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Effects of Dietary Supplementation of *Moringa Oleifera* Leaves on the Postnatal Development of the Rat Testis

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Research Article

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Abstract:

Objectives: The aim of this study was to investigate the dietary effects of Moringa oleifera leaves on the postnatal development of the rat testis and puberty time.

Methods: One hundred and twenty, 21-day-old male Wistar albino rats were used in this study. The rats were divided equally into 4 groups (n = 30 per group); as group 1 (control; fed a standard diet) and groups 2, 3 and 4 (fed a standard diet supplemented with 500, 1000, 1500 mg/kg b.w. of Moringa oleifera leaves powder; respectively). The rats were euthanized at 5, 6,7, 8, and 9 weeks of age. Testis samples were collected immediately after euthanasia and processed for light microscopy.

Results: Groups 2, 3 and 4 showed increased number of Leydig cells and germinal cells, early lumen formation and early appearance of spermatozoa in the lumen compared with the control group. However, these observations were more conspicuous in group 4 than groups 2 and 3. Group 4 reached puberty earlier and showed increased sperm production compared with other treated and control groups, suggesting that the most effective concentration of Moringa leaves powder supplementation in rats is 1500 mg/ Kg body weight.

Conclusions: Moringa oleifera leaves induce postnatal development of the testis and enhance sperm production, leading to early puberty and high fertility.

Keywords: *Development; Moringa oleifera; puberty; rat testis.*

1 Introduction

Several conditions can interfere with spermatogenesis and reduce sperm quality and production. Many factors such as drug treatment, chemotherapy, toxins, air pollution, and insufficient vitamin intake may have harmful effects on spermatogenesis and the normal production of sperm (Mosher & Pratt, 1991). A large number of plants have been tested throughout the world for the possible fertility regulatory properties (Bhatia et al., 2010). Some medicinal plants extensively used as aphrodisiac to relieve sexual dysfunction, or as fertility enhancing agents, providing a boost of nutritional value thereby improving sexual performance (Yakubu et al., 2007; Sumalatha et al., 2010; Laoung-On et al., 2021).

Moringa oleifera is a medicinally important plant, belonging to family Moringaceae. The Moringa tree grows mainly in tropical and sub-tropical areas (Sachan et al., 2024). *Moringa oleifera* is one of the leading names recently adopted in plants and drug research. It has been used over the years for different ailments, out of which only few were investigated. A large number of reports on the nutritional qualities of Moringa now exist in both the scientific and the popular literature. However, the outcome of well-controlled and well-documented clinical study is still clearly of great value (Mazumder et al., 1999; Pareek et al., 2023). *Moringa oleifera* lam has vast medicinal properties and every part is said to have beneficial properties (Mishra et al., 2011; Pareek et al., 2023).

Recently, the role of the Moringa in improving male reproduction has been the focus of interest. Previous reports have shown that Moringa fruit boosts the sperm count of men, whereas Moringa leaf extract increases the sperm count and weights of reproductive organs in male mice and rats (Cajuday and Pocsidio, 2010; Rosemary et al., 2013). Zade et al. (2013) reported that Moringa seed could increase the sexual activity of male Wistar rats. A study by Islam et al. (2024) has revealed that dietary supplementation of *Moringa oleifera* leaves improves productive and reproductive performance of Pekin duck. Furthermore, Recent studies have shown that treatment with *Moringa oleifera* leaf extract restore fertility of diabetic male rats (Jangir and Jain, 2025) and aged broiler breeder roosters (Ghadimi et al., 2024). In addition, extract of *Moringa oleifera* leaf could alleviate testicular toxicity in mice and rats (Nayak et al., 2020; Ogunlade et al., 2022).

Moringa leaves are used as a nutritional supplement and growth promoters, because they are an excellent source of protein, selenium, calcium, iron, potassium, phosphorus, bicarotene, g-tocopherol, and vitamins A, C and E (Makkar and Becker, 1997; Nambiar & Seshadri, 2001; Lakshminarayana et al., 2005; Sánchez-Machado et al., 2006; Kou et al., 2018; Pareek et al., 2023; Sachan et al., 2024). Thus, many studies have focused on the dietary effects of Moringa leaves on the sexual activity; however, there is no enough information on the dietary effects of Moringa leaves on the postnatal development of male reproductive organs. The testes are the primary male reproductive organs that act as gonads and as endocrine glands to produce sperm and hormones. It is therefore decided to investigate the dietary effects of *Moringa oleifera* leaves on the postnatal development of the testis and puberty time in a rat model.

2 Material and Methods

2.1 *Moringa oleifera* leaves powder

Moringa oleifera leaves powder was provided by Sudan Atomic Energy Commission.

2.2 Experimental Animals

One hundred and twenty, 21-day-old male Albino Wistar rats were used in this study, provided by Sudan Atomic Energy Commission. The rats were maintained under standard environmental conditions (temperature 22-24 C°; 12 h light: 12 h darkness cycle), the room was well ventilated. The rats were fed on standard diet (3:1; wheat flour: beef) and water ad libitum.

2.3 Experimental Design

The rats were randomly divided into four groups as follows:

Group 1 (control, n = 30): the rats were fed a standard diet.

Group 2 (n =30): the rats were fed a standard diet supplemented with 500 mg/kg body weight of *Moringa oleifera*.

Group 3 (n =30): the rats were fed a standard diet supplemented with 1000 mg/kg body weight of *Moringa oleifera*.

Group 4 (n = 30): the rats were fed a standard diet supplemented with 1500 mg/kg body weight of *Moringa oleifera*.

2.4. Samples Collection and Preparation

All rats were euthanized by decapitation at the end of the second, third, fourth, fifth, sixth weeks of the treatment; that is, when the rats were five, six,

seven, eight and nine weeks old (n= 6/time point). Testis samples were collected immediately after euthanasia and fixed in Bouin's solution. Tissues were dehydrated with a graded series of ethanol alcohol (70%, 90% and 100% alcohol each for two hours), cleared in xylene, and embedded and blocked in paraffin wax (Bancroft and Steven, 1990). Tissue sections of 4 μm thick were stained either with haematoxylin and eosin or with special stains as follows: 1- Masson's trichrome stain for demonstration of collagen fibers and smooth muscle fibers. 2- Van-Gieson for demonstration of collagen fibers. 3- Aldehyde Fuchsin and Verhoeff's stain for demonstration of elastic fibers. 4- Gordon and Sweet's stain for demonstration of reticular fibers. Then, sections were dehydrated through alcohols, cleared in xylene and mounted with "DPX" (Bancroft & Steven, 1990).

3 Results

Rat testes in all groups showed normal histological features; no pathological changes were observed, even with high concentration of Moringa and prolonged treatment (Figure 1). Seminiferous tubules were embedded in a fibrous stroma. Spaces between the seminiferous tubules were occupied by a loose connective tissue. This interstitial tissue contained collagen and reticular fibers, blood vessels, nerves, and several types of cells. The specific interstitial cells; cells of Leydig, were clustered in the form of groups. Each seminiferous tubule was surrounded by a lamina propria; the innermost layer was a basement membrane and the outermost layer consisted of collagenous fibers, reticular fibers, and Myoid cells (Figures 2 - 5).

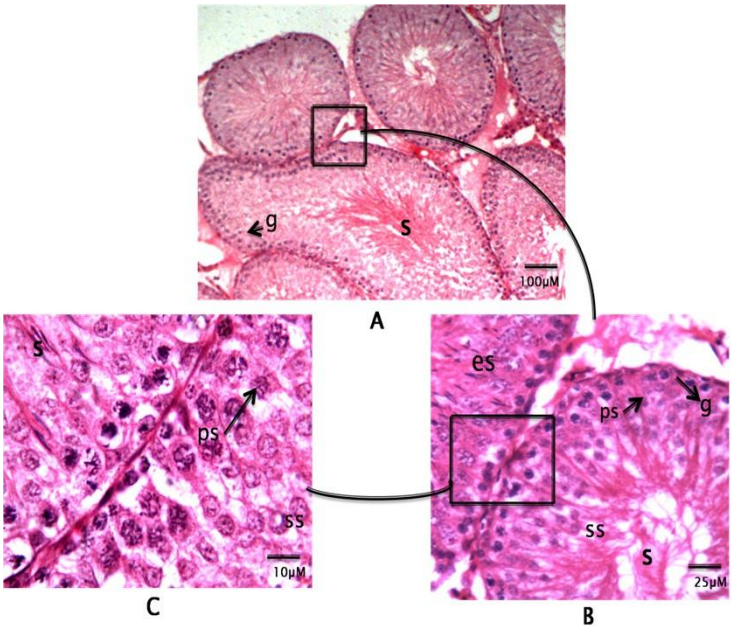

Figure 1: Group 4 rat testis at nine weeks of age, showing normal histological features. Note the spermatogonia cells (g); primary spermatocyte (ps); spherical spermatid (ss); elongated spermatid (es); spermatozoa (s). H & E stain. Scale bar 100 μm for A; 25 μm for B; 10 μm for C.

3.1 Interstitial Tissue

3.1.1 Fibrous Elements

In the present investigation, the amount of collagenous fibers in the interstitial spaces decreased gradually in all groups (Figures 2 & 3).

By seven weeks of age, increased amount of collagenous fibers was observed in the interstitial space of the control group compared with the experimental groups. From seven to nine weeks of age, no differences in the amount of collagenous fibers were noticed among the four groups.

The reticular network fibers in the interstitial space also decreased with the age. At five weeks of age, the reticular fiber network was slightly decreased in groups 3 and 4 (Figure 4) in comparing with group 2 and the control group. At six to nine

weeks of age, the control group showed an increase in the amount of the reticular fibers compared with the experimental groups (groups 2, 3, and 4), while no differences were observed among these groups (Figure 5).

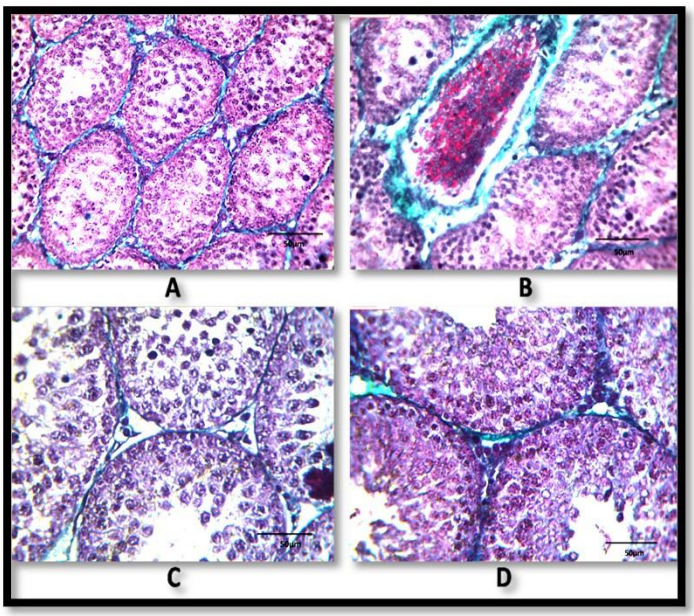

Figure 2: The rat testis of the control group and the experimental groups at five weeks of age showing increased amount of collagen fibers (green) in the control group (A) and group 2 (B) compared with group 3 (C) and group 4 (D). Masson's trichrome stain. Scale bar 50µm.

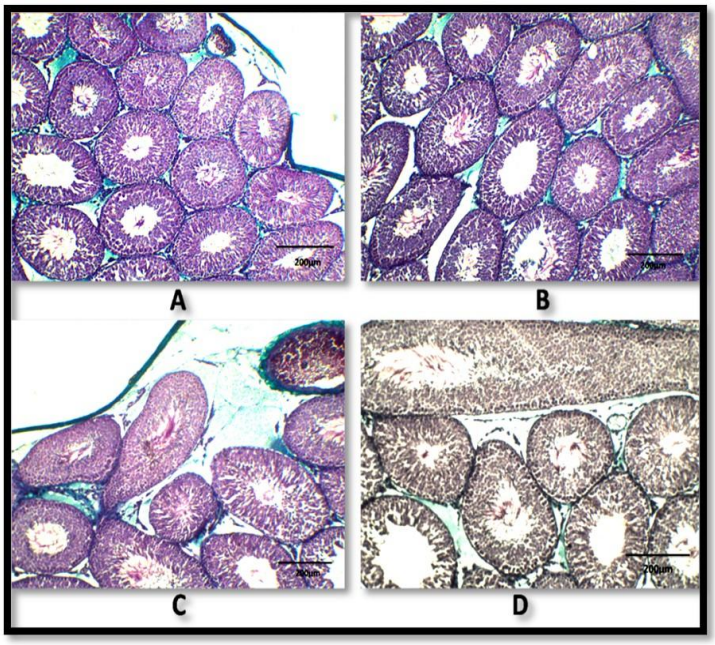

Figure 3: Collagenous fibers in the rat testis of the control group and the experimental groups at nine weeks of age. Note there are no differences in the amount of collagen fibers (green) among the four groups. A: the control group; B: group 2; C: group 3; D: group 4. Masson's trichrome stain. Scale bar 200µm.

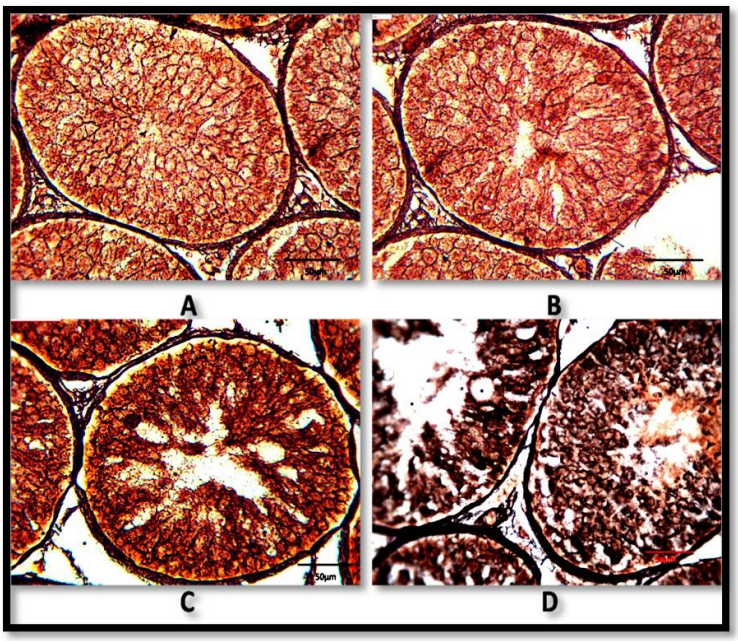

Figure 4: Reticular fibers in the rat testis of the control group and the experimental groups at five weeks of age. Note the extensive reticular fibers network (black color) in the seminiferious tubules of the control group (A) and group 2 (B), while group 3 (C) and group 4 (D) showing less amount of reticular fibers. Gordon and Sweet's stain. Scale bar 50µm.

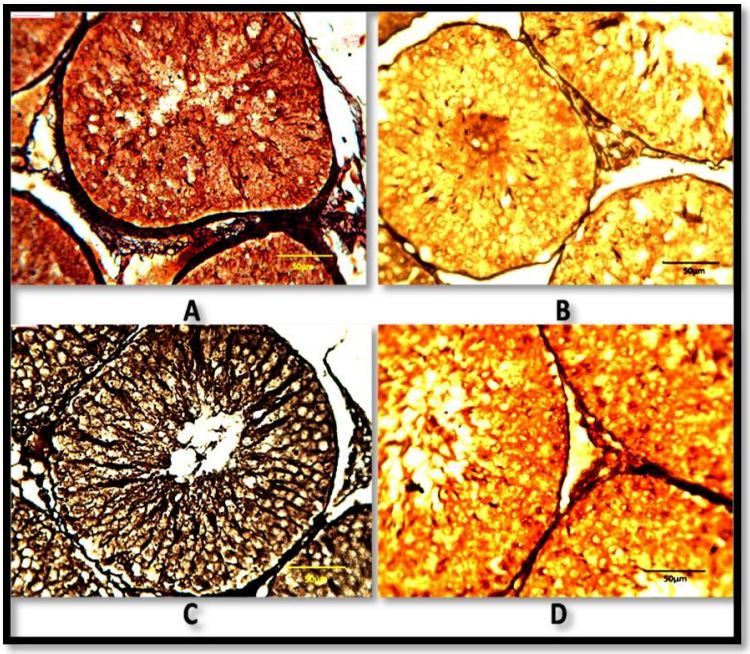

Figure 5: Reticular fibers in the rat testis of the control group and the experimental groups at nine weeks of age. Note the increased amount of reticular fibers in the interstitial spaces (black) of the control group (A) compared with group 2 (B), group 3 (C) and group 4 (D). Gordon and Sweet's stain. Scale bar 50µm.

3.1.2 Leydig Cells

Leydig cells were found in clusters or cords surrounded by small blood vessels. The number of Leydig cells increased steadily with the passage of time (Figures 6 - 8). At five weeks of age, the number of Leydig cells increased in groups 3 (5.6 ± 0.55 cells/ interstitial space) and 4 (5.6 ± 0.45 cells/ interstitial space) compared with group 2 (3.0 ± 0.6 cells/ interstitial space; $P < 0.01$) and the control group (2.6 ± 0.50 cells/ interstitial space; $P < 0.001$) (Figures6 - 8). Generally, there were no significant differences in the number of Leydig cells between the control group and group 2 ($P > 0.05$) and between group 3 and group 4 ($P > 0.05$) at the age of five to seven weeks.

At eight weeks of age, significant increases in the number of Leydig cells was recorded in group 2, group 3 ($P < 0.05$), and group 4 ($P < 0.01$) compared with the control group (Figures 7 & 8). At nine weeks of age, the highest number of Leydig cells was recorded in group 4 (16.8 ± 0.89 cells/

interstitial space), while the lowest number was noted in the control group (15.6± 0.43 cells/ interstitial space; P < 0.05). Surprisingly, the number of Leydig cells in group 1 (the control), group 2, and group 3 did not differ significantly (P > 0.05).

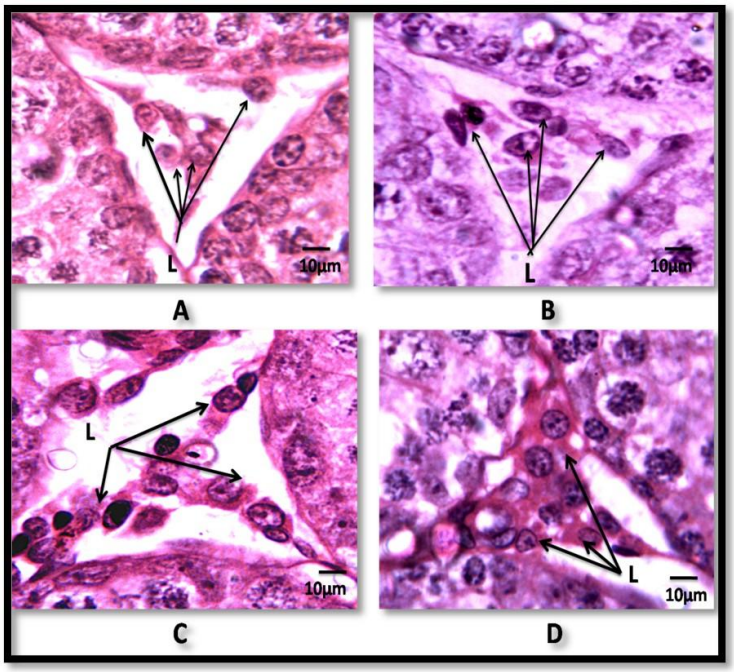

Figure 6: Leydig cells (L) in the rat testis of the control group and the experimental groups at five weeks of age. Group 1 (A) and group 2 (B) showing less number of Leydig cells than that observed in group 3 (C) and group 4 (D). H & E stain. Scale bar 10µm.

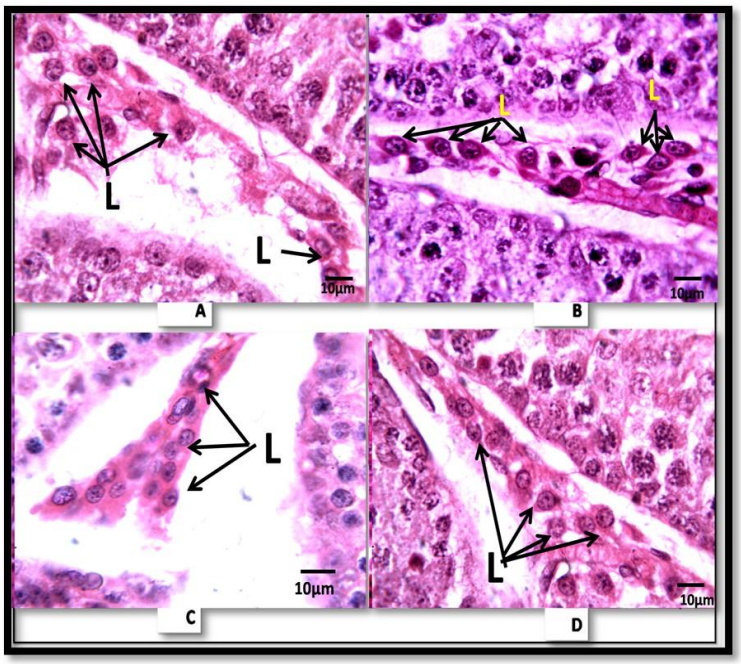

Figure 7: Leydig cells (L) in the rat testis of the control group and the experimental groups at nine weeks of age. Note decreased number of Leydig cells in group 1 (A); while group 2 (B), group 3 (C) and group 4 (D) showed increased number of Leydig cells (compare with the number of Leydig cells at five weeks of age in Fig. 6). H & E stain. Scale bar 10µm.

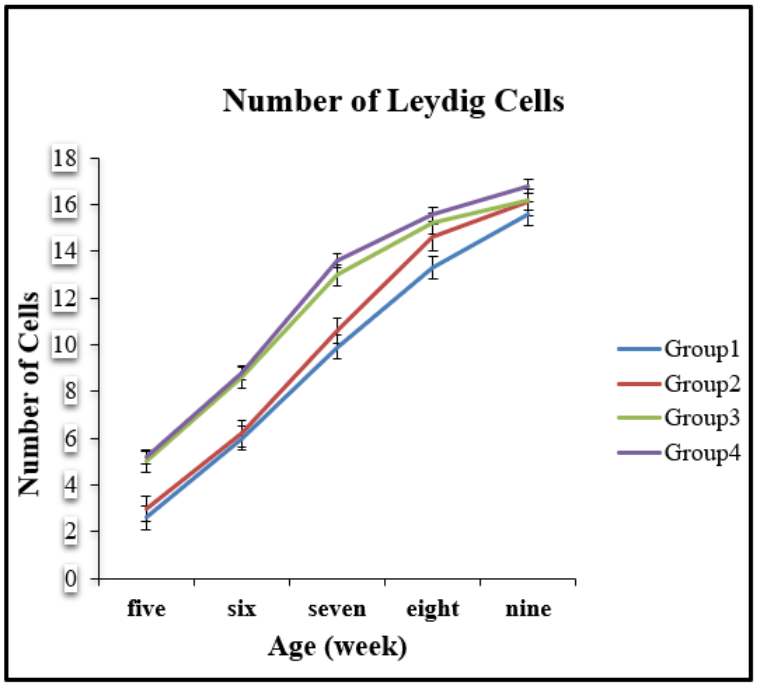

Figure 8: The Leydig cell numbers curves in the control group (group 1) and the three experimental groups; group 2 (500 mg/ Kg B.W.), group 3 (1000 mg/ Kg B.W.), and group 4 (1500 mg/ Kg B.W.). Data represent the mean ± SD.

3.2 Lamina Propria

3.2.1 Fibrous Elements

In the current study, the control group and the three experimental groups showed that the amount of collagenous fibers surrounding the basement membrane was decreased gradually (Figures 2 & 3). At five to six weeks of age the control group and group 2 exhibited increased amounts of collagenous fibers when compared with groups 3 and 4 (Figure 2); whereas at seven to nine weeks of age, no differences were observed among the four groups (Figure 3).

The network of reticular fibers varied in amount among the groups, depending on the treatments and age. Besides its presence in the area surrounding the seminiferious tubules, the reticular fibers were also found inside the seminiferious tubules (Figure 4). By six weeks of age, extensive reticular fibers network was observed in the seminiferious tubules of the control group and group 2, while groups 3 and 4 showed less amount of reticular fibers (Figure 4). Seven through nine weeks of age, there were no differences in the amount of reticular fibers network among the four groups (Figure 5).

3.2.2 Myoid Cells

The Myoid cells were arranged in a single layer around the seminiferous tubule. At five weeks of age, the Myoid cells were seen as dark, spindle-shaped cells in the control group and as flat- shaped cells in the experimental groups (Figure 9). At six to nine weeks of age; however, the Myoid cells in all groups became very dark, flat and filiform -shaped (Figure 10).

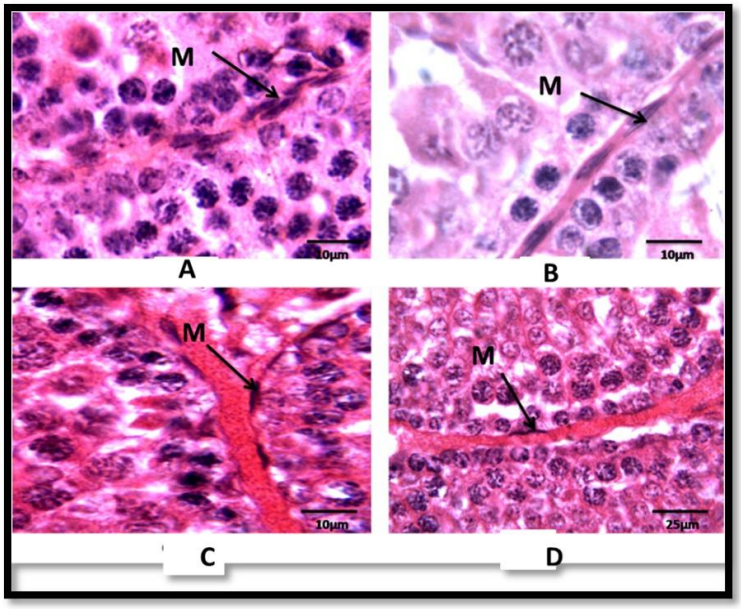

Figure 9: The Myoid cells (M) at five weeks of age. Note, spindle shaped myoid cell in the control group (A) and group 2 (B), while in group 3 (C) and group 4 (D) the myoid cells are flat. Scale bar 10µm.

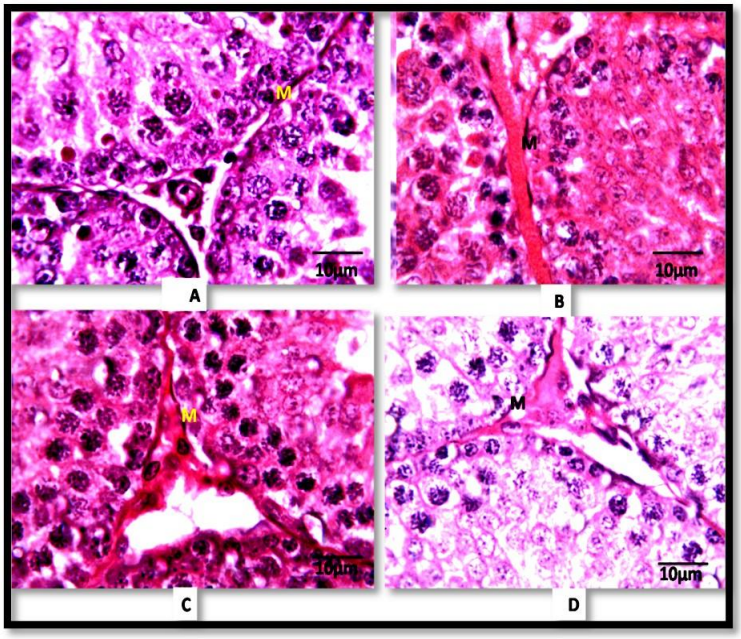

Figure 10: The Myoid cells (M) at nine weeks of age. Note, in the four groups the Myoid cells are flat. A: the control group; B: group 2; C: group 3; and D: group 4. H & E stain. Scale bar 10µm.

3.3 Lumen Formation

At five weeks of age, the control group showed a few seminiferous cords which were transformed into seminiferous tubules by acquiring lumina; however, the lumina were narrow and inconspicuous. In group 2, most of the seminiferous cords were transformed into seminiferous tubules. On the other hand, all the seminiferous tubules of groups 3 and 4 exhibited obvious central lumina (Figure 11).

At six weeks of age, most of the tubules did not acquire a define lumen in the control group, while all of the tubules in group 2 acquired central lumina. However, the central lumina were clearly seen in all seminiferous tubules of the control group at seven weeks of age.

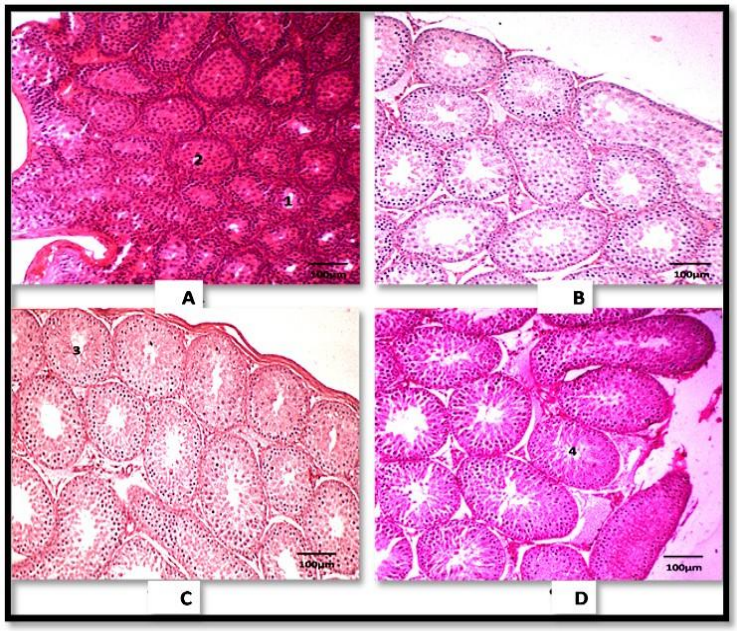

Figure 11: Luminal formation in the seminiferous tubules of the control and the experimental groups at five weeks of age. Group 1 (A) showing few seminiferous tubules having lumina (1), while there are many seminiferous tubules without lumina (2). In group 2 (B), most of the tubules have lumina. In group 3 (C) and group 4 (D) all the seminiferous tubules have lumina. H & E stain. Scale bar 100µm.

3.4 Germinal Cells

The seminiferous tubules were lined by stratified epithelium; most of the cells were sex (germinal) cells which were crowded between the Sertoli cells. The germinal cells were found in various stages of development.

Abundant spermatogonia cells were found resting upon the basement membrane of the seminiferous tubules in all groups; both type (A) sparmatogonia and type (B) sparmatogonia were seen (Figure 12). Beside the spermatogonia; primary spermatocyte, spherical spermatid and elongated spermatid were seen. They were arranged in many layers (Table 1). The number of layers varied among the groups, depending on the treatment and age.

At five weeks of age, the germinal cells were arranged in four layers in group 1 (control), four to five layers in group 2, and in six layers in groups 3 and 4 (Table 1). The control group showed a few primary spermatocytes, which were found in the middle of the seminiferous tubules. These cells were seen in different stages of cell division. At this age, secondary spermatocytes, spermatids and spermatozoa were not noticed in the control. On the contrary, secondary spermatocytes and spermatids appeared in the seminiferious tubules of the three experimental groups. In group 2, the seminiferious tubules in four out of six rats contained clusters of spherical spermatids, but neither elongated spermatids nor spermatozoa were seen. On the other hand, some seminiferous tubules in group 3 showed elongated spermatids and a number of spermatozoa, seen embedded in the cytoplasm of the Sertoli cells. In group 4, however, elongated spermatids and spermatozoa that embedded in Sertoli cells were noticed in all seminiferous tubules. In all groups, the lumina did not contain free spermatozoa (Figure 12).

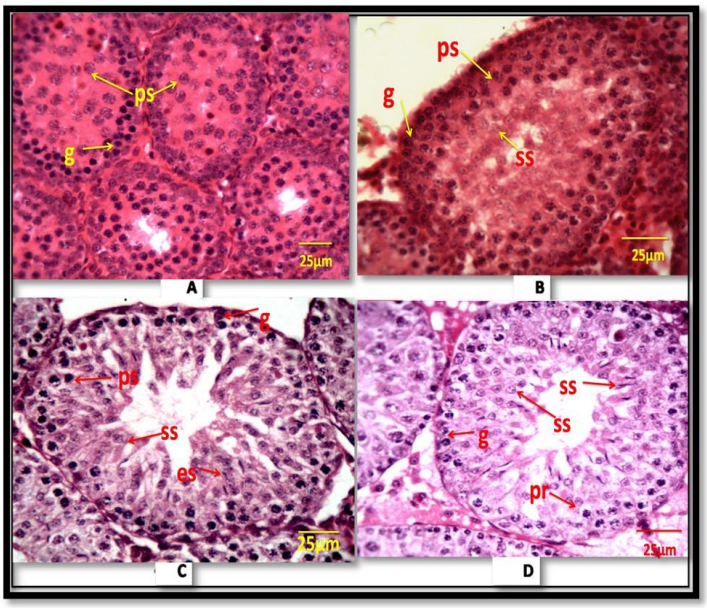

Figure 12: Germinal cells in the testis of the control and the experimental groups at five weeks of age. Note the primary spermatocytes (ps) in the middle of the seminiferous tubules of the control group (A), spherical spermatids (ss) were found in the seminiferous tubules of group 2 (B), group 3 (C) and group 4 (D); while elongated spermatids (es) were only observed in groups 3 and 4. g: spermatogonia. H & E stain. Scale bar 25 µm.

Table 1: Number of Germinal Epithelial layers in the control group (group 1) and the three experimental groups (group 2, 3, and 4).

Number of Germinal Epithelial layers					
Age(week)	**five**	**six**	**seven**	**eight**	**nine**
Group					
Group 1 (the control)	4	4-5	5	5-6	6
Group 2 (500 mg/ Kg B.W.)	4-5	5	5-6	6-7	7
Group 3 (1000 mg/ Kg B.W.)	6	6-7	7	7-8	8-9
Group 4 (1500 mg/ Kg B.W.)	6	6-7	7-8	8	8-9

At six weeks of age, the germinal cells in the controls were arranged in four to five layers (Table 1); only spermatogonia and primary spermatocytes were seen in the seminiferous tubules. In group 2 the germinal cells were arranged in five layers (Table 1). In this stage, elongated spermatids were noticed. Moreover, a few spermatozoa were seen in some tubules. These spermatozoa were embedded in the cytoplasm of the Sertoli cells. The germinal cells, in groups 3 and 4, were arranged in six to seven layers. Three out of six rats of group 3 showed free spermatozoa in the tubular lumina. In group 4, however, the free spermatozoa were visible in most of the lumina (Figure 13; Tables 1 & 2).

At seven weeks of age, the germinal cells were arranged in five layers in the control group (Table 1). The spherical and elongated spermatids and spermatozoa appeared in few seminiferous tubules. The spermatozoa were seen embedded in the Sertoli cells, but no free spermatozoa were noticed in the lumina. The germinal cells in group 2 were arranged in six layers (Table 1). In this group, most of the seminiferous tubules contained free spermatozoa (Table 2). In groups 3 and 4, the germinal cells were arranged in seven to eight layers (Table 1). The seminiferious tubules were crowded with the germinal cells and their lumina contained increased number of free spermatozoa when compared with their numbers at the age of six weeks (Figure 14).

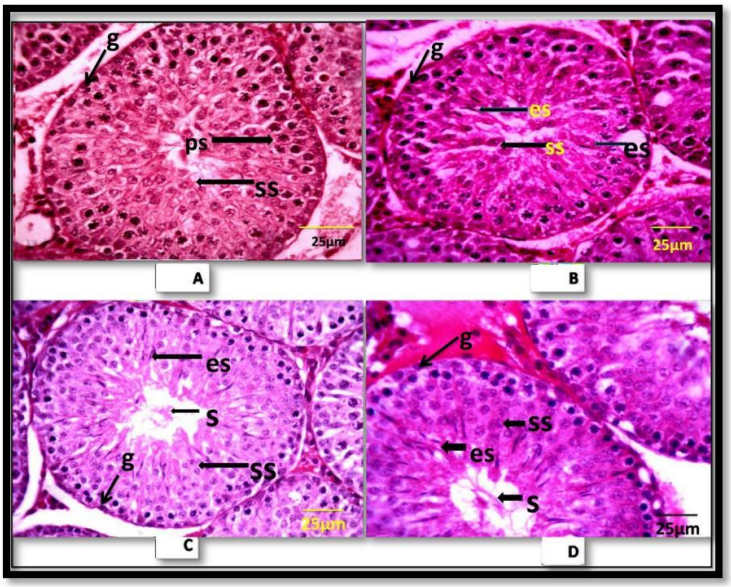

Figure 13: Germinal cells in the testis of the control and the experimental groups at six weeks of age. The seminiferous tubules of the control group (A) showing spermatogonia (g) and primary spermatocytes (ps) in different stages of cell division. Note elongated spermatids (es) in group 2 (B) and spermatozoa (s) in the lumina of group 3 (C) and group 4 (D). ss: spherical spermatids H & E stain. Scale bar 25 µm.

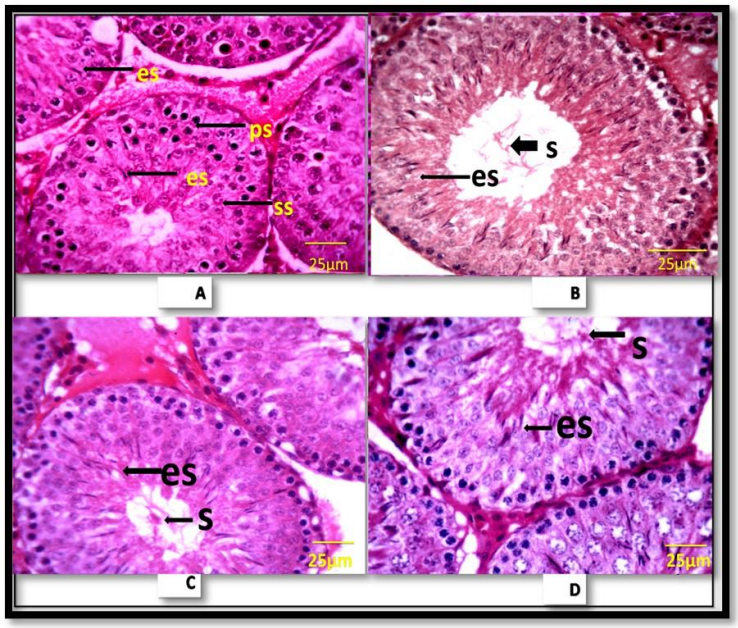

Figure 14: Germinal cells in the testis of the control and the experimental groups at seven weeks of age. Note the spherical spermatids (ss) and elongated spermatids (es) in the seminiferous tubules of the control group (A); free spermatozoa (s) in the tubular lumina of group 2 (B), group 3 (C) and group 4 (D). g: spermatogonia; ps: primary spermatocytes. H & E stain. Scale bar 25 µm.

Table 2: First appearance of the spermatozoa in the tubular lumina

First Appearance of Spermatozoa in the Lumina	
Group	**Age (week)**
Group 1 (the control)	8
Group 2 (500 mg/ Kg B.W)	7
Group 3 (1000 mg/ Kg B.W.)	6-7
Group 4 (1500 mg/ Kg B.W.)	6

At eight weeks of age, the germinal cells in the control group were arranged in five to six layers (Table 1). At this stage, free spermatozoa were seen into the lumina of most seminiferious tubules (Figure 15; Table 2). In the experimental groups, the germinal cells were arranged in six to seven layers in group 2, and seven to eight layers in groups 3 and 4 (Table 1). All tubular lumina contained free spermatozoa. An increase in the number of spermatozoa was recorded in groups 3 and 4. Group 4 showed an accumulation of spermatozoa (Figure 15).

At nine weeks of age, the germinal cells in the controls were arranged in six layers. All seminiferous tubules showed free spermatozoa in their lumina. On the other hand, the germinal cells were arranged in seven layers in group 2, eight layers in group 3 and eight to nine layers in group 4 (Table 1). At this age, the seminiferous tubules were packed with the germinal cells and spermatozoa (Figure 16). The great numbers of spermatozoa were found in group 4, followed by group 3 and group 2; respectively.

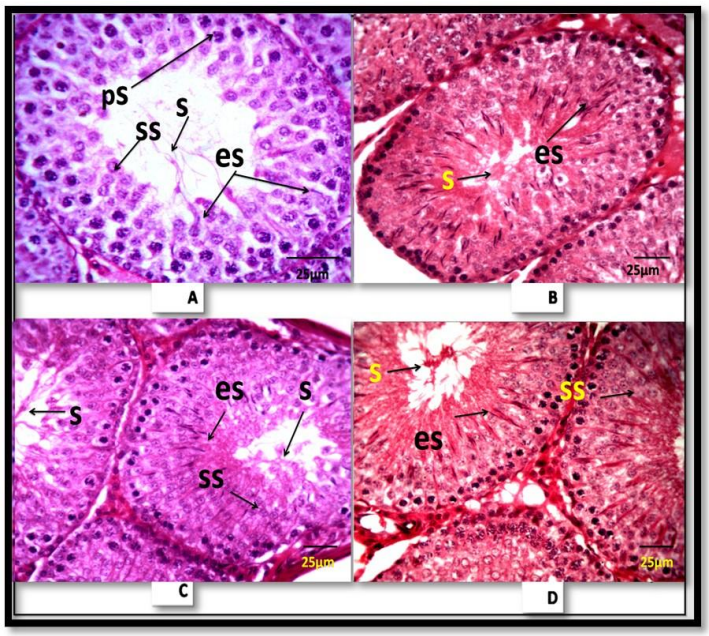

Figure 15: Germinal cells in the testis of the control and the experimental groups at eight weeks of age. The seminiferous tubules of the control group (A) showing few spermatozoa (s) in their lumina, whereas group 2 (B), group 3 (C) and group 4 (D) show increased number of spermatozoa (s). Note the accumulation of spermatozoa in the tubular lumen of group 4. ps: primary spermatocytes; ss: spherical spermatids; es: elongated spermatids. H & E stain. Scale bar 25 µm.

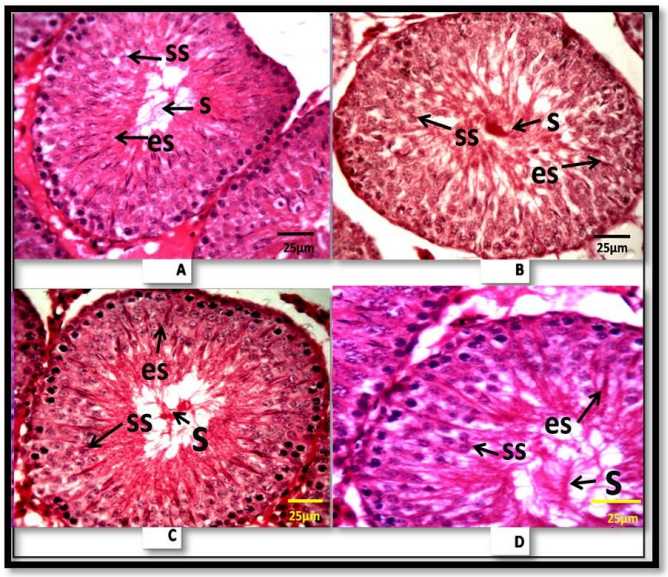

Figure 16: Germinal cells in the testis of the control and the experimental groups at nine weeks of age. The seminiferous tubules were packed with the germinal cells and spermatozoa (compare with Figure. 15). ss: spherical spermatids; es: elongated spermatids; s: spermatozoa. H & E stain. Scale bar 25 µm.

4 Discussion

This study focused on the dietary effects of *Moringa oleifera* leaves on the morphology of rat testis prepuberty. The histological features of the rat testis at different stages of development were observed and related to the concentration of Moringa.

The testis showed normal morphological features in all rats fed on a diet supplemented with *Moringa oleifera*, regardless of the concentration. This observation indicates that using *Moringa oleifera* leaves as a nutritional supplement, even in high concentrations (1000 &1500mg/ Kg B.W), has no side effects on the testis morphology. This result is in agreement with the finding of Adedapo et al. (2009) who reported that dietary supplementation with *Moringa oleifera* is safe in rats.

The current study revealed that the amount of reticular and collagen fibers in the interstitial spaces and lamina propria was variable depending on the concentration of *Moringa oleifera* and age. The amount of fibrous tissues was decreased gradually with the increase in Moringa concentration. A remarkable reduction in the fibrous tissues was noticed in group 4, particularly at nine weeks of age. However, the interstitial cells (Leydig cells) were found in an increased number in groups 2, 3, and 4 compared with the control (group 1). Furthermore, the number of Leydig cells was greatly affected by Moringa concentration and age. Large numbers of cells were recorded in groups 3 and 4 at nine weeks of age, suggesting that Moringa could induce Leydig cells proliferation. This finding concurs with the results of previous studies in mice (Cajuday and Pocsidio, 2010; Zade et al., 2013).

This study has shown that the myoid cells surrounding the seminiferous tubules underwent gradual flattening in all groups. This finding is in agreement with Leeson and Leeson (1963), who reported that the flattening of myoid cells has a positive correlation with age. Moreover, the myoid cells appeared more flattened in groups 2, 3, and 4 than those seen in group 1, suggesting the role of Moringa on flattening of myoid cells. Moringa may affect the myoid cell directly or indirectly through its effect on the seminiferous tubule diameter. This suggestion in accord with Palombi et al. (1992) who interpreted the increase in flattening of myoid cells as a result of increased tubular diameter. In addition, reduction in the fibrous tissues in rats fed on *Moringa oleifera* leaves, indicated earlier, may also be due to increased tubular diameter.

At five weeks of age, groups 3 and 4 showed conspicuous lumina in all seminiferous tubules, while group 2 exhibited some seminiferous tubules without lumina. On the other hand, the lumina in the control group appeared just beyond six weeks. These observations indicate that high concentration of *Moringa oleifera* may have induced lumen formation. The current result supports the finding of Cajuday and Pocsidio (2010), who observed early lumen formation in mice treated with *Moringa oleifera* leaf powder.

Germinal cells were arranged in layers; the number of layers varied, depending on the age and Moringa concentration. At each age, groups 2, 3,

and 4 showed increased number of germinal cells compared to the control group. Furthermore, the current investigation has shown that spermatogonia diameter and number of primary spermatocytes and spermatids were greatly affected by Moringa concentration. Effect of Moringa on secondary spermatocytes was not detected because they were rarely seen; this could logically be due to their short life (Keele et al., 2006). Generally, most crowded tubules with the germinal cells were seen in group 4, indicating that high concentration of *Moringa oleifera* induces the spermatogenesis. This result is in line with the finding of Zade et al. (2013), who reported that *Moringa oleifera* seeds increase the numbers of mature Leydig cells, spermatocytes and spermatids in Wister albino rats, suggesting that different parts of Moringa plant could be effective in improving male fertility.

At five weeks of age, spherical and elongated spermatids and spermatozoa embedded in Sertoli cells were seen in all testis samples taken from rats fed on diet supplemented with high concentration of Moringa; i.e. group 3 (1000 mg/Kg B.W) and group 4 (1500mg/ Kg B.W). On the other hand, elongated spermatid and spermatozoa embedded in Sertoli cells appeared, for the first time, at six and seven weeks of age in group 2 and the control group; respectively. At six weeks of age and thereafter, the spermatozoa were clearly seen into the tubular lumina of 50% of group 3 and 100% of group 4. However, group 2 and the rest of group 3 exhibited free spermatozoa into the lumina at seven weeks of age. On the contrary, the control group did not show free spermatozoa in the lumina before eight weeks of age. These observations indicate that using high concentration of *Moringa oleifera* leaves as a nutritional supplement induces spermatogenesis and accelerates appearance of elongated spermatids and release of spermatozoa into the tubular lumen. Presence of sperm in the seminiferous tubules is considered as a sign of puberty (Sukkar and Nasrat, 2006). Taken together, puberty occurred earlier in groups 3 and 4 (i.e. at 6 weeks of age) than in group 2 and the control group (i.e. at 7 and 8 weeks of age; respectively). At eight and nine weeks of age, the tubular lumina of groups 3 and 4 were very crowded with the spermatozoa, suggesting that *Moringa oleifera* leaves increase the sperm production and concentration, which indicates high fertility (Oyeyemi et al., 2008; Mohlala et al., 2023). Interestingly, although group 4 and 50 % of group 3 rats reached puberty at the same time, group 4 rats showed increased number of spermatozoa in the tubular lumina compared with those of group 3. In general, this phenomenon was observed from 6 weeks of age (puberty time) onwards, revealing that the most effective concentration was 1500 mg/ Kg body weight of *Moringa oleifera* leaves powder.

overall, the present study reveals that using *Moringa oleifera* leaves as a nutritional supplement increases Leydig cells number and induces lumen formation, spermatogenesis and spermiogenesis in rats; resulting in speeding up puberty and improving fertility.

5 Conclusions

Based on the findings of this study, it can be concluded that dietary supplementation of *Moringa oleifera* leaves powder induces postnatal testicular development and enhances sperm production in rats, leading to early puberty and high fertility. Future research should focus on the effects of Moringa on the excurrent ducts and male accessory glands in a rat model. In addition, further research is needed to identify the effects of Moringa on the fertility of domestic animals as well as humans.

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