Alexandria Journal of Veterinary Sciences
www.alexjvs.com

AJVS. Vol. 59 (1): 134- 142. Oct. 2018
DOI: 10.5455/ajvs.4340

Does camel milk have a positive impact on rat reproductive functions?

Shereen B. Gad, Abdel-Haseeb H. Fayed, Mona H. Hafez, Aya H. Abdel-Hafeiz*
Department of Physiology, Faculty of Veterinary Medicine, Alexandria University, Egypt

Key words:
Camel milk, Ovary, Testes, Oxidative stress.

***Correspondence to:**
ayavet172@gmail.com

ABSTRACT

Camel milk considered one of the most valuable food sources for nomadic people and has been consumed for centuries due to its nutritional values and medicinal properties. Camel milk has unique composition that differs from other ruminants' milk. It contains high minerals; vitamins and insulin contents but have low protein; cholesterol and sugar. This may give it its medicinal properties. Two experiments were done toinvestigate1-if camel milk has a positive impact on some aspects of reproductive functions and fertility in adult male rats, and 2- its effect on puberty in one month old male and female rats. In the first experiment twenty adult 6 months old male rats were used and were allocated into 2 equal groups (10 rats each). The control adult male rats were received (1 ml saline / kg BW) and treated adult male rats were received camel milk (1ml /kg BW) for 2 months by gastric gavage. Males from both groups were allowed to undergo monogamous pair mating. In the second experiment we studied the effect of camel milk on the puberty of one month old male and female rats. Control group [n=10(5male+5female)] were received (1ml /rat) and treated group [n=10 (5male+5female] were received camel milk (1ml saline /rat) for 1 month by gastric gavage. Results showed that camel milk administration caused a significant ($p \leq 0.05$) increase in antioxidant markers (GSH and SOD), sperm motility%, alive sperm%, sperm concentration, serum estrogen, progesterone levels, serum and testicular testosterone level and improves the conception rate, while, it caused a significant ($p \leq 0.05$) decrease of the sperm abnormalities%. Histologically, camel milk treated mature rats showed a significant ($p \leq 0.05$) increase in the diameter and the lumen space of seminiferous tubules with complete spermatogenic layers in mature and immature male rats. Treated immature female rats revealed significant ($p \leq 0.05$) increase of tertiary growing follicles and the number of uterine glands. In conclusion camel milk administration caused a positive impact on male rats reproductive functions and fertility and enhanced the puberty of both immature male and female rats.

1. INTRODUCTION

Most population may not have been too familiar with camel milk. In Sahara, butter of fresh camel milk is widely used as a base for medicines. Cosmetics or pharmaceuticals can be developed from camel milk. Camel milk can treat a series of metabolic and autoimmune diseases. Chronic pulmonary tuberculosis can be treated with raw camel milk (Agarwal et al., 2008). Camel milk contains high minerals; vitamins and insulin contents but have low protein; cholesterol and sugar (Alavi et al, 2017). This may give it its medicinal properties. Additionally, camel milk has antioxidant properties such as vitamins in high concentrations such as vitamin B2, C, E and A and many trace elements e.g., magnesium and zinc (Zakaria et al., 2016and 2018) and manganese (Kamal, 2012). Camel milk alleviates oxidative stress status and free radicals production in aluminum chloride (Al-Hashem, 2009), cadmium (Al-Hashem et al, 2009) and Lead toxicity in rats (Zakaria et al., 2018). Lee et al. (2007) reported that Mn^{2+} can stimulate specific puberty-related hormones and suggested that it may facilitate the normal onset of puberty; also, demonstrated that Mn^{2+} is a direct stimulator of prepubertalGnRH/LH secretion and may facilitate the normal onset of male and female puberty.

Magnesium in camel milk helps in the absorption and metabolism of different vitamins e.g., C, B and E and acts as antioxidant (Zakaria et al., 2016).

Magnesium deficiency impairs reproductive functions without any concomitant generation of oxidative stress (Tremellen, 2008) though its importance in maintaining balance between oxidants and antioxidants is well recognized (Keenoy et al., 2000). Zinc is an essential trace element for the activity of large number of enzymes and for the living organisms and found in large quantity in camel milk (Ozdemir and Inanc, 2005). Zn can block cellular deterioration by antioxidant system activation (Ozdemir and Inanc, 2005 and Zakaria et al., 2016). Zn plays a significant role in testis development and sperm physiological functions (Colagar et al., 2009 and Zakaria et al., 2016).

The present study was conducted to determine the effects of camel milk on some aspects of reproductive functions and fertility in adult male rats and puberty in one month old male and female rats.

2. MATERIAL AND METHODS and

2.1.Milk samples: Camel milk samples were collected in the morning from camels farm in Matrouh Governorate, Milk was collected by hand milking from a healthy she camel. The samples were collected from January till March 2017 aseptically in sterile screw capped plastic bottles, placed in a cool box containing ice packs and immediately transported to the laboratory and kept frozen at -20 ºC till analysis for its major constituents with averages (3.72% Fat, 3.54 % Protein, 5.11% Lactose , 9.41% solid non fat (SNF) and 0.12 % Minerals) by lactostar, Funke Cerbber Milk Scan 2008 (Labortechnik, 2105, Germany, Berlin).

2.2. Experimental animals and protocol: In the first experiment twenty mature male albino rats aged 6 months and with an average weight 200 ± 20 g. The rats were purchased from the Faculty of Agriculture, Alexandria University, Egypt. This experiment was conducted at the Department of Physiology, Faculty of Veterinary Medicine, Alexandria University. Rats were provided with food pellets (23% crude protein, 5%crude fat, 3.35% crude fiber and energy not less than 3000 Kcal/ Kg) (Elfagr company, Alexandria-Cairo Desert Road, Egypt) and water ad-libitum. The rats were housed in 40 x 30 x 20 cm wire cages (5 rats / cage) and acclimatized to our laboratory conditions for 2 weeks before the experimental procedures. The male rats were divided randomly into 2 groups (10 rats each). (1) control group: adult male rats were received (1 ml saline / kg BW) and (2) treated group: adult male rats were received camel milk (1ml /kg BW) as referred by(AL-Hashem, 2009); treatment was conducted for 2 months by gastric gavage. At the end of the 2 months male rats from control and treated groups were allowed to undergo monogamous pair mating. Individual males from each group mated with one previously proven fertile female. Pregnancy was confirmed through vaginal smear test hat revealed presence of estrus cornified cell and rat sperms (first day of pregnancy) and pregnant females were separated and placed in individual breeding cages 7 days before expected day of parturition and the number of pregnant females was estimated in relation to the total number of the females used in the experiment to calculate the conception rate. After delivery of each pregnant female, pups were collected and counted to calculate the litter size. In the second experiment we studied the effect of camel milk on the puberty of one month old male and female rats. (1) Control group (n=10) (5 males + 5 females) were received (1ml saline /Kg B W) and (2) treated group (n=10) (5 males + 5 females) were received camel milk (1ml /Kg B W); treatment was conducted for 1 month by gastric gavage.

2.3. Blood sampling: After twenty four hours from the last treatment in immature rats and after twenty four hours from the end of the fertility trial in mature male rats, all rats in the two experiments(males and females)were anesthetized by ether and blood was collected from the retro-orbital vein of the eye by using heparinized micro hematocrit tubes. After that all rats were sacrificed by decapitation then a midline abdominal incision was made. The testes and ovaries were dissected free, blotted, examined with the naked eye then examined histologically. Also, the epididymi were collected for sperm characteristics estimation. Blood samples were collected in clean test tubes, left for 2 hours at room temperature, refrigerated overnight at 4ºC and then centrifuged at 3000 r.p.m for 15 min to separate serum. Clear serum samples were stored at -20ºC until analyzed for testosterone, estrogen, progesteroneand some antioxidant markers.

2.4. Epididymal sperm analysis: Sperm motility, alive sperm and sperm abnormalities percents and sperm concentration were estimated according to the method described by Bearden and Fuquay (1980).

2.5.Oxidative stress and antioxidant markers: Malondialdehyde (MDA), **Reduced glutathione (GSH)** and **Superoxide dismutase (SOD)** levels in both testicular homogenate and serum were determined colorimetrically by using assay kits obtained from Bio-Diagnostic Company, Egypt according to the methods described by Ohkawa et al. (1979), Beutler et al. (1963) and Nishikimi et al. (1972) respectively.

2.5.1. Sex steroid hormonal assay: Serum estrogen, progesterone and testosterone and testicular testosteronewere determined by indirect enzyme immunoassay assay Kit (Monobind, 100 North point Drive, Lake Forest, CA 92630 USA) according to the method described by Al-Otaibi et al., (2015),Pedersen, et al., (2003) and Tietz (1995) respectively. The sensitivities of the kits, were 25 pg/ml., 0.938 ng/mL and 0.05 ng/ml respectively.

2.6. Histological examination: The reproductive organs (testes of adult and immature male rats and ovaries and uterine horns of immature female rats) were collected from all male and female rat groups, grossly examinedand rapidly fixed in 10% neutral formalin for 24 hrs. After fixation, tissue specimens were processed through the conventional paraffin embedding technique. 5 µm thick sections were obtained from paraffin blocks, stained with hematoxylin and eosin (Bancroft et al., 2013) and examined under light microscope.

2.7. Statistical analysis: Values are represented as means ±standard errors. The variables were tested using independent t-test and fisher's exact test. The level of significance was set at P≤ 0.05. Parametric analyses were carried out using the Statistical Analysis System software (SAS, 2011).

3. RESULTS:

3.1. Epididymal sperm analysis:

As illustrated in table (1) the % of sperm motility, the % of alive sperm and sperm concentration increased significantly (P≤0.01) in camel milk treated group compared to control group (95.0 ± 5.00vs 55.0 ± 9.35), (90.8 ± 1.24vs 66.0 ± 3.45) and (105 ± 13.4vs 46.8 ± 3.98), respectively. While the % of sperm abnormalities decreased significantly (P≤0.01) in camel milk treated group compared to control group (5.40 ± 0.75vs13.0 ± 1.14).

3.2. Oxidative stress and antioxidant markers:

As revealed in table (2) testicular and serum antioxidant markers (GSH) and SOD increased significantly (P≤0.05) in camel milk treated group compared to control group (758 ± 44.6 vs 645 ± 13.4); (925 ± 40.5 vs 723 ± 11.0), respectively. Camel milk treated group showed a significant (p≤ 0.05) decrease in the serum oxidative stress marker (MDA) compared to control group (22.8 ± 2.29vs 41.1 ± 1.22) but testicular MDA had a non significant (P≤ 0.05) variations between groups.

3.3. Sex steroid hormonal assay:

As recorded in table (3) both serum and testicular testosterone levels increased significantly (P≤0.05) in camel milk treated mature and immature groups compared to the control groups (2.78 ± 0.32 vs 1.37 ± 0.21, 2.69 ± 0.50 vs 0.35 ± 0.02, 0.95 ± 0.06 vs 0.65 ± 0.06 and 0.95 ± 0.06 vs 0.65 ± 0.06 respectively) . Moreover, serum estrogen and progestrone levels increased significantly (P≤0.01) in camel milk treated female immature rats compared to control group (89.40 ± 6.00vs48.00 ± 4.77 and 4.02 ± 0.68 vs 1.42 ± 0.20).

3.4. Conception rate and litter size:

As illustrated in table (4) the conception rate of camel milk treated group increased significantly (P≤0.05) compared to control group (60% (6/10) and 20% (2/10), respectively), but the litter size had a non significant (P≤0.05)variation between groups.

3.5. Histological examination:

As recorded in table (5) the number of spermatogenic layers, diameter of seminiferous tubules and thickness of spermatogenic layers showed a significant (p≤0.05) increase in the testes of camel milk treated mature and immature groups compared to control groups (7.80±0.37 vs 5.60±0.40, 380±20.1 vs 302±14.6 and 245±9.19 vs 165±5.11) and (3.60±0.24, 214±5.22 vs 122±4.04 and 2.20±0.37, 173±3.96 vs 69.8±8.15) respectively.

The testes of control mature rats showed normal seminiferous tubules which lined with spermatogenic cells arranged from the basement membrane as the following; primary, secondary and tertiary spermatogonia, spermatid till free sperms within the lumen (Fig. 1). The testes of rats treated with camel milk showed normal seminiferous tubules with normal spermatogenic cell lining. Interestingly, the diameter of seminiferous tubules was increased. Moreover, the lumen was increased and numerous free sperms were seen within it (Fig. 2).

Regarding the immature control rats, the testes revealed small size seminiferous tubules which lined with non-cohesive spermatogenic cells with large nucleus and the lumen was completely unclear (Fig. 3). While, the testes of immature rats treated with camel milk showed complete spermatogenic layers with marked increase in both the lumen space and free sperms within it (Fig.4).

The ovaries of immature control female rats showed predominance of primordial, primary and secondary follicles and the uterus showed normal endometrial mucosa with few uterine glands (Fig. 5and7respectively). While, the immature female rats treated with camel milk revealed an interesting increase of tertiary growing follicles in their ovary and uterine glands within the endometrium in comparison with non treated control animals (Fig.6and 8respectively).

Table (1): Effect of administration of camel milk for 2 months on epididymal sperms parameters of mature male rats.

	Groups	
Parameters	Control(1ml saline /kg BW)	Camel milk(1ml /kg BW)
Sperm motility %	55.0 ± 9.35	95.0 ± 5.00[a]
Alive sperm %	66.0 ± 3.45	90.8 ± 1.24[a]
Sperm concentration 10^6/ml	46.8 ± 3.98	105 ± 13.4[a]
Sperm abnormalities %	13.0 ± 1.14[a]	5.40 ± 0.75

Values are means ± standard errors. Means without a common superscript in the same row differ significantly ($P \leq 0.01$). N = 10.

Table (2): Effect of administration of camel milk for 2 months on Testicular and serum Glutathione (GSH), Malondialdehyde (MDA), Superoxide dismutase (SOD) in mature male rats.

	Groups	
Parameters	Mature control (1ml saline /kg BW)	Mature treated with camel milk(1ml /kg BW)
Testes		
MDA, nmol/ g tissue	61.7 ± 2.50*	53.3 ± 3.55*
GSH, mmol/g tissue	645 ± 13.4	758 ± 44.6*
SOD, U/g	266 ± 2.14	300 ± 2.17**
Serum		
MDA, nmol/ml	41.1 ± 1.22*	22.8 ± 2.29
GSH, mmol/ml	723 ± 11.0	925 ± 40.5*
SOD, U/ml	132 ± 4.17	186 ± 3.17*

Values are means ± standard errors. Means having superscript * in the same row significantly different at ($P \leq 0.05$) while that of ** are significantly different at $P \leq 0.01$).
MDA: Malondialdehyde. GSH: Glutathione reduced. SOD: Superoxide Dismutase. N = 10.

Table (3): Effect of camel milk on serum estrogen, progesterone and testosterone and testicular testosterone in rats:

Parameters	**Groups**			
	Mature control(1ml saline /kg BW/ 2 months) [a]	Mature treated with camel milk(1ml /kg BW/ 2 months) [a]	Immature control (1ml saline /rat/ 1 month)[b]	Immature treated with camel milk(1ml /rat/ 1 month)[b]
Serum testosterone, ng/ml	1.37 ± 0.21	2.78 ± 0.32*	0.65 ± 0.06	0.95 ± 0.06**
Testicular testosterone, ng/g	0.35 ± 0.02	2.69 ± 0.50**	0.23 ± 0.03	0.72 ± 0.07**
Serum estrogen, pg/ml	-	-	48.00 ± 4.77	89.40 ± 6.00**
Serum progesterone, ng/ml	-	-	1.42 ± 0.20	4.02 ± 0.68*

Values are means ± standard errors. Means with superscript* in the same row differ significantly at ($P \leq 0.05$), while that of ** are significantly different at ($P \leq 0.01$). $N^a = 10$. $N^b = 5$.

Table (4): Effect of administration of camel milk for 2 months in male rats on conception rate and litter size.

Parameters	**Groups**	
	Mature control (1ml saline /kg BW)	Mature treated with camel milk(1ml /kg BW)
Conception rate, %*	20 (2/10) [a]	60 (6/10)
Litter size, no.**	6.50 ± 0.50	6.17 ± 0.48

* Fisher's exact test for conception rate, P = 0.17. ** Values are means ± standard errors. Means without a common superscript in a row differ significantly ($P \leq 0.05$). N = 10.

Table (5): Histological examination of seminiferous tubules of mature and immature male rats**:**

Parameter	Groups			
	Mature control(1ml saline / Kg B W/ 2 months) [a]	Mature treated with camel milk(1ml /Kg B W/ 2 months) [a]	Immature control(1ml saline /rat/ 1month)[b]	Immature treated with camel's milk(1ml / rat/ 1month)[b]
Number of spermatogenic layers	5.60±0.40	7.80±0.37*	2.20±0.37	3.60±0.24*
Diameter of seminiferous tubules (µm)	302±14.6	380±20.1*	173±3.96	214±5.22*
Thickness of spermatogenic layers (µm)	165±5.11	245±9.19*	69.8±8.15	122±4.04*

Values are means ± standard errors. Means with superscript* in the same row differ significantly at ($P \leq 0.05$). $N^a = 10$. $N^b = 5$.

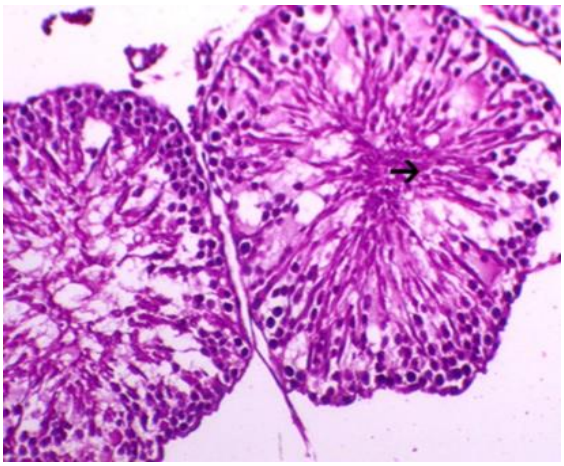

Fig. (1): Testis of control mature rat showing normal seminiferous tubules lined with normal spermatogenic cells layer and demonstrating presence of free sperms within their lumens (arrow), H&E, X 200.

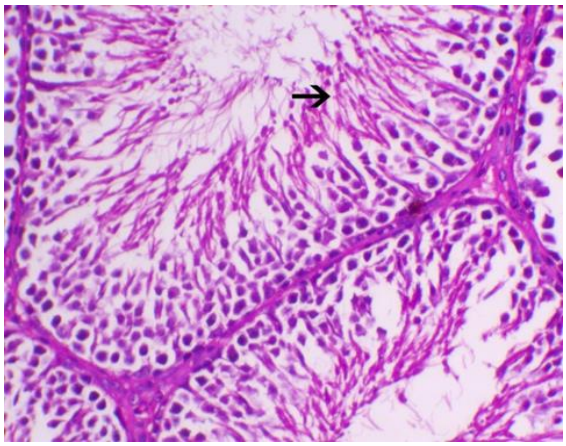

Fig. (2): Testis of mature rat treated with camel milk showing normal seminiferous tubules with normal spermatogenic cells layer with marked increase the lumen diameter (arrow indicates free sperms), H&E,X200.

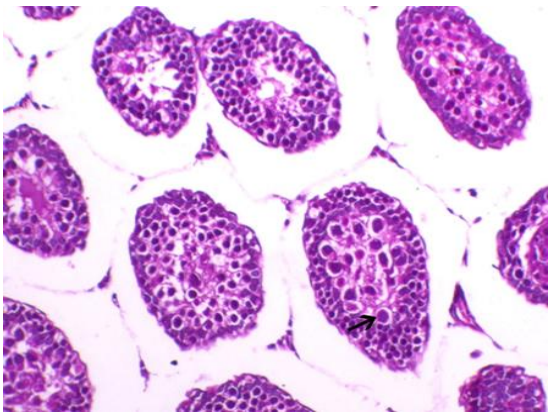

Fig. (3): Testis of control immature male rats showing marked number of seminiferous tubules within the inactive stage (arrow), H&E,X200.

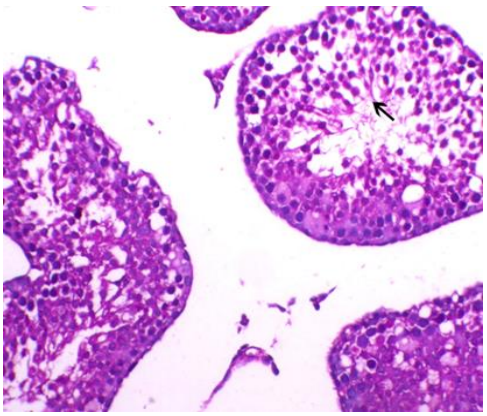

Fig. (4): Testis of immature male rats treated with camel milk showing increase lumen area with few noticeable spermatids (arrow), H&E,X200

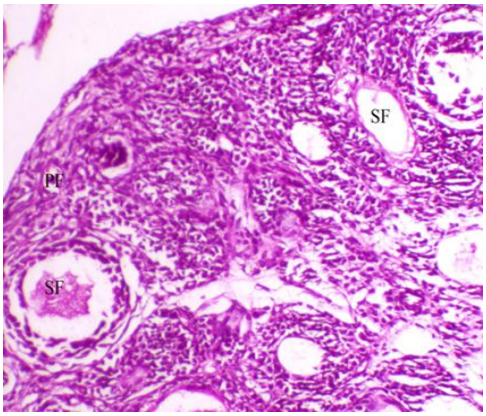

Fig. (5): Ovary of control immature female rats showing presence of primordial follicles and (PF) secondary follicles (SF) H&E,X200.

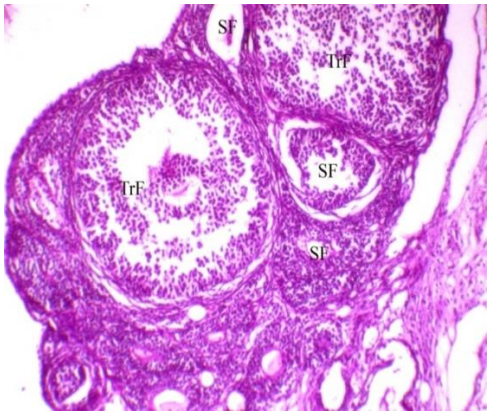

Fig. (6): Ovary of immature female rats treated with camel milk showing presence of primordial follicles (PF), secondary follicles (SF) & Tertiary growing follicles (TF), H&E,X200.

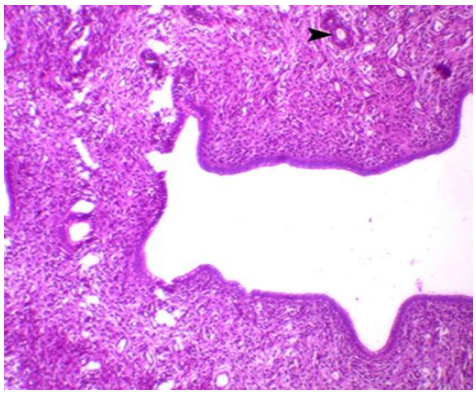

Fig. (7): Uterus of control immature female rats showing presence of few number of uterine glands (arrowhead), H&E,X200.

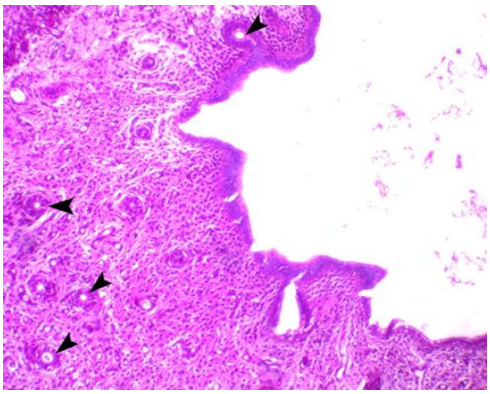

Fig. (8): Uterus of immature female rats treated with camel milk showing numerousnumber of uterine glands (arrowhead), H&E,X200.

4. DISCUSSION:

In the present study, treatment of the mature male rats with camel milk resulted in a significant improvement of the epididymal sperm quality and improvement of seminiferous tubules parameters revealed by a significant increase the number of spermatogenic layers, diameter of seminiferous tubules and thickness of spermatogenic layers in immature male rats this could be due to increase in testosterone levels, (Zakaria et al., 2016 and 2018). Spermatogenesis and maintenance of the histology and the function of the accessory sex glands in the male need testosterone (Odabas and Kanter, 2008). Treatment of male rats with camel milk caused a significant increase in testicular and serum levels of testosterone and this finding could be based on the large quantity of Zn found in camel milk (Zakariaet al., 2016); and this finding agree with Shahraki et al. (2015) who reported that zinc is necessary to maintain normal serum testosterone. Inadequate zinc levels prevent the pituitary gland from releasing luteinizing and follicle stimulating hormones, which stimulate testosterone production. The nuclear receptors for steroids are all zinc finger proteins. Low amounts of zinc in the diet lead to decrease semen volumes and serum testosterone concentrations. Spermatogenesis and the development of the primary and secondary sex organs in the male can be adversely affected by Zn deficiency (Egwurugwu et al., 2013). Zinc is also involved in synthesis of hormones that are important for reproduction. Their deficiency affects both steroid (Boland, 2003) and thyroid (Abdollahi et al., 2013) hormone production. Zinc is involved in steroidogenic enzymes cytochrome P_{450}, 17α-hydroxylase and cytochrome P_{450} side-chain cleavage (Ceylan et al., 2008). Zinc is also involved in the secretion and

function of male hormone testosterone through the enzymes that control the arachidonic acid cascade (Boland, 2003). Thus, zinc plays an essential role in sexual development and spermatogenesis (Ceylan et al., 2008). Moreover, zinc provides sperm membrane integrity, increases sperm motility and regulates the spiral movements of the sperm tail (Hernández-Meléndez et al., 2015). Zinc ions are of great significance for the maturation process of rat sperm cells during their passage through the epididymis. It was reported that high zinc concentration is associated with enhanced sperm cell motility. Intracellular mitochondrial zinc ions play a crucial role for sperm cell motility, while loosely bound or free zinc ions in the seminal plasma exert a secondary role on human sperm cell motility (Egwurugwu et al., 2013). Furthermore, the present findings could be attributed to the presence of magnesium in camel milk that helps in the absorption and metabolism of different vitamins e.g., C, B and E and acts as antioxidant (Zakaria et al., 2016). Depletion of intracellular Mg^{2+} is known to affect all functions dependent on this ion, including glycolysis, protein synthesis, respiration, and reproduction (Wong et al., 2001). It is reported that the number of spermatogoniaA, preleptotene spermatocytes, mid pachytene, spermatocytes and step 7 spermatid increased in Mg^{2+} treated rats. There have been reports that Mg^{2+} deficiency induced morphological changes up to 40% of the spermatids (Chandra et al., 2013). Additionally, these finding could be due to the high quantity of Mn^{2+} in camel milk (Kamal, 2012) and these data agree with the results of Lee et al., (2006) who demonstrated that Mn^{2+} is a direct stimulator of prepubertal GnRH/LH secretion and may facilitate the normal onset of male puberty.

Treatment of the adult male rats with camel milk caused improvement of the oxidative stress status of rats evident by a significant decrease in serum MDA and a significant increase in the levels of testicular, and serum activity of GSH and SOD this could be attributed to the antioxidant nutrients constituents of camel milk. Magnesium is known to reduce oxidative stress and enhance vitamin E and C absorption (Al-Ayadhi and Mostafa, 2013), moreover, zinc increases total glutathione, GSHPx, and SOD levels, furthermore, zinc can prevent cell damage through activation of the antioxidant system (El Heni, et al., 2011 and Zakaria et al., 2016). In addition, vitamin E has been suggested to enhance glutathione levels (Klevay, 2003). Together, high levels of Mg, Zn and vitamin E in camel milk might help to increase glutathione biosynthesis and enzymes production and so, to decrease the oxidative stress (Zakaria et al., 2016 and 2018). Treatment of adult mature male rats with camel milk for two months caused a significant increase in their fertility evident by a significant increase the conception rate and treatment of immature female rats caused enhancement of their puberty indicated by improvement of the ovarian follicles to secondary and tertiary with increase the number of uterine glands this could be based on the presence of a high quantity of zinc in camel milk, this finding agree with Sunar, (2009) who reported that zinc has a key role in the physiology of the reproductive system and zinc deficiency in the diet in particular leads to hypogonadism (Sunar, 2009). Sunar, (2009) reported that zinc deficiency led to an inhibition in LH and estrogen levels. Moreover, Gales, et al., (2014) found that magnesium protects, in part, the ovary against the reduction in the number of normal follicles and against the increasing number of atretic follicles. Also, our finding could be attributed to richness of camel milk with Mn as reported by Kamal (2012) which agree with the results of Lee et al.(2007) who reported that reported that Mn^{2+} stimulated LH release in prepubertal female rat, and this effect was due to stimulation of GnRH secretion induced by Mn that may facilitate the normal onset of puberty.

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