), tunica vaginalis (TV) (black arrows) (H&E).

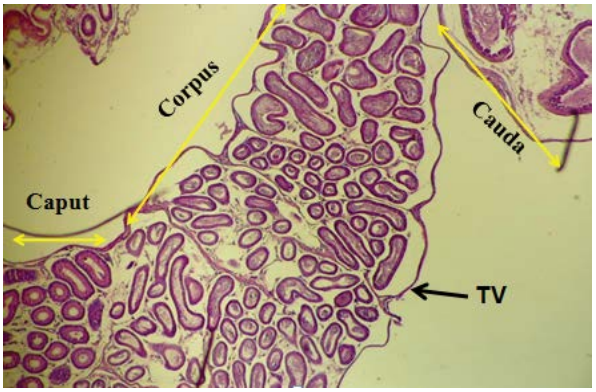

Figure 4: Representative photomicrographs showing the Hematoxylin and eosin (H&E) stained caput, corpus and caudal epididymis of the treated group. Epithelium height in different segments is found to be highest in the caput and lowest in cauda. Section also revealed epididymis lumen with great quantity of sperms. Tunica vaginalis (TV) (H&E).

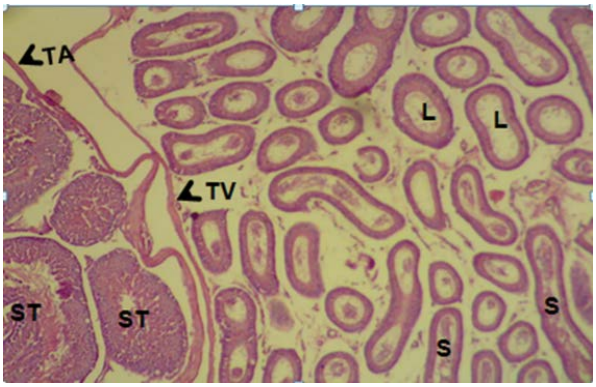

Figure 5: Photomicrographs illustrating the epididymal caput of six-week age male mice (at puberty) from treated group. The epididymal caput observed with sperms (S) in its lumen (L). Section also revealed seminiferous tubules of tests (ST), tunica albuginea (TA) and tunica vaginalis (TV) (head arrows) (H&E).

Discussion

About 15% of the couples suffer from infertility whose 30% of the causes are related to women and almost 30% are a male factor causes [24] 10% to both sexes and 25% of the cases are related to unidentified reasons. Waters and his colleagues 2006 cited that male infertility

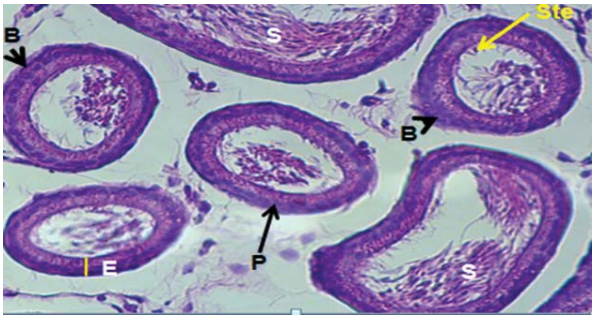

Figure 6: Histological section of caput epididymis of a treated animal shows the lumen of the epididymal duct contains large amount of sperm cells (S) and is lined by a pseudostratified columnar epithelium (E) with apical stereocilia (Ste) (yellow arrow). The epithelium shows numerous tall cells, the principal cells (P)with deeply stained nucleus. Principal cells extended from the basement membrane to the lumen. The pyramid-shaped cells, basal cells (B) has nucleus showing a densely stained chromatin (H&E).

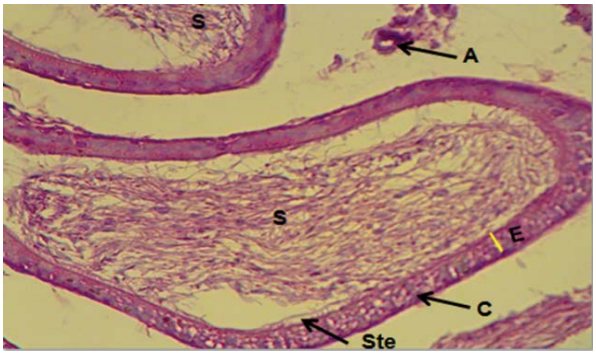

Figure 7: Histological section of cauda (tail of epididymis) of the treated group revealed a great quantity of sperms (S). The predominant cells in the tail region (clear cells) (C), the stereocilia (Ste) are much shorter in the cauda segment. Epithelium (E), arteriole (A) (H&E).

participate to 50% of all infertility problems. In fact, male infertility caused by many factors such as infection, varicocele, ducts obstruction, exposure to toxins as well as radiation, while genetic lesions include single-gene defects, chromosomal aneuploidies, micro-deletions in addition to rearrangements [25,26].

Meanwhile, Hull, 1986 investigated that the main cause of male infertility is defective sperm function which accounting for about 27% of all infertile couples. Performance of sperms,disorder in production and damage in the spermatogenesis are among the commonest causes of men infertility [27,28]. Aitken RI, et al. (1988) reported that the chemical nature of the damage to the sperm plasma membrane is responsible for this abnormal state indicating the important role of lipid peroxidation in the causes of men infertility [29]. The possibility that damage to the plasma membrane of the sperm resulted from lipid peroxidation indicated by a failure to exhibit sperm-oocyte fusion suggested the relationship between the appearances of such defects and the increased production of reactive oxygen species (ROS) by the sperm [30]. ROS may bind with biomolecules and change their structure. Major cell damages result from the ROS are induced alteration of polyunsaturated fatty acids in membrane lipids, essential proteins and DNA [31,32].

In fact, free radicals and reactive oxygen species are strongly associated with oxidative stress (OS). However, many factors likeinflammation, obesity, cigarette smoking as well aspollutants are important factors associated with defect inspermatogenesis process and sperm production [33].

The potential toxic effects on sperm performance, quality as well as function by high levels of reactive oxygen species production in the male reproductive tract has become an actual concern [34]. When antioxidant capacity of seminal plasma is less than ROS productionit results in oxidative stresses (OS) which is strongly harmful to sperm [35]. The mechanisms by which OS causes infertility areOS damages the sperm plasma membraneresult ininhibition of the sperm motilityas well as reduces its ability to fuse with oocyte. The second mechanism is that OS could directly damage sperm DNA, affecting the paternal genomic contribution to the embryo [36]. Moreover, high levels of ROS production result in apoptosis of germ cells and may speed up this process leading to DNA damage in addition to poor outcome of fertility [35].

However, as mentioned by Turner and his colleagues 2007 the epididymis providing a good microenvironmentfor maturation of sperm andacquisition of its motility as well as enhances the fertilizing potentialandeliminatedefective sperms [37,38]. The epididymis tubule linked to the testis and serves to store sperm produced [39]. The epididymis also plays a key role in sperm protection, transport and concentration. In fact, all these processes depend on and rogenhormone [38]. Meanwhile, during epididymis transit, the spermsexposed tothe threaten of OS that impaired spermatogenesis, and when this process impaired, it causes damage in the cytoplasmic extrusion mechanisms and the sperms are elaborate from the germinal epithelium carrying (surplus residual cytoplasm).Thus, sperms that are released during spermiation are supposed to be defective, immature and malfunctioning [35]. The retention of residual cytoplasm by sperm is thought to be positively correlated with reactive oxygen species generation (Aitken and Roman, 2008). In fact, these sperms have a high content of polyunsaturated fatty acids (PUFAs) rich membrane, plasmalogens and sphingomyelins [40-42].

Marty and his colleagues 2003 cited that pregnant females' exposures toxenobiotics and environmental factorsacts as endocrine disruptorsresult in reduce androgenic signalling and decreased synthesis of the testosterone hormone. However, androgens are necessary for the development as well as maintenance of the epididymis. Thus, perinatal exposures to xenobiotics make this organ as potential target for these toxic effects, which can influence male fertility [43].

Robaire B, et al. (2000) reported that there are many other substances could also damage the male reproductive system such as chemicals and plastics, new anti-neoplastic drugsand herbal products(alkylphenols) pesticides, fungicides and cleaning agents [43].

However,idiopathic male infertility has an unexplained reduction in normal spermogram values and semen quality [44,45]. In fact, up to twenty-three percentage of male infertility is idiopathic [26]. Sullivan R (2004) reported that these men may suffer frompost-testicular defects whichresult in ejaculationwith normalsperm morphology butless fertilization capacity [45]. Mean while, some molecules are participated into sperm development, sperm maturation process and sperm-oocyte recognition during its passage through epididymal duct such as protein P34H. Such molecules appear to be a key to the causes of the idiopathic male infertility [44]. In addition, Hamada and his colleagues 2011 cited that"epididymis could be involved inmany cases of male infertility including the pathophysiology's that affects sperm maturation, which is a key event in the of fertilization process.

Additionally, any changing and disruption of the epididymal microenvironment through congenital abnormalities, temperature, protein concentration as well as intrinsic alterations in pH might be acritical factor that may causes male post-testicular infertility [44].

In general, the antioxidant protects gonadal cells and mature spermatozoa from consequence of OS damage [46,47], since an antioxidant is a molecule that inhibits the oxidation of other molecules by controlling or preventing the excess free radicals [48]. However, because sperms lack the (cytoplasmic enzyme systems) they are unable to repair the harmful induced by ROS [49].

Mean while, there has been an increased focus on the role of OS associated with male infertility [50]. In fact, antioxidant intake can support endogenous antioxidants [51] they inhibit the promulgation of ROS production [52] and protect DNA from oxidative damage and could enhance sperm quality as well as improve fertilityin men [53].

Traditional medicine in developing countries is necessary for population health [54]. As cited by Aboua Y, et al. (2009) reported that the flavonoid rich plant has been used to prevent OS" [55]. Badami S, et al. (2003) also improved that natural compounds rich in antioxidant like polyphenols, minerals and vitamins have a potential action to inhibit the generation of ROS or scavenge free radicals [31]. Thus, these plant materialscan protect male reproductive organ function and important for men fertility.

However, this studyrevealed that there is an enhancementin the sperm amount in capute, corpus and cauda epididymis, which may be due to the presenceof phenolic compound in Aloe vera that hasantioxidant effects on lipid peroxidation [32]. Shahraki A, et al.(2014) also investigated that Aloe vera has an antioxidative effect, andcould enhance spermato genesis process through its positive effect on testosterone levels as well as histological features oftestis[56].

Aloe vera consist of an essential antioxidant vitaminsuch as A and C, vitamins in addition to B group like (thiamin, niacin, B2 (Riboflavin), B12,) and folic acid [57]. In fact, a potent antioxidative compound was isolated from a methanolic extract of *Aloevera Barbardenis Miller* [58]. These antioxidant vitamins can explain the result of this study.

A significant increase in the amount of sperm in capute, corpus and cauda epididymis agreed with previous study of Mohammad Baqir, et al. (2014) using low dose of fresh Aloe vera extract and improved that this plant could enhanced sperm parameters especially sperm motilityandviability [59]. Furthermore, fresh Aloe vera extract reinforce and enhanced the histological features of male mice testis[60]. Jafaribarmak M, et al. (2012) reported in their study reported that Aloe vera extract can increase the seminiferous tubules diameter, and enhancement in testicular tissue [32,61]. Thus, Rodriguez F, et al.(1988) recommended in their study to add Aloe veraextracts in the dilution of semen for the artificial fertilization of sheep [62].

The histological observations of the caput, corpus and cauda epididymis showed increased numbers of sperms inside lumen of epididymis tubules. These finding is agree with previous studies of Jasem E, et al. (2011), who improved an increase in testes weight of rats givenanAloe vera extractin addition to a significant increase in sperm count and motility, and decrease in sperm abnormalities in compare with control group [63].

However, the rising in sperm concentration might beresult from an increase in sperm production in testes, since the epididymis can provideimportant information on recent testicular events. In fact, Aloe vera extracts improved spermatogenesis process because it is rich in many compounds such asmucopolysaccharides, sterols, enzymes and prostaglandins (PGs). Some of these PGs such as PGD2, PGE2, and

PGF2a has been involved in the regulation of testicular testosterone production suggesting that PGs might has a rapport in male fertility physiology. Moreover, analysis of testosterone level between groups treated with Aloe vera has increased remarkably [64]. In fact, this hormone plays key role in maintenance of epididymis structure and function in addition to its necessities for the process of sperm maturation throughout epididymalduct [65].

Briefly Aloe vera enhance sperm quality due to its potential spermatogenic activity because it has antioxidant and chemical compounds and may be useful for improve male fertility. Another study also improved that use of Aloe vera powder in a dose of 60 mg/kg b.w. result in an increased in the litter size of rabbits and the fertility rate. This study suggested that Aloe vera gel consumption could increase sperm amount by enhances spermatogenesis and could be used for boost fertility [66].

It's so important to mention here that there are many researchers suggested that Aloe vera extract reduced sperm count and motility and has potential antifertility. In fact, those researchers didn't filter Aloe vera extract as an important step to remove anthraquinones, the potentially bioactive compounds in this plant [67], because this plants composed of two parts; the first one is the pericyclic cells, which is found belowAloe vera leaf skinthat produce a yellow latex named an (Aloe juice). The main active compound of latex is anthraquinones, including barbaloin, isobarbaloin, Emodin as well as aloins A, and B [68]. The other layer, located in the inner central area ofAloe vera leaf, constitute ofparenchymal cellsproduces mucilaginous, a clear slightly viscousfluid named as inner gel or Aloe gel, these gel posses' polysaccharides in addition to the three malic acid acylated carbohydrates [67].

However, Aloe latex, yellow saps (rich in anthraquinones) is cytotoxic while polysaccharide material obtains from the inner gel is not toxic [69,70]. The toxic effect of Aloe vera whole leaf extract may be because of the anthraquinones generated by oxidation of low molecular weight (LMWF) component derived from this plant leaves like a loin [71]. The cytotoxic effects could also result from the production of ROS by redox cycling induced by anthraquinones of the LMWF fraction. It is apparent that the LMWF obtained has cytotoxic activities [72]. For this reason and because of the widespread using of commercial Aloe vera has encouraged scientiststo scientifically assess these products since it contains the anthraquinones which associatedwith considerable risks. Therefore, it is so important to filter Aloe vera extract to remove anthraquinones.

Conclusion

From results of this study we can conclude that consumption of low dose of Aloe vera gel extract showed normal histological structure of male mice epididymis and enhancement of the sperm amount inside its lumen and can be a good candidate for manufacturing fertility drugs.

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