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Impact of Aloe Vera gel aqueous extract-supplemented yogurt on reproductive performance of male rats

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Abstract

Anciently, Aloe Vera (AV) is used as a medicinal plant for the treatment of various health problems. Recently, AV is considered as a functional raw material in the food technology of dairy products. The current study aims to investigate the effect of AV gel aqueous extract and/or yogurt consumption on male reproductive performance represented by gonadosomatic index (GSI), sperm quality, serum testosterone levels and testicular histology. The plain yogurt was prepared using fresh cow's milk and starter cultures. *Aloe vera* yogurt was prepared to contain AV (10 or 20 %). Twelve groups of rats orally fed with distilled water or AV gel aqueous extract and/or yogurt for 7 and 14 days separately. After 7 days of feeding, GSI percent has been decreased after feeding AV gel aqueous extract and recovered after 14 days. However, after 7 days, sperm concentration has been increased in rats received AV gel aqueous extract 10% and declined significantly after administration of AV gel aqueous extract and yogurt mixture but this decline has been recovered after 14 days. Rats received yogurt alone showed significant decrease of sperm motility percent as well as disturbance of sperm morphology represented by high abnormal sperm percent. On the other side, level of testosterone is declined significantly in all 7 days-treated groups and recovered after 14 days except in rats received AV gel aqueous extract 20% and yogurt mixture. Further, histological examination of the testes showed deleterious changes in normal testicular architecture after 7 days administration in rats received AV gel aqueous extract 10 and 20% as well as rats treated with AV gel aqueous extract 10% for 14 days however, rats treated with AV gel aqueous extract 20% showed partial recovery after 14 days. On contrary, mixture AV gel aqueous extract 10 or 20% with yogurt disclosed recovery of deteriorated testicular tissue after 7 and 14 days. Therefore, it can be concluded that the AV gel aqueous extract and/or yogurt has dose- and time dependent effect on male reproductive performance.

Keywords: *Aloe Vera*; Yogurt, Sperm; Testes; Reproductive performance.

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INTRODUCTION

All over the world, infertility affects about 15% of couples, about 40 to 50% of them are related to male factors (Singh et al., 2001). In the animal farms, the male is half of the herd so, special care must be concerned in the development of animal resources and production. The main role of the male is reproduction to guarantee the survival of offspring and continuation of milk and meat production. Testes are the primary organ of the male reproductive system carrying two essential biological processes; spermatogenesis and steroidogenesis (O'Donnell et al., 2017). Unfortunately, those biological processes are sensitive and adversely affected by infectious and inflammatory conditions ending with temporary or permanent infertility. Although chemotherapy and antibiotics were traditional strategies for disease control worldwide, they have the danger of bacterial resistance and introducing high risk to human health (Fair and Tor, 2014). Therefore, male reproduction care and improvement must be a primary concern by exploring novel alternative strategies of ameliorative substances with natural sources without side effects. *Aloe vera* has been used by mankind for several thousand years in the treatment of different disorders due to the medicinal value of the inner gel of its succulent leaves so, it is sometimes termed as a “miraculous” or “wonder” plant. AV applications have been recorded in ancient civilizations of India, Egypt, Greece, Rome, and China. At past, the Egyptians called AV as the "plant of immortality "and it is cultivated around the graves till now. The Chinese called it their “elixir of youth “or” Magical potion of youth "(Ahlawat and Khatkar, 2011; Nuriye et al., 2015). The name of Aloe derived from “alloeh (k)” (Arabic) or “allal” (Hebrew) or “aloes” (Greek); which means "shining bitter substance "and “vera” means, "true , veritable "(Park and Lee, 2006; Shrestha et al., 2015). AV is a stemless or short-stemmed plant with triangular, fleshy with serrated edges leaves, the leaves color varies from bright green to gray color arranged in a rosette shape at the stem (Ali et al., 2012; Jiang et al., 2013; Misir et al., 2014; Iwi, 2014). It mainly grows in tropical and subtropical areas; south and east Africa are considered the home of AV, then it was cultivated in warm climatic areas of Asia, Europe, and America (Ahlawat and Khatkar, 2011). Previously, AV is placed in the family Liliaceae but recently it is belonging family Aloaceae (Upton 2012). *Aloe Vera* contains about 75 nutrients, in addition to 200 active compounds including amino acids, sugars, enzymes, vitamins, minerals, saponins, anthraquinones, lignin, and salicylic acid (Misir et al., 2014). In addition, polysaccharides, anthraquinones, prostaglandins, phytoestrogens (such as beta-sitosterol), cholesterol, Aloe Emodin, and fatty acids like campstrols are the most important compounds of AV so, the existence of various chemical substances in AV plant may affect sex hormones (Joseph and Raj, 2011; Poorfarid et al. 2013). Consequently, AV is well recognized for its therapeutic purposes which are supported by many reported beneficial applications including immunomodulatory, wound and burn healing, hypoglycemic, anti-cancer, gastro-protective, antifungal, and anti-inflammatory properties. All these valuable therapeutic properties of AV have been employed for several commercial applications (Maana., et al 2018). However, the research on the influence of AV/yogurt on male fertility is scarce and its effect on male reproductive pattern is not well investigated, Therefore, the goal of the current study is exploring the influence of

AV and/or yogurt administration on male reproduction monitored by GSI, assessment of some semen parameters, serum testosterone evaluation and examination of testes histology.

MATERIALS AND METHODS

Experimental animals

Seventy-two adult male albino rats weighing 250-300 g were randomly distributed into six groups (12 for each). Group I (control "Ctrl"); rats were administrated distilled water (1ml/kg bw), group II was administered plain yogurt **(Y),** group III was administered 10% AV gel aqueous extract (AV 10%) (Lee and Lucey, 2010), group IV was administered 20% AV gel aqueous extract (AV 20%) (Lee and Lucey, 2010), group V was administered yogurt + 10% AV gel aqueous extract (AVY 10%), and group VI was administered yogurt + 20% AV gel aqueous extract (AVY 20%). Each group subdivided into first and second subgroups (6 animals each) which were treated separately for 7 and14 successive days, respectively. All administrations were carried out orally using a drenching tube. All animals used in this study were subjected to the handling according to the international guiding principles for biomedical research involving animals as outlined by the Council for International Organization of Medical Sciences and the International Council for Laboratory Animal Science (CIOMS and ICLAS, 2012).

Aloe Vera gel aqueous extraction and yogurt preparation

Aloe vera gel was extracted from collected AV leaves according to Noor et al., (2008) with slight modifications. The gel was diluted by distilled water (10 and 20 g of AV added to 100 ml distilled water) and mixed in the blender to get the final concentration of 10% and 20%. Yogurt was prepared from pasteurized fresh cow milk containing 3% (v/v) of Commercial yogurt culture, AV gel aqueous extract with 10% and 20% concentrations were added and mixed with milk and starter which previously pasteurized (90°C for 20 min) for Aloe vera yogurts preparation.

Sample collection

After 7 and 14 days, rats were anesthetized by diethyl ether and blood samples were collected from retro-orbital venous plexus, then centrifuged at 3000 rpm for 15 minutes, sera were collected and kept at -20^{O}C until the evaluation of testosterone hormone. Also, testis and epididymis were removed and weighed for GSI calculation according to Kumari and Singh (2013), at the same time, epididymal semen was collected in prewarmed physiological saline at 37°C (D'souza, 2003) for seminal analysis. Finally, testes were dissected and preserved in 4% paraformaldehyde for histological examination.

Epididymal semen analysis

Immediately after semen collection and dilution, epididymal sperm concentration was evaluated by dilution with sodium bicarbonate solution and formalin then sperms were counted by Neuber's hemocytometer as described by Srinivasulu, and Changamma, (2017). Onedrop of semen was placed on a prewarmed, dry, and clean slide for assessment of sperm motility according to Seed et al., (1996). At the same time, Sperm abnormalities were monitored by mixing one drop of diluted semen with Eosin stain according to Wyrobek and Bruce, (1978). Finally, one drop of semen was placed on a prewarmed dry and clean slide, then mixed with one drop of Eosin-Nigrosine stain for evaluation of sperm vitality (dead and live percent) according to Esteso et al., (2006).

Evaluation of testosterone

Serum testosterone levels were measured using enzyme-linked immunosorbent assay (ELISA) kits according to manufacturer's instructions (Cal biotech, El Cajan, CA, USA), Catalogue NO. (TE373S) by using a microplate reader (Infinite 50, Männedorf, Switzerland) at wavelength 450 nm (Chen et al., 1991).

Histological examination

After testicular excision, they were sliced and fixed in 10% neutral-buffered formalin for at least 24 h. The specimens were then immersed in tap water and dehydrated in ascending dilutions of ethanol (70-100%), cleared in xylene, and embedded in paraffin wax at 56°C in a hot air oven for 24 h. Serial sections of 5 µm thick were cut using a rotary microtome then were processed for hematoxylin and eosin (H&E) staining (Bancroft and Gamble, 2002).

Statistical analysis

Results were analyzed statistically by Graph pad prism 5 (GraphPad Software, San Diego, California USA). Data were expressed as (mean ± standard error of the mean (SEM)) and differences between groups were analyzed by using one-way analysis of variance (ANOVA), Values of $P<0.05$ were considered significant compared to control rats.

RESULT

Gonadosomatic index (GSI)

After 7 days of treatment, GSI ratio was decreased significantly in AV 20% and AVY 20% groups compared with that treated with plain yogurt as shown in fig. 1A. On the other side, GSI ratio was recovered to control level after 14 days of treatment. Further, Y, AV 20% and AVY 10% groups showed significant decrease in GSI ratio compared with AVY 20% (Fig 1B).

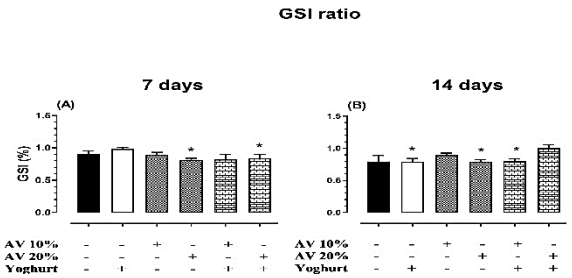

Fig. 1: Gonadosomatic index (GSI) ratio of control and treated rats with AV gel aqueous extract with or without plain yogurt administration. (A) GSI ration after 7 days administration, (n=6), * Significant with Y ($P<0.05$). (B) GSI ration after 14 days administration, (n=6), * significant with AVY 20% ($P<0.05$).

Epididymal sperm count and motility

Figure 2 illustrated epididymal sperm count as a response of treatment with AV gel aqueous extract (10 and 20%) alone or mixed with yogurt. After 7 days, rats received mixture of extract and yogurt showed significant decrease in epididymal sperm count compared with control and AV gel aqueous extract 10%-treated group as shown in fig. 2A. On contrary, epididymal sperm count has been recovered to control level after 14 days of treatment with AV gel aqueous extract 10 and 20% as well as AV gel aqueous extract 10%+yogurt. (Fig. 2B).

Figure 2C dented the deleterious effect of plain yogurt on epididymal sperm motility which has been decreased significantly in corresponding group compared with control after 7 days of treatment. Additionally, rats administered with AV gel aqueous extract 10%, AV gel aqueous extract 10%+yogurt and AV gel aqueous extract 20%+yogurt showed significant increase of epididymal sperm

motility compared with yogurt-treated rats. However, after 14 days of treatment, yogurt-treated rats showed recovery of epididymal sperm motility, at the same time, rats treated with AV gel aqueous extract 10% and AV gel aqueous extract 10%+yogurt disclosed significant increase of epididymal sperm motility compared with those treated AV gel aqueous extract 20% (Fig. 2D).

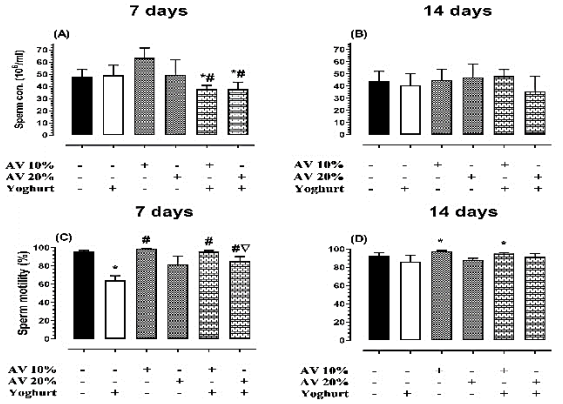

Fig. 2: Epididymal sperm concentration and motility of control and treated rats after administration of AV gel aqueous extract or mixed with plain yogurt. (A) and (B) denotes epididymal sperm concentration after 7 and 14 days of treatment, respectively, (n=6), * Significant with Ctrl, # Significant with AV 10%. (C) represents percent of epididymal sperm motility after 7 days of administration, (n=6), * Significant with Ctrl, # Significant with Y and ∇ Significant with AV 10%. (D) Epididymal sperm motility after 14 days of administration, (n=6), * Significant with AV 20%.

Sperm morphology

After 7 days of treatment, there was slight increase of abnormal sperm percent in rats treated with plain yogurt and those fed with yogurt mixed with AV gel aqueous extract 10 and 20%. However, after administration of AV gel aqueous extract alone (10 or 20%), treated rats showed slight decrease of abnormal sperm percent compared with control (Fig. 3A). On contrary, prolonged treatment period (14 days) revealed recovery of abnormal sperm percent in rats received plain yogurt and yogurt mixed with AV gel aqueous extract 20% indicating adverse effect of yogurt on sperm morphology (Fig 3B).

Abnormal sperm percent showed no change in dead sperm percent after 7 days of treatment in all treated rats compared with corresponding control (Fig 3C). Likewise, after 14 days of administration, there was no change in dead sperm percent among all treated groups compared with control. (Fig. 3D).

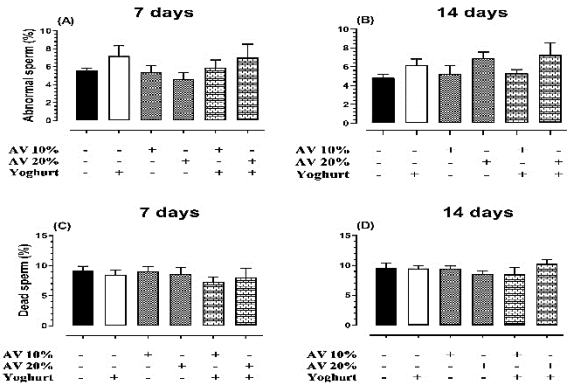

Fig. 3: Abnormal and dead sperm percent of control and treated rats. (A) and (B) abnormal sperm percent after 7 and 14 days of treatment, respectively (n=6). (C) and (D) denotes dead sperm percent after 7 and 14 days of treatment, respectively, (n=12).

Serum testosterone level

Administration of AV gel aqueous extract and yogurt for 7 days revealed significant decline in serum testosterone level in all treated groups compared with corresponding control; this decline was markedly observed in group treated with AV gel aqueous extract 20%+yogurt (Fig. 4A). However, prolonged administration period showed recovery of serum testosterone level in all treated rats except in those received AV gel aqueous extract 10%+yogurt showed non-significant decrease in serum testosterone level compared with control and significant

decrease compared with rats treated with AV gel aqueous extract 10 and 20%.

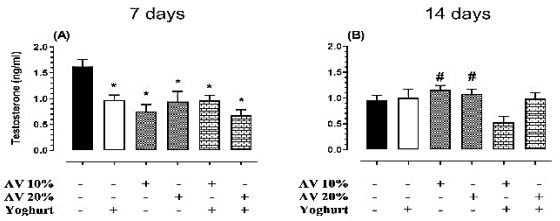

Fig. 4: Serum testosterone level in control and treated rats in response to administration of AV gel aqueous extract after (10 or 20%) and/or yogurt for 7 (A) and 14 days (B), (n=6). * Significant with Ctrl, # Significant with AVY 10%.

Histological examination

Fig 5A and B revealed the normal testicular structure of CTRL group after 7 and 14 days of treatment, respectively. Testicular tissue is composed of seminiferous tubules lined with round and well-stained spermatogonia or germ cells. Like control, rats received yogurt only for 7 and 14 days showed normal testicular architecture with seminiferous tubules lined by germ cells (Fig 5C and D).

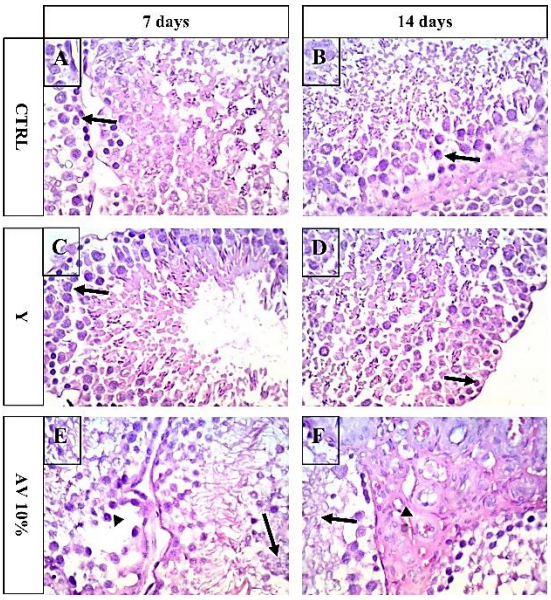

Fig. 5: Photomicrography of testicular structure of normal and treated rats after 7 and 14 days of AV gel aqueous extract 10% or plain yogurt administration. (A) Testicular tissue of CTRL group after 7 and (B) after 14 days of treatment. Normal testicular tissue composed of seminiferous tubules lined with round and well stained spermatogonia or germ cells (arrow). (C) Testicular tissue of rats received yogurt only for 7 days and (D) for 14 days show normal testicular architecture with seminiferous tubules lined by germ cells (arrow). (E) Testicular tissue of rats treated with AV gel aqueous extract 10% discloses deleterious effect represented by marked testicular degeneration and necrosis (arrow) and vacuolation (arrowhead) in seminiferous tubules after 7 days of administration. (F) Testicular tissue of the same rats after 14 days of treatment with degeneration and necrosis (arrow), seminiferous tubules showed marked edema (arrowhead). (400X, H&E).

However, the administration of AV gel aqueous extract alone at a concentration of 10% disclosed deleterious effect represented by marked testicular degeneration and necrosis and vacuolation in seminiferous tubules after 7 days of administration (Fig 5E).

In addition to degeneration and necrosis, seminiferous tubules showed marked edema after 14 days of AV gel aqueous extract 10% administration (Fig 5F). The adverse effects also appeared after administration of a higher concentration of AV gel aqueous extract 20% for 7 days in the form of germ cells degeneration associated with edema and vacuolation (Fig 6A). Although, degeneration represented in testicular tissue after 14 days of AV gel aqueous extract 20% administration, some improvement in the lining germ cells appeared in one layer (Fig 6B). Combination of AV gel aqueous extract 10% and yogurt for 7 and 14 days showed recovery of AV gel aqueous extract-induced alteration in testicular tissue (Fig 6C and D respectively). Seminiferous tubules appeared with normal lining germ cells. Like the previous group, rats received a mixture of AV gel aqueous extract 20%+yogurt for 7 days showed recovery of degenerated seminiferous tubules which appeared with normal spermatogonia with

the presence of giant cells within the lumen of seminiferous tubules (Fig 6E). Whereas, after 14 days, there was a partial recovery of degenerated tissue with the appearance of a single layer of germ cells with other degenerated and necrosed layers (Fig 6F).

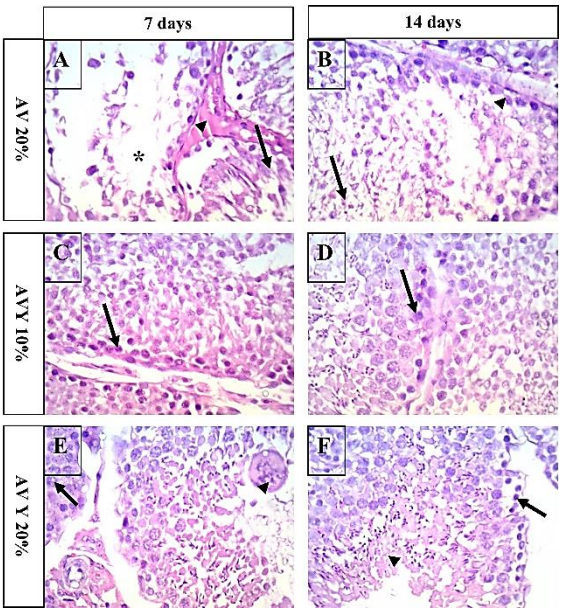

Fig. 6: Photomicrography of testicular structure of treated rats after 7 and 14 days of AV gel aqueous extract alone or mixed with yogurt administration. (A) Testicular tissue rats received AV gel aqueous extract 20% after 7 days discloses germ cells degeneration (arrow) associated with edema (arrowhead) and vacuolation (asterisk) (Fig). (B) Testicular tissue of the same treated rats after 14 days shows degeneration in testicular tissue (arrow), some improvement in lining germ cells appeared in one layer (arrowhead). (C) Testicular tissue of rats treated with AV gel aqueous extract 10%+yogurt and (D) for 14 days shows recovery of AV-induced alteration in testicular tissue and seminiferous tubules appeared with normal lining germ cells. (E) Testicular tissue of rats treated with AV gel aqueous extract 20%+yogurt for 7 days shows recovery of degenerated seminiferous tubules which appeared with normal spermatogonia (arrow) with presence of giant cells within the lumen of seminiferous tubules (arrowhead). (D) Whereas Testicular tissue of rats treated with AV gel aqueous extract 20%+yogurt for 14 days shows partial recovery of degenerated tissue with appearance of single layer of germ cells (arrow) with other degenerated and necrosed layers (arrowhead). (400X, H&E)

DISCUSSION

The current study aims to investigate the influence of plain yogurt or yogurt mixed with AV gel aqueous extract in 10 and 20% concentration on the male reproductive performance by assessment of GSI ratio, some semen parameters and evaluation of serum testosterone level as well as histological examination. To evoke its deleterious effect on sperm viability, yogurt may require prolonged administration and 14 days not enough to disclose its influence. However, impaired effect of yogurt is marked on sperm motility and elevation of abnormal sperm percent. The literature and studies about the adverse effect of yogurt on semen quality are scarce but our results are different from the previous. So, an advanced study is required to investigate the real role of yogurt in time and dose dependent as well as to investigate its action pathway either centrally through the hypothalamus, LH, and FSH hormones or peripherally on testicular tissue. On contrary, most previous studies showed improvement effect of yogurt on male reproduction due to the probiotic bacterium -lactobacillus- induce modulation of local gastric intestinal immunity leading to activation of metabolic pathways that restore tissue homeostasis (Poutahidis et al., 2007 and Leikcvich et al., 2013). In addition, the administration of yogurt for a long time ending with larger testes, higher testosterone levels, and higher sperm count (Poutahidis et al., 2014). Surprisingly, dairy products including yogurt contain some hormones that disturb the physiological endocrine and reproductive function (Servos et al., 2005). The most important hormones found in milk and dairy products are prolactin, estrogens, progesterone, corticosteroids, and insulin-like growth factor1 (IGF-1). These hormones may have a major suppression impact on male reproduction or carcinogenic activity

associated with some active metabolites of estrogen and IGF-1 (Meyer et al., 2017; Mourupoju and Sundaresan, 2018). Hence, these results explain the current findings and why yogurt suppresses sperm motility, viability and reduces testosterone hormone level. Moreover, our findings match with those of Aravindakshan et al., (2004) who found a decrease in epididymal weight, sperm count, and motility after administration of exogenous estrogen-containing compounds.

Like yogurt, AV gel aqueous extract administration in 10 % for successive 7 days disclosed non-significant increase of sperm count, motility while, 20% concentration revealed no change on abnormal and dead sperm percent. However, AV gel aqueous extract effect was clear on the testosterone hormone; after 7 days of AV administration, testosterone dropped markedly especially in rats treated with AV gel aqueous extract 20%+yogurt confirming synergetic suppressive effect of both supplement on male reproduction. Although serum testosterone level recovered after 14 days of treatment, AV gel aqueous extract 10%+yogurt supplement failed to restore normal serum testosterone level. We expect this contrasting and controversial impact of AV gel aqueous extract coming through the central pathway via the hypothalamus and pituitary gland, but this needed advanced study to be proven. In addition, presence of substances such as prolactin, estrogens, progesterone, corticosteroids, and insulin-like growth factor1 (IGF-1) which characterized by suppressive effect on male reproduction (Mourupoju and Sundaresan, 2018).

Our results agree with the findings of Shairi et al., (2009) and Karimi et al., (2012) who confirmed that alcoholic extract of AV for thirty days reduced testosterone level associated with low LH and FSH hormones level. In addition, AV has an estrogenesis activity so, it has an important role in increasing estrogen (Moshtaghi et al., 2010). Subsequently, exposure to exogenous estrogen causes structural and functional changes in the male reproductive system such as decreases sperm concentrations, motility, and plasma testosterone (Goyal et al. 2003, Sharpe et al. 2003), reduces Sertoli cell number (Atanossova et al. 2005), and gene expression (Adachi et al. 2004). Moreover, administration of synthetic form of estrogen hormone (Diethylstilbestrol) ending with disorders of reproductive tract development in male result from disturbance of androgen-estrogen balance. These disorders are represented by distension of efferent ducts with adverse changes of their epithelial cells and prevention of overgrowth of rete testis (Revas et al., 2003). This supports our hypothesis regarding the central, not local effect, of AV gel aqueous extract on reproductive performance as well as, some compounds in AV gel aqueous extract such as coumaric acid may stimulate the bio-activity of testicular macrophages which in role stimulates nitric oxide production via stimulating the release of cytokines and suppress the conversion of cholesterol to pregnenolone through inhibition of P450 cytochrome activity,thereafter, reducing testosterone hormone level (Chrousos, 2007; Budai et al., 2013; Klein-Wieringa et al., 2013). This explanation agrees with current histological findings which showed giant cells in testicular tissue in rats received AV gel aqueous extract. Moreover, AV gel aqueous extract flavones (A type of phytoestrogens) exert their effect through modulation and inhibition of metabolizing enzymes including 17-Beta-hydroxy steroid hydrogenase and aromatase which constitute a link in the testosterone

production pathway (Krazeisen et al., 2001; Whitehead and Lacey, 2003).

Previous studies confirmed that phytoestrogens of AV gel aqueous extract have a suppressive role in the hypothalamus leading to the reduction of gonadotropin-releasing hormone (GnRH) production, and thus, deteriorating the hypothalamic-pituitary-gonadal axis (Selvage et al., 2004). Thereafter, inhibition of LH hormone (responsible for stimulating testosterone release from Leydig cells) and FSH hormone (responsible for spermatogenesis). As expected, the combination of yogurt and AV gel aqueous extract exerts a synergetic anti-androgen effect as shown in the present results, since both are rich in phytoestrogens as mentioned before. Hence, dietary phytoestrogens enhance apoptosis of germ cells which in turn decrease sperm production. This effect is independent of the hypothalamic-pituitary-testicular axis and is due to the disruption of estrogen's actions in the testis (Stephen Assinder et al., 2007).

CONCLUSION

AV gel aqueous extract has variable effect on sperm quality in dose- and time dependent manner however, it acts as suppressive agent on serum testosterone level. Further, plain yogurt has anti-androgenic impact and synergetic suppressive effect on male reproduction when it supplemented in combination with AV gel aqueous extract.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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