Original Article

Effect of Moringa Oleifera Leaf Extract on the Histology of Epididymis of High Fat Diet Exposed Adult Albino Rats

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ABSTRACT

Introduction: Infertility is one of the major obesity hazards. High fat diet has been found to affect epidydimal sperm count, motility, and morphology. Moringa Oleifera was used in some studies to alleviate some fertility-related problems.

Aim of the Work: To study the effect of high fat diet on the histological structure of the epididymis of adult albino rats and the possible protective role of moringa oleifera.

Material and Methods: Thirty male adult albino rats were divided into three groups, Group I (control group): was further subdivided into two subgroups IA: negative control & subgroup IB: rats were fed on standard diet and received moringa oleifera 400 mg/kg/day. Group II (high fat diet group):received high fat diet for eight weeks. Group III (Moringa Olreifera group): received high fat diet together with moringa oleifera 400mg/kg/day for eight weeks. At the end of experiment, all rats were sacrificed. Epididymis was bilaterally harvested, weighted and processed for both light & transmission electronic microscopic examination.

Results: H&E sections of epididymis of the high fat diet group showed irregular tubules, with flattened epithelium and significant decrease in sperms' content in the lumen of most tubules. Many clear and halo cells were observed. EM sections showed degenerated principal cells, dark irregular basal cells with many vacuoles. In moringa oleifera group, H&E sections showed rounded to oval tubules with increase in sperms' content in their lumina. No halo cells were detected. EM sections showed principal cells with many mitochondria, few vacuoles and clear demarcations between cells. Basal cell with oval nuclei and clear cells with granules were detected.

Conclusion: High fat diet had degenerative effects on the histology of epididymis but moringa oleifera had a significant role in alleviating these hazards.

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Key Words: Diet, epididymis, highfat, moringa.

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INTRODUCTION

Obesity is one of the worldwide health risks that is rapidly increasing to extents reaching epidemic levels[1,2]. Moreover, multiple chronic problems, including type-2 diabetes, hypertension, cancer, cardiovascular diseases & male infertility are usually linked with obesity[3].

Regarding male fertility problems, it has been reported that high fat diet-induced obesity negatively affects testicular cellular structure, spermatogenesis, sperm motility and sex hormonal levels with subsequent reduction of fertilization rate in rats[4,5,6]. Recent studies reported significant decrease in the daily sperm production which was attributed to a notable decrease in spermatozoa and spermatids number[2].

Moringa oleifera is a tropical plant whose pharmaceutical use has greatly expanded nowadays. It is rich in calcium, potassium, phosphorous, vitamins A & D, iron and essential amino acids, omega 3 oils as well as some antioxidants including β-carotene, vitamin C, flavonoids[7,8]. Moreover, it has been reported that it has anti-inflammatory effects[9]. In some countries, moringa oleifera seeds are used in treating sexual inadequacy[10]. Obembe *et al*., 2015 reported that long term exposure of male albino rats to moringa oleifera resulted in a marked increase in their sperm count[9].

In addition, moringa oleifera was reported to improve sexual performance and libido[11]. Some studies attributed the stimulatory effect of moringa oleifera on testis and epididymis, due to the presence of flavonoids. Flavonoids are antioxidants that have a protective effect against oxidative stress testicular impairments[10]. Other studies observed increase in the weight of the testis and epididymis, following moringa oleifera administration in mice[12].

Hence the aim of this study was to study the effect of high fat diet on the histological structure of the epididymis and the possible protective role of moringa oleifera.

MATERIAL AND METHODS

Moringa Oleifera Extract

The plant leaves were purchased from Pure Life co. Egypt. The plant aqueous extract was prepared by mixing 1 gm of dried and powdered leaves with 10 ml boiling water for 5 minutes. The mixture was then filtered twice through filter paper into a sterile tube. The aqueous extract solution

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(100 mg/ml) was freshly prepared and stored at 4℃ for up to 5 days[13].

Types of Animals Diet

Standard diet: in the form of 420 kcal/100g diet; total protein = 18%; total fat = 9%. It has been used for control group

High-fat diet: in the form of 515 kcal/100g diet; total protein = 18%; total fat = 39%[14]. It has been used for high fat diet groups.

Animals

Thirty male adult albino rats weighing 200 - 220 gm were obtained from the animal house of the Faculty of Medicine Ain Shams Research Institute (MASRI). Rats were kept in ordinary wire-mesh cages in a room temperature of 21 ±3 ℃. They were provided with standard rat diet and granted free access to water. They were housed for a week preceding the experiment in order to accommodate to the experimental conditions. This experiment followed the guidelines of the Committee of the Animal Research Ethics (CARE) at Faculty of Medicine, Ain Shams University.

Experimental design

Animals were randomly divided into three groups, ten rats in each:

Group I (control group): was further subdivided into two subgroups (five rats in each):

- Subgroup IA: rats were fed on standard diet and were considered as negative control.
- Subgroup IB: rats were fed on standard diet and received moringa oleifera 400 mg/kg/day via gastric tube for 8 weeks[15].

Group II (high fat diet group): ten rats received high fat diet for 8 weeks.

Group III (Moringa Olreifera group): ten rats received high fat diet together with moringa oleifera 400 mg/kg/day for 8 weeks.

At the end of the experiment, all rats (no dead rats) were weighed then anesthetized using ether inhalation and then sacrificed. The epididymis of both sides were excised in each rat.

Histological techniques

The body of right epididymis of each rat was cut longitudinally into two halves. One half was fixed in 10% neutral-buffered formalin, dehydrated, then embedded in paraffin blocks. Sections of 5µm thickness were subsequently cut and stained with H&E then examined by the light microscope[16].

The other right epididymal half was cut in 1mm3 thick parts, fixed in 4% glutaraldehyde to be processed for transmission electron microscopic examination (TEM). Semithin sections were stained with Toluidine blue and examined by the light microscope. The ultrathin sections from selected fields were stained with Uranyl Acetate and Lead Citrate[17], examined and photographed by Philips 201-transmission electron microscope of Electron Microscope Unit, Faculty of Science - Ain Shams University.

Semen smear preparation

The tail of the left epididymis of each rat was immediately cut and squeezed in a Petri dish in order to obtain fresh semen samples[18]. A drop from the obtained epididymal content from each rat was put on a grease-free slide and stained by 1% eosin stain[19]. The slides were air dried then sperms' morphology was examined.

Morphometric studies

The sperm count as well as the height of the tubular epithelium were measured in Eosin-stained semen smear and H&E-stained sections respectively. Measurements were done in randomly chosen five fields/section in five different sections for every rat in each group at magnification x400[20] using image analyser Leica Q win V.3 program installed on a computer in the Histology Department, Faculty of Medicine, Ain Shams University. The computer was connected to a Leica DM2500 microscope (Wetzlar, Germany).

Statistical analysis

Body weight, tubular epithelial thickness in H&E-stained sections and sperm count in Eosin-stained semen smear sections of each rat in each group were collected. Data analysis was performed using MedCalc® Version 11.1.1.0 for Windows (MedCalc Software, Belgium) and Microsoft Office Excel 2010 (Microsoft, USA). Analyses of Variance (ANOVA test) and student t-test were done. Data were presented as mean ± standard deviation (SD), and were considered to be highly significant when $P \leq 0.001$, significant when $P \leq 0.05$ and insignificant when $P > 0.05$.

RESULTS

Microscopic Results

Group I (Control group): Histological examination of the H&E-stained sections of body of epididymis of rats of subgroups IA and IB revealed almost similar results. The sections showed rounded to oval tubules filled with numerous sperms (Figure 1). These tubules were lined by pseudostratified epithelium composed mainly of principal cells and some clear cells. The principal cells appeared columnar with projecting apical stereocilia at their luminal side. The basal cells were seen resting on the basement membrane and the clear cells were observed with their pale stained cytoplasm (Figure 2). Narrow interstitial connective tissue space was seen in-between the adjacent tubules (Figures 1,2).

Semithin sections further clarified the epididymal epithelial cells. Regularly arranged columnar principal cells with basal oval nuclei were obviously seen with

numerous well demarcated apical stereocilia and supranuclear dark granules. The apical region of the principal cell exhibited enormous secretory apparatuses (Figures 3,4). Basal cells with oval nuclei were seen resting on the basement membrane (Figures 3,4). Few clear cells with multiple apical dark granules were also seen interspersed in-between the principal cells and resting on the tubular basement membrane (Figure 4). Spindle shaped smooth muscle fibers with elongated oval nuclei were seen surrounding each tubule in a circular manner (Figures 3,4). Blood vessels and connective tissue fibers were observed among neighboring tubules (Figure 3).

Transmission electron microscopic sections showed principal cells having regular rounded euchromatic nuclei with thin rim of peripheral heterochromatin, numerous mitochondria and numerous projecting stereocilia. The apical secretory apparatuses were seen consisting of supranuclear large longitudinally oriented Golgi complex, numerous vesicles, and large microvesicular bodies (Figure 5). Basal cells with small oval euchromatic nuclei were seen resting on the basement membrane (Figure 6).

Eosin-stained semen smears showed unstained viable sperms with hooked head, neck and tail connected to each other (Figure 7).

Group II (high fat diet group): Histological examination of the H&E-stained sections of the body of epididymis of rats of group II showed markedly irregular tubules, with moderate sperms' content in most of them that was apparently decreased as compared to that of the control group (Figures 8,9). Moreover, some tubules appeared almost devoid of sperms enclosing only cellular debris (Figures 8,9). In some areas, tubules appeared adherent to each other with very narrow interstitial tissue space (Figure 8) while in other areas they appeared widely separated with accumulation of connective tissue fibers in between some of them (Figure 9). Interconnected tubules were also encountered (Figure 8). Some tubules showed an apparent decrease in their tubular epithelial thickness as compared to the control group, with loss of principal cells' stereocilia in many areas (Figure 10). Many clear cells were noticed (Figure 10). Halo cells with small dense rounded nuclei surrounded by clear cytoplasm appeared in-between the principal cells near the lumen in many tubules (Figures 9,11). Moreover, vacuolated tubular cells were observed in numerous tubules (Figure 11).

Semithin sections revealed marked irregularity in the arrangement of principal cells with rounded to oval nuclei and dispersed dark granules (Figure 12). Some basal cells appeared with regular oval nuclei while others had irregular ones (Figure 12). Many clear cells with their characteristic dark granules were seen extending and approaching the lumen (Figure, 12,13). Some halo cells were also observed (Figure 13). Interstitial spaces were seen occupied by dense connective tissue fibers studded with cellular infiltration (Figures 13,14) among which were macrophages with dark irregular nuclei in addition to some binucleated ones (Figure 14).

Examination of TEM sections further clarified the irregularity in principle cells' nuclei where some of them were irregular in outline (Figure 15) and some were fragmented with ill-defined outline (Figure 16) and few were almost regular with well-defined nucleolus (Figure 17). Some dispersed dark granules not confined to the supranuclear region (Figures 15,16) and numerous elongated mitochondria were also detected in many principal cells (Figures 16,17). Discontinuous stereocilia and many apical vacuoles were observed (Figure 15). Basal cells with oval hyperchromatic nucleus and others with dark irregular nuclei were observed near the basement membrane (Figure 17). Numerous clear cells were encountered with oval nuclei and their characteristic electron dense granules (Figure 15). Halo cells with oval nuclei and surrounded by their clear cytoplasm with few organelles were also observed near the basement membrane (Figure 16). Other irregular halo cells with distorted nuclei surrounded by a clear cytoplasmic rim were seen near the lumen (Figure 15).

Eosin- stain semen smears sections showed nonviable dark stained sperms, most of which had detached head with coiled tail. Some sperms appeared fragmented with bifid tails (Figure 18).

Group III (Moringa Oleifera group): Histological examination of the H&E-stained sections of epididymis of rats of group III showed that most tubules were filled with numerous sperms (Figure 19), while some appeared still enclosing cellular debris (Figure 20). Some tubules appeared adjacent to each other with relatively narrow interstitial tissue in between (Figure 19). However, others had intervening dense connective tissue (Figures 19,20). Some tubules regained their normal tubular epithelial thickness (Figure 20) while others still showed apparent decrease in their thickness as compared to the control group with loss of stereocilia (Figures 20,21). Regarding the tubular epithelial cells, few clear cells were seen among the principal ones however, no halo cells were detected (Figure 20).

Semithin sections clarified the shape and arrangement of tubular epithelial cells. In some areas, nuclei of principal cells were almost uniform in shape and arrangement (Figures 22,23) with supranuclear dark granules (Figure 23). In others, they appeared variable and irregular in shape (Figures 22,24) and some of them appeared binucleated (Figure 24). The observed clear cells with their characteristic granules were detected either near the basement membrane (Figure 22) or in the middle of the tubular epithelium (Figure 24). The interstitial tissue among some tubules was studded with connective tissue fibers, cellular infiltrates and congested blood vessels (Figure 23).

Transmission electron microscopic sections showed some principal cells with oval and others with slightly irregular nuclei (fFigures 25,26). Electron dense granules regained their supra nuclear site, and uniform

stereocilia were observed (Figure 25). Numerous mitochondria were seen in the cytoplasm of principle cells (Figures 25,26). Clear demarcation between neighboring cells was also observed (Figure 26). Clear cells with oval nuclei and multiple electron dense granules were encountered near the basement membrane (Figure 27) and among principal cells in the middle of tubular epithelium (Figure 26). Basal cells with oval nucleus were seen near the basement membrane (Figure 27).

Eosin- stain sperm smears sections showed that most of the sperms appeared non stained (alive) with normal hooked head, neck and tail connected to each other (Figure 28). However, some nonviable sperms appeared darkly stained (Figures 28,29) and some of which were viable having pyriform head and coiled tail (Figure 29).

Statistical Results (Table & Histogram1)

Statistical analysis of the morphometric results of the present study showed that rats which received high fat diet in group II had highly significant increase in body weight as compared to the control group (Histogram1A). Meanwhile, a highly significant decrease in both; epididymal tubular epithelial thickness (Histogram 1B) and sperm count (Histogram 1C) was detected compared to the control group.

On the other hand, treatment by Moringa Oliefera in group III revealed non-significant change in body weight as compared to the high fat group II, however, it was still highly significantly increased as compared to the control group (Histogram1A). Moreover, highly significant increase in both; epididymal tubular epithelial thickness (Histogram 1B) and sperm count (Histogram1C) was detected as compared to the high fat group II. However, the epithelial thickness was still significantly decreased, and the sperm count was non-significantly changed compared to the control group (Histograms, 1B,1C respectively).

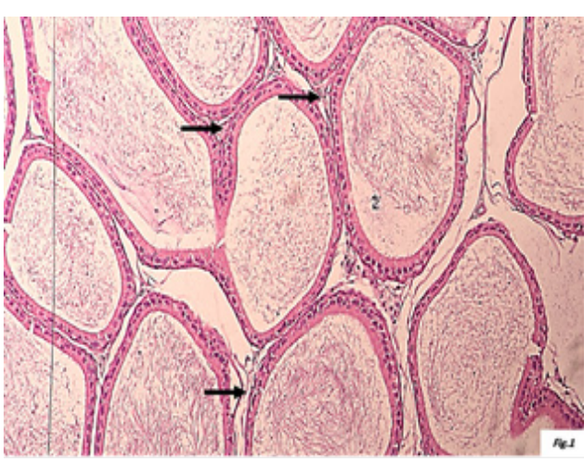


Fig. 1: Showing rounded to oval tubules of the body of epididymis filled with numerous sperms. Notice the narrow interstitial connective tissue space in between (↑). (Control group, H&E x 100)

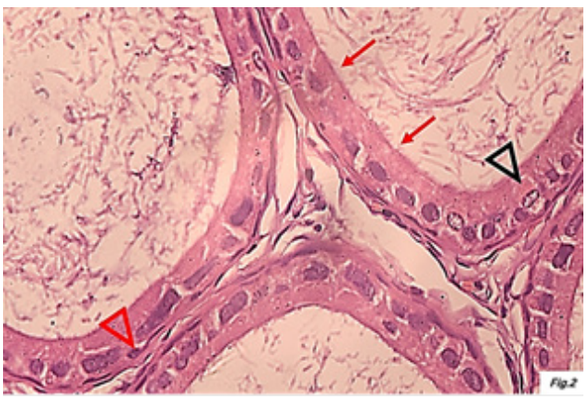


Fig. 2: Showing the tubular epithelial lining the body of epididymis formed of columnar principal cells with projecting stereocilia (↑) and rounded clear cells (black Δ) with pale stained cytoplasm. Basal cells (red Δ) can be also noticed. (Control group, H&E x400)

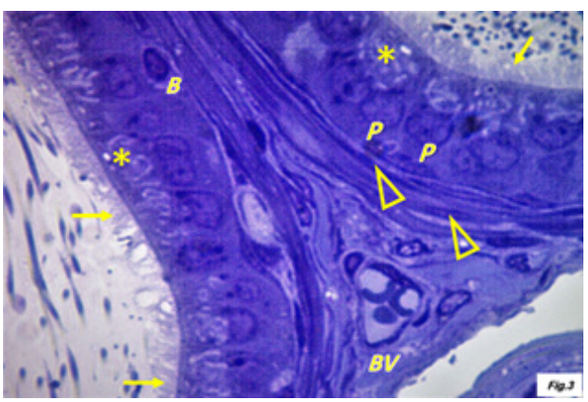


Fig. 3: showing the tubules of the body of epididymis with regularly arranged columnar principal cells with oval nuclei (P) and numerous apical stereocilia (↑). Secretory apparatuses can be clearly seen in the apical parts of each principal cell (*). Notice the basal cell with oval nucleus (B) resting on the basement membrane. Note also the connective tissue fibers containing blood vessels (BV) between the tubules. Circularly-oriented smooth muscle fibers can be seen circumscribing the tubules (Δ). (Control group, Toluidine blue, x1000)

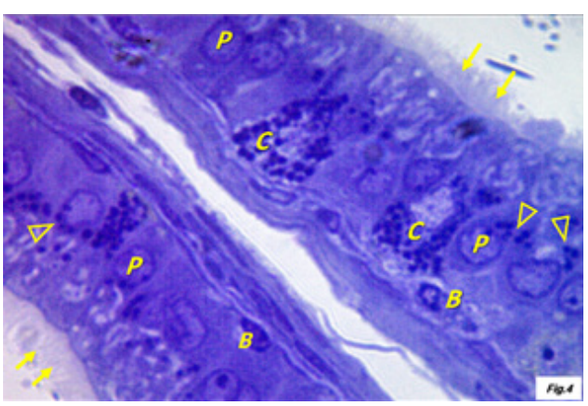


Fig. 4: showing the pseudostratified epithelium of the body of epididymis containing clear cells (C) with numerous dark granules resting on the tubular basement membrane. Notice the regularly arranged columnar principal cells with basal oval nuclei (P), supranuclear dark granules (Δ) and apical stereocilia (↑). The basal cells (B) can also be seen resting on the basement membrane. (Control group, Toluidine blue, x1000)

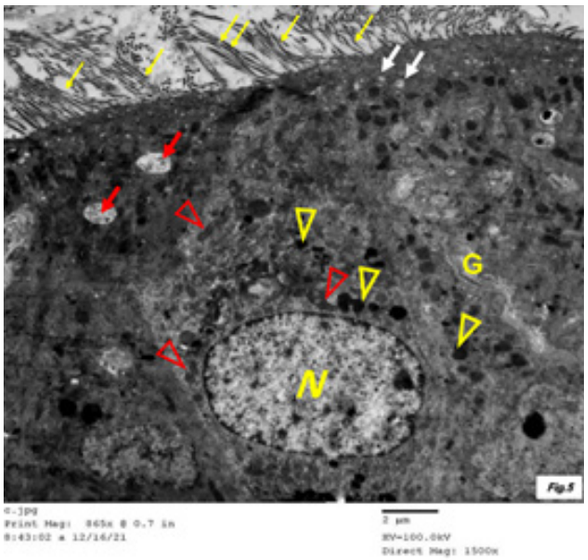


Fig. 5: showing principal cells of the body of epididymis with regular rounded euchromatic nucleus (N) with thin rim of peripheral heterochromatin. Longitudinally-arranged Golgi complex (G), Numerous vesicles (white↑) and large microvascular bodies (red↑) can be seen. Notice the numerous mitochondria (red Δ) and supranuclear dark granules (yellow Δ), and apical stereocilia (yellow↑).(Control group, Uranyl acetate & lead citrate, x1500)

Fig. 6: showing a basal cell in the body of epididymis with oval nucleus (Nb) near the basement membrane. (Control group, Uranyl acetate & lead citrate x2000)

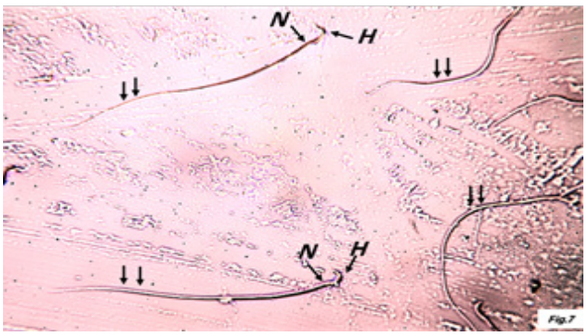


Fig. 7: Semen smear from the body of epididymis showing unstained viable sperms with hooked head (H), neck (N) and tail (↑↑) connected to each other. (Control group, Eosin Stain x400)

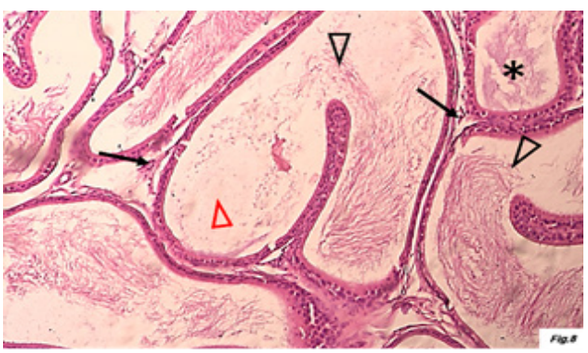


Fig. 8: Showing the body of epididymis with irregular tubules, enclosing apparently moderate sperms' content. Some tubules appear almost devoid of sperms (red Δ) and others containing only cellular debris (*). Notice the narrow interstitial tissue space (↑) and the interconnected tubules (black Δ). (Group II, H&E x100)

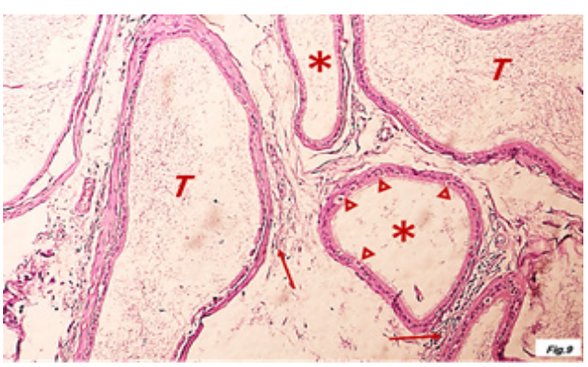


Fig. 9: Showing the body of epididymis containing widely separated irregular tubules (T) with moderate content of enclosed sperms. Notice a tubule almost devoid of sperms, enclosing only cellular debris (*). Halo cells can be noticed (Δ) among the epithelial lining near lumen. Note also the connective tissue fibers concentrated in between some tubules (↑). (Group II, H&E x100)

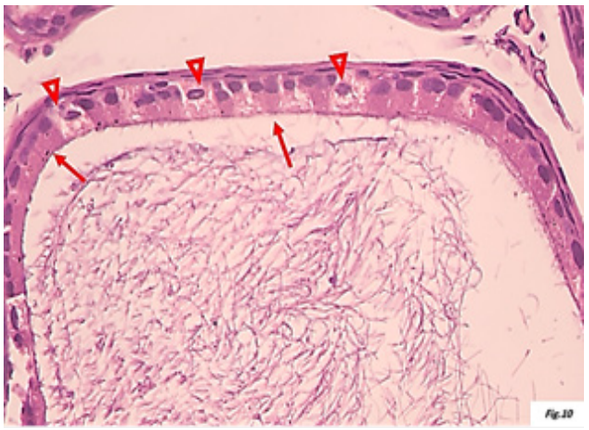


Fig.10: showing a tubule of the body of epididymis with apparent decrease in tubular epithelial thickness. Notice the clear cells (Δ) in the tubular epithelium and focal loss of stereocilia (↑). (Group II, H&E x400)

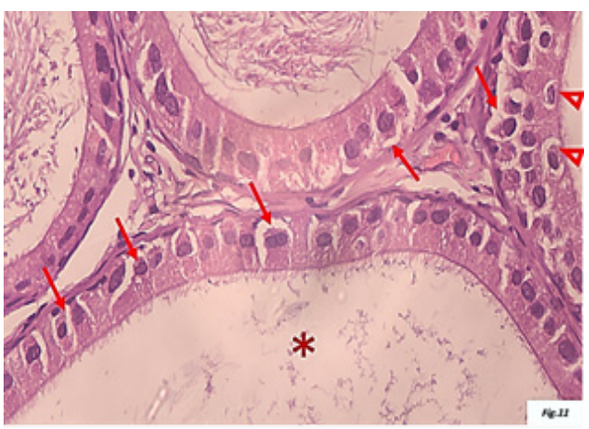


Fig. 11: Showing many cytoplasmic vacuolation in principal cells (↑) and halo cells (Δ) near the lumen of the body of epididymis . Notice the cellular debris inside the lumen of a tubule (*). (Group II, H&E x400)

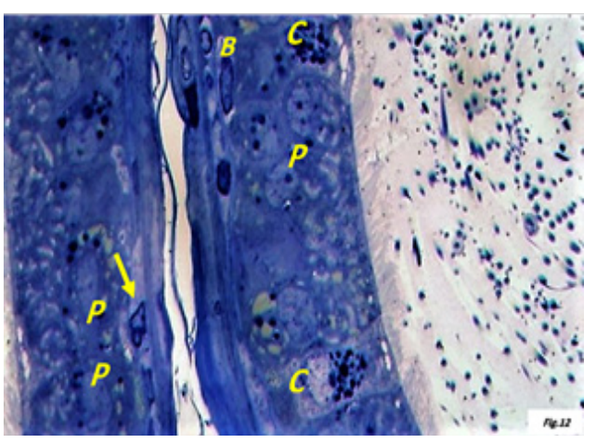


Fig. 12: Showing irregularly arranged rounded to oval nuclei (P) of principle cells of the tubular epithelium of the body of epididymis . Notice the basal cell with regular oval nucleus (B) and another with irregular one (↑). Note also the clear cells (C) with their characteristic dark granules approaching the lumen. (Group II, Toluidine blue, x1000)

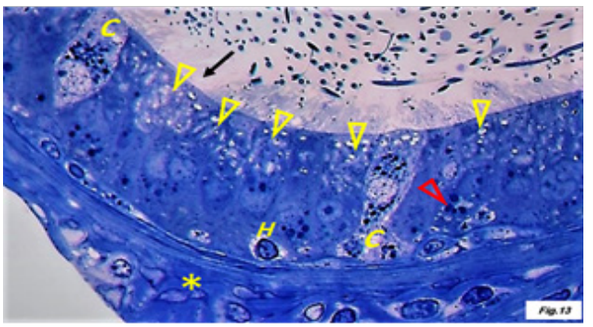


Fig. 13: Showing clear cells (C) among the tubular epithelium of the body of epididymis with their characteristic apical dark granules and halo cell (H) with clear cytoplasmic rim. Notice the dispersed dark granules (red Δ) and the widespread apical vacuoles (yellow Δ). Note also the dense intertubular connective tissue with cellular infiltration (*). Notice the focal loss of stereocilia of some principal cells (↑). (Group II, Toluidine blue, x1000)

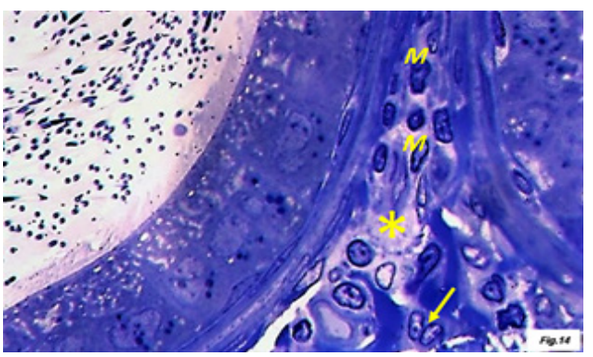


Fig. 14: the body of epididymis showing macrophages (m) with dark irregular nuclei in the interstitial tissue (*). Notice the binucleated one (↑). (Group II, Toluidine blue, x1000)

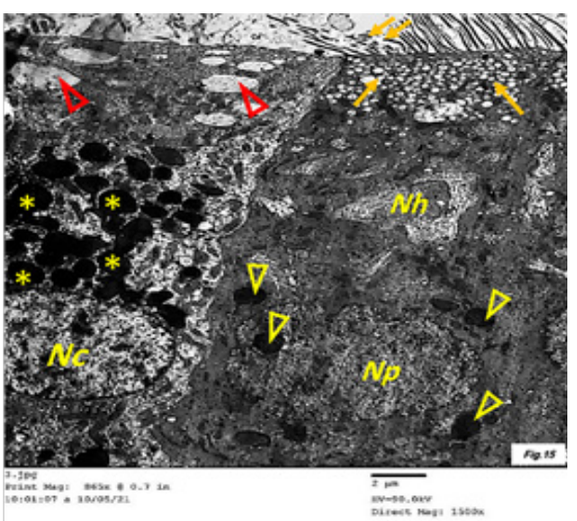


Fig. 15: Showing the tubular epithelium of body of epididymis containing clear cell, with oval nucleus (Nc), numerous electron dense granules (*) and large apical vacuoles (red Δ) at the luminal surface. Notice the adjacent irregularly outlined principal cell nucleus (Np) with surrounding dark granules (yellow Δ), numerous apical vacuoles (↑) and discontinuous stereocilia (↑↑). An irregular halo cell can be seen with distorted nucleus (Nh) surrounded by a clear cytoplasmic rim. (Group II, Uranyl acetate & lead citrate x1500)

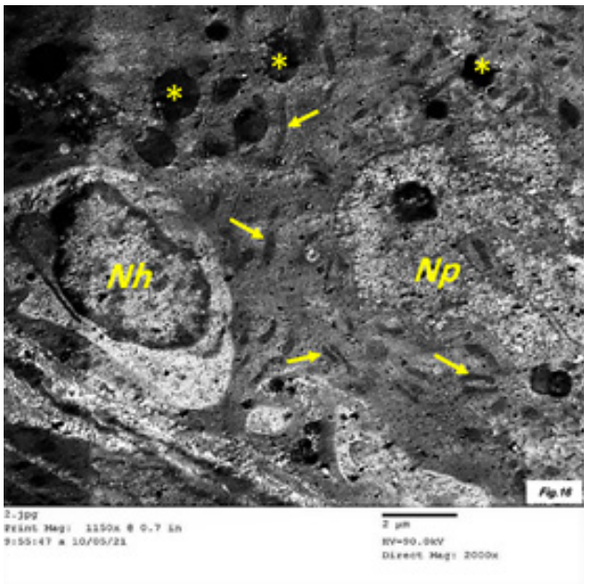


Fig. 16: showing a principal cell of the body of epididymis having fragmented nucleus (Np) with ill-defined outline with numerous surrounding elongated mitochondria (↑) and dark granules (*). Notice the adjacent halo cell with oval nucleus (Nh) and surrounding clear cytoplasm observed near the basement membrane. (Group II, Uranyl acetate & lead citrate, x2000)

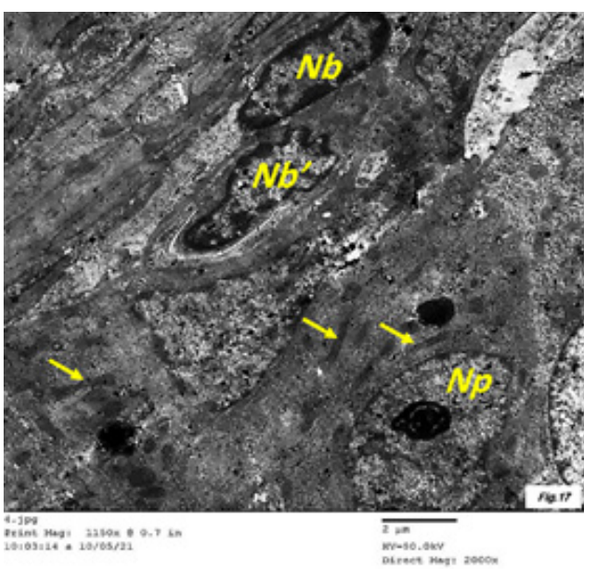


Fig. 17: Showing the tubular epithelium of the body of epididymis with almost regular principal cell nucleus (Np) with well-defined nucleolus and numerous surrounding elongated mitochondria (↑). Notice the adjacent two basal cells near the basement membrane, one with oval hyperchromatic nucleus (Nb) and the other with dark irregular nucleus (Nb'). (Group II, Uranyl acetate & lead citrate, x2000)

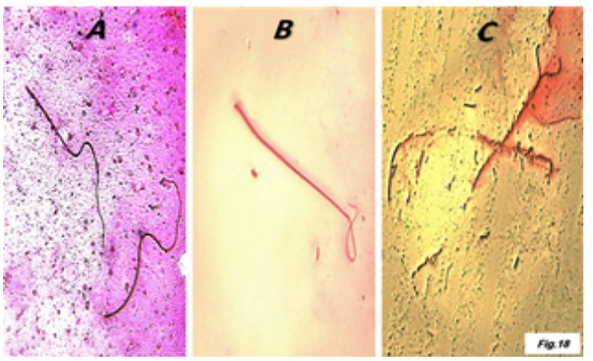


Fig. 18: Eosin- stained semen smear from the body of epididymis showing darkly stained nonviable sperms in three different fields. A: shows sperms with detached head, B: a sperm with detached head and coiled tail and C: fragmented sperms with bifid tail. (Group II, Eosin Stain x400)

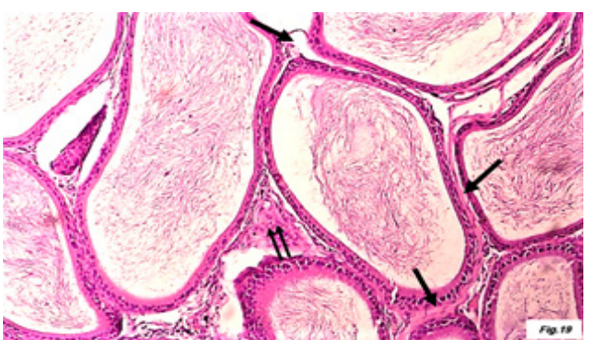


Fig. 19: Showing adjacent tubules of the body of epididymis filled with numerous sperms with intervening narrow interstitial tissue containing dense connective tissue (↑). Notice focal areas of increased interstitial connective tissue (↑↑). (Group III, H&E x100)

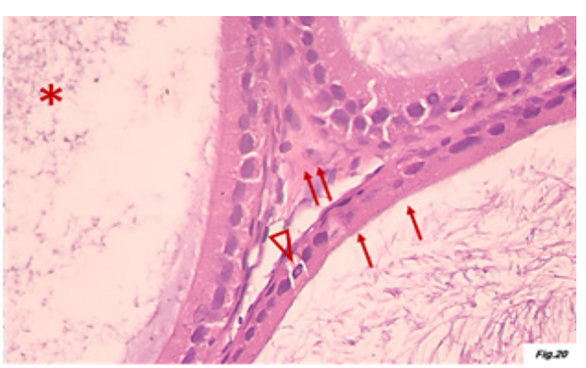


Fig. 20: Showing a tubule of the body of epididymis enclosing cellular debris (*). Notice the adjacent tubule showing apparent decrease in epithelial thickness with loss of stereocilia (↑). Note also the clear cells (Δ) seen among the principal ones in tubular epithelium and the dense connective tissue among tubules (↑↑). (Group III, H&E x400)

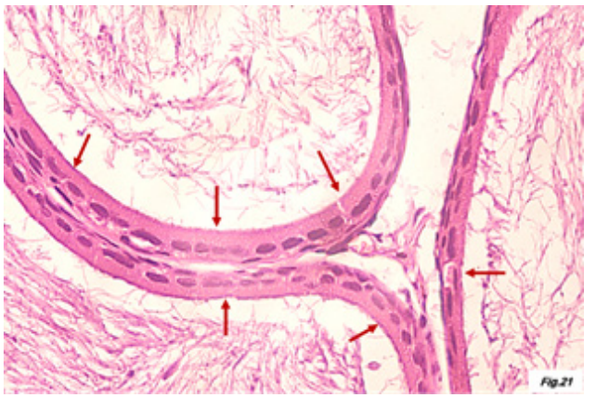


Fig. 21: Showing tubules of the body of epididymis with apparent decrease in their epithelial thickness with loss of stereocilia (↑) in some areas. (Group III, H&E x400)

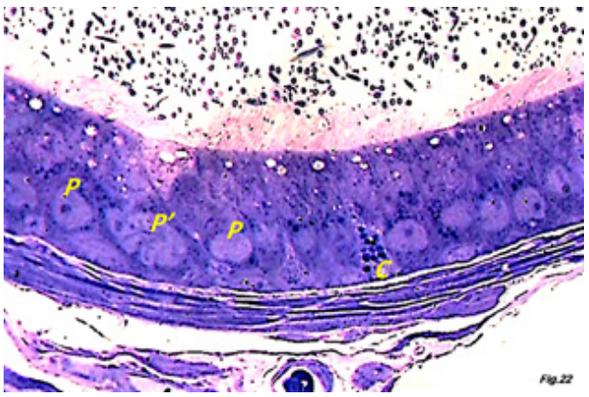


Fig. 22: Showing the epithelium lining the tubules of the body of epididymis having some irregular principal cell nuclei (P') among uniform ones (P). Notice the clear cell (C) near the basement membrane. (Group III, Toluidine blue, x1000)

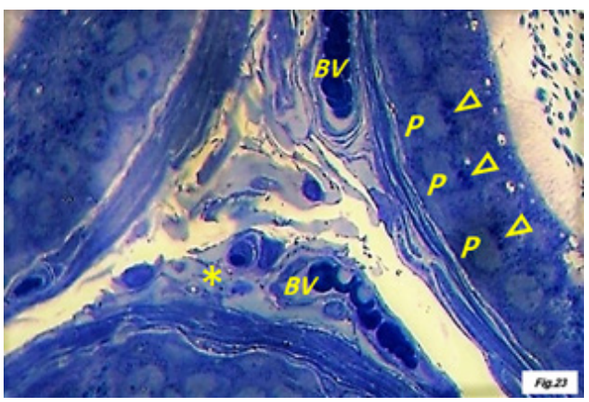


Fig. 23: Showing tubular epithelium of the body of epididymis having principal cells nuclei (P) with almost uniform shape and arrangement with supranuclear dark granules (Δ). Notice the dense connective tissue with cellular infiltrates (*) and congested blood vessels (BV) seen among the tubules. (Group III, Toluidine blue, x1000)

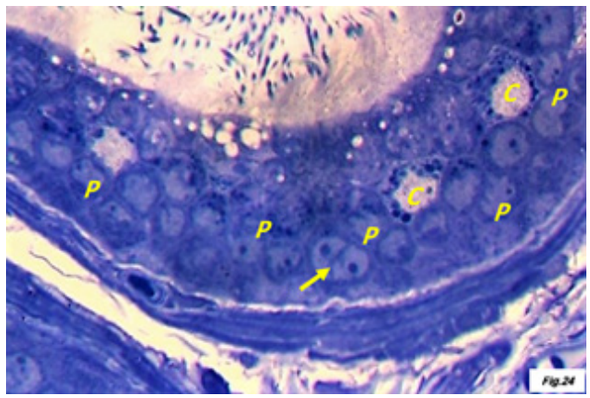


Fig. 24: Showing tubular epithelium of the body of epididymis having variable shaped principal cell nuclei (P) with intervening clear cells (C) with their characteristic granules. Notice the binucleated principal cell (↑). (Group III, Toluidine blue, x1000)

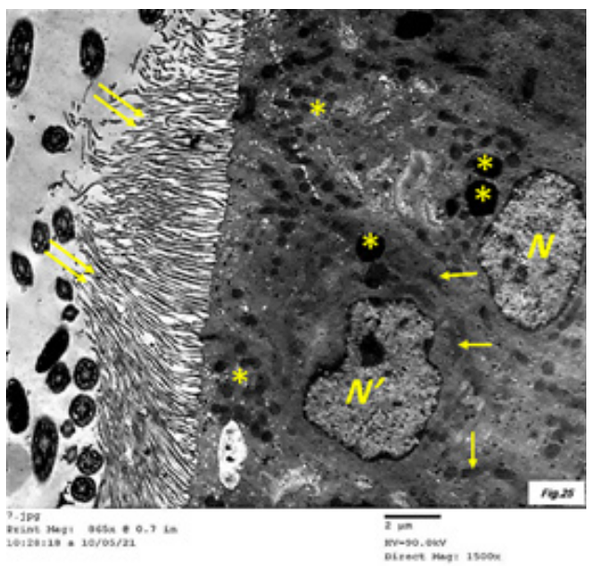


Fig. 25: Showing the principal cells exhibiting oval (N) and slightly irregular (N') nuclei with surrounding numerous mitochondria (↑) , supra nuclear electron dense granules (*) and uniform stereocilia (↑↑). (Group III, Uranyl acetate & lead citrate, x1500)

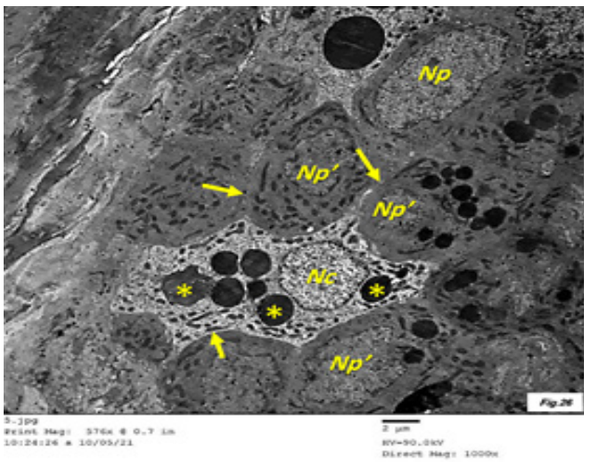


Fig. 26: The tubular epithelium of the body of epididymis showing principal cells with oval (Np) and others with slightly irregular (Np') nuclei. Notice the clear cells with oval nuclei (Nc) & multiple electron dense granules (*) among principal cells in the middle of tubular epithelium. Note also the clear demarcation between them the cells (↑). (Group III, Uranyl acetate & lead citrate, x1000)

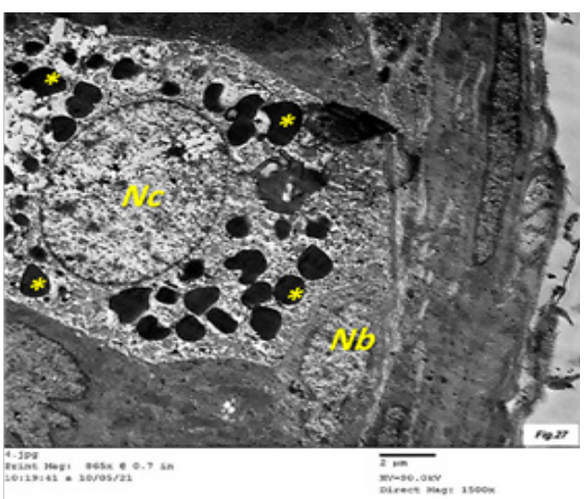


Fig. 27: The tubular epithelium of the body of epididymis showing Clear cell with oval nuclei (Nc) and multiple electron dense granules (*) near the basement membrane. Notice the adjacent basal cell with oval nucleus (Nb). (Group III, Uranyl acetate & lead citrate, x1500)

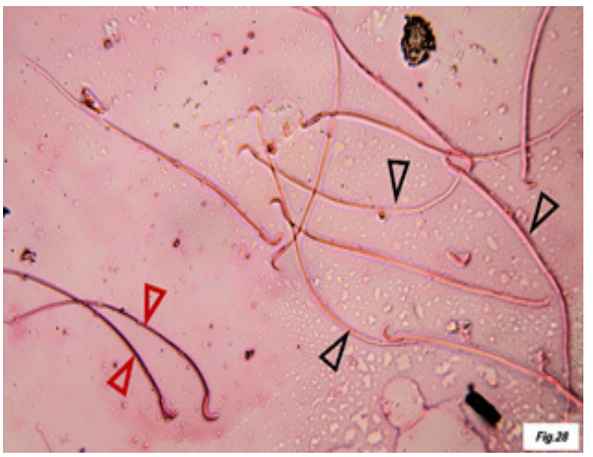


Fig. 28: Eosin- stained semen smear from the body of epididymis showing many non stained viable sperms (black Δ) with normal hooked head, neck and tail connected to each other. Notice also the two darkly stained nonviable sperms (red Δ). (Group III, Eosin Stain x400)

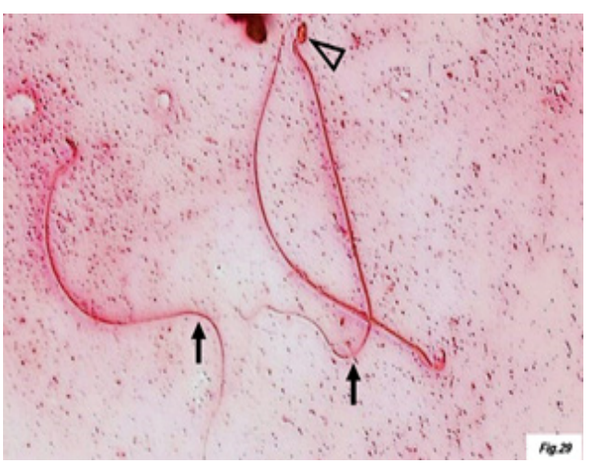
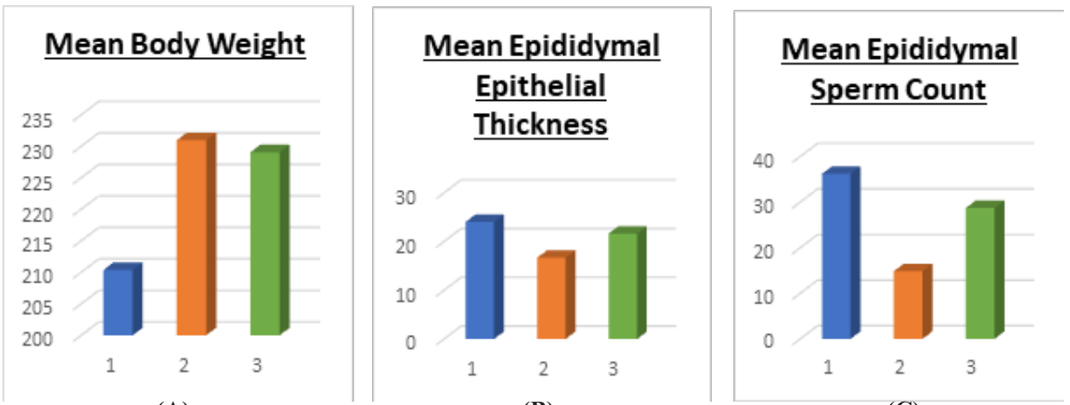


Fig. 29: Eosin-stained semen smear from the body of epididymis showing dark stained nonviable sperms. Notice the coiled tails (↑) and the pyriform head (Δ). (Group III, Eosin Stain x400)

Table 1: Showing body weights of rats, epididymal tubular epithelial thickness and epididymal sperms' number done by (student "t" test):

	Group I (Control Group)	Group II (High Fat Diet Group)	Group III (Moringa Olreifera Group)
Mean Body Weight of rats (in gms)	210.34 ±3.98	230.95±4.83*	229.045 ±3.06*
Mean Epididymal Tubular Epithelial Thickness (in µm)	24.03 ±0.99	16.65 ±1.4*	21.62 ±2.13*$
Mean Epididymal Sperms' Number	36.17 ±5.42	14.83 ±3.43*	34.67 ±9.85$

Values are expressed as Mean ± SD.



(A) **(B)** **(C)**

Histogram 1: Demonstrating the morphometric comparison between the three groups as regards; (A) body weight of rats, (B) epididymal tubular epithelial thickness and (C) epididymal sperm count in semen smear.

DISCUSSION

It has been reported that increased body mass index (BMI) associated with obesity has profound effects on male reproductive parameters with subsequent deterioration of fertility[21]. Recently, it has been stated that obesity can be experimentally induced by high fat diet[2]. Thus, in the present study, we used high fat diet as a way of induction of obesity in order to demonstrate its effect on the histology of epididymis and evaluate the possible protective role of Moringa Oleifera on these effects.

Results of examination of sections of epididymis of rats that received high fat diet in the current study showed obvious irregularity in the tubular shape with flattening of their lining epithelium which was further documented by the statistically highly significant decrease in the tubular epithelial thickness. This was in accordance with Merve etal.,2019 who recorded similar decrease in tubular epithelial thickness accompanied with high fat diet[22]. Epididymal insult induced by bisphenol-A administration also led to flattening of tubular epithelial lining[23].

Further examination of tubular epithelial lining in the present study revealed irregular arrangement of principal cells with loss of the supranuclear arrangement of their granules and its replacement by an irregular dispersed arrangement. These granular changes could be explained by the results reported by Ahmed *et al*., 2008 who declared that hypoandrogenic status was usually associated with notable affection and decrease in the number of micropinocytotic vesicles of epididymal principal cells[24].

Moreover, widespread apical vacuoles and discontinuous stereocilia were frequently detected in principal cells of this group. Similarly, previous research reported large principal cell vacuoles with degenerated stereocilia in rats fed on high fat diet[22,25]. Principal cells were reported to play a major role in synthesis, secretion and absorption within the epididymis[26]. Owing to their large surface area available for molecular interaction and their different transport systems and membrane pumps, principal cells' stereocilia help to keep the proper volume of the epididymal lumen[25]. Therefore, the noted loss of stereocilia in this group could further clarify the epididymal insult caused by high fat diet with expected impact on its function.

Principal cells have been also proven to play a vital role in the epididymal defense mechanism as they work on phagocytosis of the cellular debris and abnormal particles within the epididymis[27]. Therefore, the principal cells' histopathological changes noted in the current study may account for the accumulation of cell debris noted in many tubules.

As for the basal cells, the current study revealed that some of them appeared with irregular nuclei. It has been stated that basal cells are responsible for the formation of prostaglandins in order to regulate water and electrolyte transport by the principal cells[26]. Moreover, they have a protective role against reactive oxygen species thus protecting the maturing sperms[28]. This might contribute to explaining the decrease in the number of sperms in epididymal tubules noted in the H&E-stained sections of this group. This was further revealed by the highly significant decrease in sperm count compared to the control group.

Another finding in the present study that may further reveal the high fat diet associated epididymal insult is the frequently encountered clear cells and that most of them were seen extending to approach the lumen of tubules. The main function of these cells is endocytosis of different particles such as necrotic sperms from the lumen of the epididymis[29]. Clear cell hyperactivity was also observed by Ahmed *et al*., 2008 who explained it as being a defense mechanism attempting to remove the accumulated luminal cell debris[24]. This might be the most probable explanation for the observed luminal position of clear cells in this group.

Additionally, halo cells were also frequently observed in high fat diet group especially near the lumen as well. Halo cells were assumed to be lymphocytes that had a role in the male reproductive immunological

function[23,27]. and stimulation of these cells could be a result of inflammatory reaction, since it is the main immune cell in the epididymis[30].

Inter tubular interstitial spaces of this group varied from being apparently narrow between adherent tubules in some areas to being markedly wide in other areas where the tubules appeared widely separated with intervening dense connective tissue fibers. This wideneing was accompanied by congested blood vessels and cellular infiltration including macrophages which were similar to basal cells. It has been reported that epididymal inflammatory status was accompanied by widened interstitium in between the tubules with cellular infiltrations and congestion of blood vessels[31,32]. It has been previously observed that some macrophages structurally similar to the basal cells were seen near the tubular epithelium[26]. They might be the progenitors of the basal cells and stimulators for renewal of degenerated epididymal cells[27].

Moreover, many tubules had marked decrease in their sperms' content and others were almost devoid of sperms enclosing only cellular debris. Similarly, some studies reported an association between obesity and decreased sperm concentration and poor semen quality[21,33]. This was further clarified by the Eosin-stained semen smears in this group which revealed that numerous sperms were darkly stained denoting that they are non-viable. Moreover, most of the observed sperms appeared with detached head, coiled or bifid tail or even totally fragmented. Sperm morphology has been reported to be an important parameter in the evaluation of male fertility[34]. Many studies recorded hazardous impacts of high fat diet and obesity on sperm structure and function. Among these recorded effects were the decrease in sperm motility[22,35,36], increase in sperm DNA fragmentation index[22] , decrease in sperm capacitation and binding ability[5,37], with subsequent decrease in fertilization rate[35,36]. It has been demonstrated that Obesity directly affects spermatogenesis leading to impairment in the physical and molecular structure of sperms, resulting in adverse effect on sperm maturation in epididymis[38].

On the other hand, examination of epididymal sections of rats fed on high fat diet and treated simultaneously with Moringa Oleifera revealed that many tubules regained their normal epithelial thickness which was further reinforced by the obtained statistically highly significant increase in comparison to the high fat diet group. Principal cells appeared with almost uniform nuclei with restoration of their supranuclear arrangement of dark granules. Similarly, it was reported that administration of Moringa Oleifera leaf powder in diabetic rats led to restoration of the morphological features of testicular seminiferous tubules as well as restoration of the normal thickness of epididymal tubular lining[39].

Moreover, most tubules in this group were filled with numerous sperms apart from few others which still enclosed cellular debris. Statistical analysis revealed highly significant increase in sperm count compared to that of group II. Additionally, Eosin-stained semen smears also revealed that most of the sperms appeared unstained denoting their viability with normal hooked head, neck and tail connected to each other. Few sperms were still seen with some morphological abnormalities.

Moringa Oleifera extract has been found to enhance the sexual behavior and vigor of male rats [10, 40]. It has been also reported that it enhances testicular spermatogenesis with subsequent increase in sperm count in both testes and epididymis[10], in addition to an increase in the epididymal weight[12]. Moringa Oleifera plant contains; steroids, alkaloids, flavonoids, phenolics and tannins[40]. Steroids have been stated to be the main causes of sexual behavior enhancement[41], while the noted enhancement of spermatogenesis has been attributed to the antioxidants present in the leaves of this plant[12]. Both antioxidants and flavonoids have the ability to ameliorate oxidative stress-related testicular impairments[42]. Knowing that the sperm cytoplasm contains only low levels of antioxidant enzymes, hence the antioxidant system of the epididymis and its enhancement by Moringa Oleifera treatment plays a vital role in sperm protection thus favoring the reproductive process[43].

Among the findings that could also reveal the improvement induced by Moringa Oleifera was the positional regression of clear cells where most of them lost their luminal approach and were detected either near the basement membrane or in the middle of the tubular epithelium. Moreover, halo cells were no longer frequently detected compared to the high fat diet group.

Last but not least, the current study revealed that Moringa Olifera did not cause significant decrease in body weight. It was just a protective measure against the hazards of high fat diet on the epididymal tissue.

CONCLUSION

High fat diet led to marked histopathological epididymal changes. However, Moringa Oleifera greatly improved such changes. Thus, it could be considered as a promising protector for ameliorating obesity induced hazards on other organs which could be an issue for future research.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

دراسه هستولوجيه لتأثير مستخلص أوراق المورينجا أوليفيرا على البربخ في النظام الغذائي عالي الدهون في ذكور الجرذان البيضاء البالغة

شيريـــن عـــادل سعـــد، مها مصطفي احمد زكريا، ماري رفعت إسحق

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المقدمه: يعتبر العقم من أكبر مخاطر السمنة ، حيث يؤدي ذلك إلى انخفاض عدد الحيوانات المنوية واضطراب بنية الخصية. تم استخدام المورينجا أوليفيرا في بعض الدراسات للتخفيف من المشاكل المتعلقة بالخصوبة.

الهدف من العمل: دراسة تأثير النظام الغذائي عالي الدهون على التركيب النسيجي للبربخ في الجرذان البيضاء البالغة والدور الوقائي المحتمل للمورينجا أوليفيرا.

المواد والطرق المستخدمه: تم تقسيم ثلاثين من الجرذان البالغة من الذكور إلى ثلاث مجموعات ، المجموعة الأولى (المجموعة الضابطة): تم تقسيمها إلى مجموعتين فرعيتين IA: المجموعة الضابطة السلبية والمجموعة الفرعية IB: تم تغذية الجرذان بنظام غذائي قياسي وتلقيت المورينجا أوليفيرا ٤٠٠ مجم / كجم /يوم. المجموعة الثانية (مجموعة النظام الغذائي عالي الدهون): تناول نظام غذائي عالي الدهون. المجموعة الثالثة (مجموعة المورينجا أوليفيرا): تلقت نظام الغذائي غني بالدهون مع مورينجا أوليفيرا ٤٠٠ مجم / كجم / يوم لنفس فترة التجربة. بعد ٨ أسابيع ، تم التضحية بجميع الفئران الجرذان. تم حصاد البربخ من الجهتين ووزنه ومعالجته للفحص المجهري الضوئي و المجهر الالكتروني النافذ.

النتائج: أظهرت شرائح H&E لمجموعة النظام الغذائي عالي الدهون في البربخ نبيبات غير منتظمة ، مع ظهارة نسيج طلائي مفلطح وانخفاض الحيوانات المنوية في تجويف معظم النبيبات. كما لوحظت العديد من الخلايا الصافية والهالة. أظهر الفحص الالكتروني خلايا أساسية متدهورة وخلايا قاعدية داكنة غير منتظمة مع العديد من الفجوات. في مجموعة المورينجا أوليفيرا ، أظهرت شرائح H&E نبيبات دائرية إلى بيضاوية مع زيادة الحيوانات المنوية في تجويفها. لم يتم الكشف عن خلايا الهالة.كما أظهر الفحص الالكتروني الخلايا الأساسية مع العديد من الميتوكوندريا ، وعدد قليل من الفجوات والفواصل بين الخلايا. تم الكشف عن الخلايا القاعدية ذات النوى البيضاوية والخلايا الصافية ذات الحبيبات.

الخلاصة: النظام الغذائي عالي الدهون له تأثير تنكسي على أنسجة البربخ ولكن المورينغا أوليفيرا لها دور مهم في التخفيف من هذه المخاطر.