Effect of Flaxseed or Turmeric Oils on Reproductive Parameters and Serum Oxidative Stress Markers of Male Albino Rats (*Rattus Rattus*)

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ABSTRACT

Background: Phytoestrogens are estrogen analogues of plant origin, allowing them to act as agonists at estrogen receptors.

Objectives: This study was done to discover the effects of flaxseed and turmeric oils on male rats' fertility.

Methodology: Eighteen male albino rats are divided into three groups (6 rats each). Group 1 (Control group) rats received distilled water 1 mL/kg orally); group 2 (Flaxseed oil group) rats received 1 mL/kg flaxseed oil orally;group 3 (Turmeric oil) rats received 1 mL/kg turmeric oil orally. After 30 days, blood samples, epididymis, and testis were collected for biochemical analysis, sperm characteristics and histological examinations.

Results: Flaxseed oil induced a significant decline ($p<0.05$) in % of body weight change relative to the control group. Flaxseed and turmeric oils groups showed significant declines ($p<0.05$) in gonadosomatic index and sperm motility, concentration, and viability, with a significant increase in sperm abnormalities. Besides, Flaxseed and turmeric oils groups showed significant decreases ($p<0.05$) in the serum testosterone levels with significant increases ($p<0.05$) in serum prolactin, FSH, and LH levels. Flaxseed and turmeric oils consumption significantly decreased ($p<0.05$) plasma CAT and SOD while they significantly increased ($p<0.05$) plasma MDA relative to the control group. The testis of rats ingested flaxseed and turmeric oils showed degenerated seminiferous tubule**s**, with a diminished number of mature spermatozoa in the tubular lumen, reduced diameter of seminiferous tubules and intact basement membrane.

Conclusion: Feeding flaxseed and turmeric oils consumption to adult male rats induced hormonal disturbances with non-uniformities in sperm characteristics.

Keywords: Flaxseed oil, Turmeric oil, Phytoestrogen, Rats, Sex hormones, Sperm characteristics

INTRODUCTION

Presently, many men have erectile dysfunction and infertility. This is for many reasons, including some foods which contain Phytoestrogens.

Flaxseed is the flax plant's seed (*Linum usitatissimum* L., family Linaceae) widely used worldwide due to its biologically active constituents exhibiting several health benefits. Flaxseed oil is a rich source of polyunsaturated fatty acids; it contains 54-59% linolenic acid (18:3n-3, ALA) and lignans (secoisolariciresinol diglycoside-SDG) [(1,2,3)]. Additionally, it is a rich source of phytoestrogens [(4)].

Flaxseed oil has several medicinal characteristics include anticancer, antioxidant, antiviral, bactericidal, anti-inflammatory, and antiatherosclerotic properties [(1)]. It also possesses hypoglycemic and cholesterol lowering effects [(5)] allowing it to reduce the risk of insulin resistance, obesity, and dyslipidemia [(6)].

Turmeric (*Curcuma longa*, family Zingiberaceae) is widely cultivated for its rhizomes. It is a widely distributed perennial herb mainly in sub-tropical and tropical regions. It is used as coloring agent in food, confectionery, and pharmacy industry. Turmeric powder, extracted from plant rhizomes, is mainly constituted of curcumin.

It possesses various pharmacological actions, including anti-inflammatory [(7)], antioxidant [(8)], hypolipidemic [(9)], and anti-cancerous [(10)]. Curcuma extract possesses substantial anti-spermatic and antifertility activity in male albino rats [(11)].

Despite all the benefits that have been mentioned, the question arises here dose flaxseed or turmeric oils have a negative effect on male fertility?

MATERIALS AND METHOD

Flaxseed oil was bought from Harraz Herbal Drugstore, Cairo, Egypt. Turmeric oil was purchased from El-Captain Company, Cairo, Egypt.

Experimental protocol

This study was performed on eighteen male albino rats (Rattus rattus) weighing (120-150g). Rats were purchased from the animal farm of El-Nile Com. for Pharmaceutical Product, Cairo, Egypt. Rats were kept in cages (three rats per cage) under control and standard conditions of normal light/dark cycle, humidity, and temperature throughout the experimental duration. Water ad libitum and food were accessible during the investigation period. The experiment was done at the Faculty of Science, Al-Azhar University, Cairo, Egypt. The rats were divided into three groups (n=6) after acclimatizing for one week as follows:

Group I: Control group, rats in this group eceived distilled water orally (1 ml/kg).

Group 2: Flaxseed oil group, rats in this group received flaxseed oil orally (1 ml/kg) [(12).]

Group 3:Turmeric oil group, rats in this group received turmeric oil orally (1 ml/kg) [(13)].

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Biological measurement

Rats' body weights were measured before the oils consumption and at the end of the experiment to calculate the % change in body weight compared to the control group.

Blood samples collection

After the experiment (30 days), blood samples were withdrawn from rats' retro-orbital sinuses. Serum and plasma were frozen after separation until analysis.

Gonadosomatic index

Immediately after scarification, the testes from rats in all groups were dissected; the testes' weight was recorded, and the testes' relative indices to body weights were calculated. Testes samples were preserved in Bouin's fluid, while the epididymites were collected for semen analysis.

Biochemical analysis

Hormonal assay

Testosterone was measured using an ELISA kit, LDN Labor Diagnostika Nord Gmbh & CO, Germany. Follicle-stimulating hormone (FSH) was measured using an ELISA kit, Kamiya Biomedical Company, USA. Luteinizing hormone (LH) was measured using an ELISA kit, Abnova, Taiwan. Prolactin hormone was measured by using an ELISA kit, Assay Genie, Ireland.

Antioxidant analysis

Superoxide dismutase (SOD), catalase (CAT), and lipid peroxide measured as malondialdehyde (MDA) were determined using colorimetric kits purchased from Biodiagnostic Co., Dokki, Giza, Egypt.

Sperm collection and evaluation

The total number of sperm retrieved after 4 hours of incubation in normal saline (volume=1 ml, 35–37^{o}C) was calculated after the left caudal epididymis was detached. Using a hemocytometer chamber, the traditional approach was used to calculate the sperm concentration.

Sperm shape

One drop of 1 % Eosin-Nigrosin was added to the sperm solution and left for 30 minutes. Smears were prepared and allowed to air dry on that described by **Narayana *et al.*** [14]; 1000 sperm/rat were screened and divided into normal and other aberrant types.

Histopathological examination

Histopathological examination was made on sections from each group's testes after staining with hematoxylin and eosin (H&E). Zeiss light microscope and an Image Pro-Express Analysis System were used to examine the testes' numerous properties concerning the tunica albuginea's condition, seminiferous tubules, different cells in the interstitial tissues and seminiferous epithelium.

Ethical approval:

All the experimental procedures were carried out according to the principles and guidelines of the Ethics Committee of the of Faculty of science, Al-Azhar University, Cairo, Egypt conformed to "Guide for the care and use of Laboratory Animals" for the use and welfare of experimental animals, published by the US National Institutes of Health (NIH publication No. 85–23, 1996).

Statistical analysis

Results were presented as mean ± SD (standard deviation). One-way analysis of variance (ANOVA), and the LSD test were used in the statistical. SPSS (version 27) was used to analyze the results. The significance level was considered if $p<0.05$.

RESULTS

Biological measurement and gonadosomatic index

The flaxseed oil group showed a significant decline in the % of body weight change and gonadosomatic index relative to the control rats. Regarding the turmeric oil group, there was a significant reduction in gonadosomatic index relative to the control group Table (1).

Hormonal assay

Table (2) revealed that flaxseed and turmeric oils groups showed significant declines ($p\leq0.05$) in the serum testosterone concentration concurrent with significant increases ($p\leq0.05$) in prolactin, FSH, and LH levels relative to the control group.

***Sperm* characteristics and shape**

Flaxseed and turmeric oil groups showed significant decreases ($p\leq0.05$) in sperm motility, concentration, and viability, with a significant elevation in sperm abnormalities relative to the control group. In addition, there were significant declines in sperm motility and viability in the flaxseed oil relative to the turmeric oil group Table 3. The sperm abnormalities include sperm without hook heads, amorphous shape heads, banana shape heads, small heads, large heads and abnormal tails Figure 1.

Histopathological results

The testes of the rats in the control group showed normal histological arrangements of spermatogonia in the seminiferous tubules with Sertoli cells resting on intact basement membranes Figure 2 (A&B). Testis of the flaxseed oil group rats showed degenerated seminiferous tubule**s**, with a diminished number of mature spermatozoa in the tubular lumen, reduced diameter of seminiferous tubules and intact basement membrane Figure 2 (C&D). The testis of turmeric oil group rats showed the same changes as the flaxseed oil group with exfoliated cells in the tubular lumen Figure 2 (E&F).

Antioxidant markers

Flaxseed and turmeric oil group revealed significant decreases ($p\leq0.05$) in plasma CAT and SOD levels, with a significant increase in MDA level relative to the control group Figure 3.

Table (1): Effect of flaxseed and turmeric oils on % of body weight change and gonadosomatic index in male rats.

Parameters	Control	Flaxseed oil	Turmeric oil
Body weight change %	218.50 ±10.86	199.83 ±9.43 [a] - 8.54%	216.17 ± 10.42 -1.06%
Gonadosomatic index	1.37 ± 0.04	1.16 ± 0.05 [a] -15.32%	1.19 ± 0.08 [a] -13.14%

Significance was considered at p≤o.o5. Results were presented as mean ± SD (n = 6). [a] Significant versus control.

Table (2): Effect of flaxseed or turmeric oils on serum levels of reproductive hormones (testosterone, prolactin, FSH, and LH) measured in male rats.

Parameters	Control	Flaxseed oil	Turmeric oil
Testosterone	4.70 ± 0.42	2.78 ± 0.21 [a] - 40.85 %	2.86 ± 0.26 [a] -39.14 %
Prolactin	4.45 ± 0.36	6.39 ± 0.56 [a] 43.59 %	6.24 ± 0.38 [a] 40.22 %
FSH	25.87 ± 2.09	39.83 ± 2.36 [a] 53.96 %	38.58 ± 1.55 [a] 49.13 %
LH	10.31 ± 1.06	15.46 ± 1.03 [a] 49.95 %	14.79 ± 1.45 [a] 43.45 %

Significance was considered at p≤o.o5. Results were presented as mean ± SD (n = 6). [a] Significant versus control.

Table (3): Effect of flaxseed or turmeric oils on sperm characteristics (sperm motility, concentration, viability, and abnormalities) determined in male rats.

Sperm characteristics	Control	Flaxseed oil	Turmeric oil
Motility (%)	53.17 ± 4.88	22.00 ± 2.00 [a] -58.62 %	35.50 ± 4.46 [a, b] -33.23 %
Concentration (10^6/ml)	46.17 ± 4.83	26.83 ± 2.48 [a] -41.88 %	29.00 ± 1.41 [a] -37.18 %
Viability (%)	66.83 ± 6.88	33.67 ± 4.18 [a] -49.61 %	42.00 ± 4.29 [a, b] -37.15 %
Abnormalities (%)	9.95 ± 1.46	34.17 ± 3.97 [a] 243.41 %	31.50 ± 3.29 [a] 216.58 %

Significance was considered at p≤0.05. Results were presented as mean ± SD (n = 6). [a] Significant versus control, [b] Significant versus flaxseed oil group.

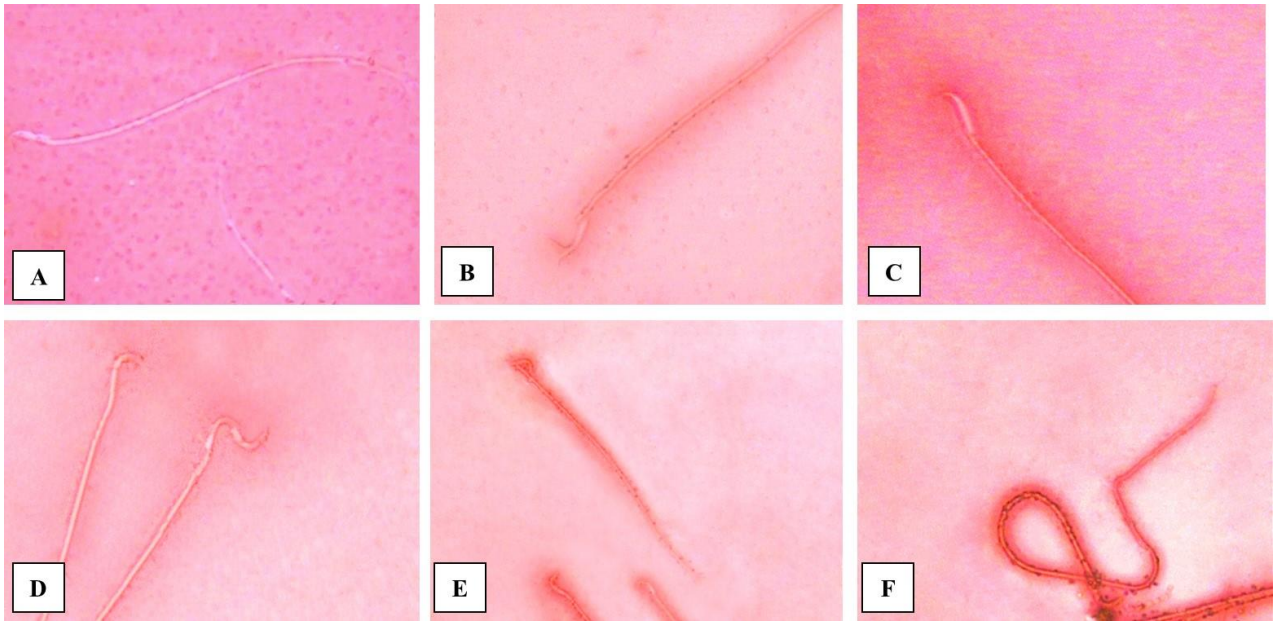

Figure (1): Photomicrographs illustrating sperm morphology normal and various sperm defects (stained by Eosin-Nigrosin stain, X 400). A- Normal lives; B- Normal dead; C- Without hock; D- On the right amorphous shape and on the left normal dead; E- Amorphous shape; and F- Abnormal tail.

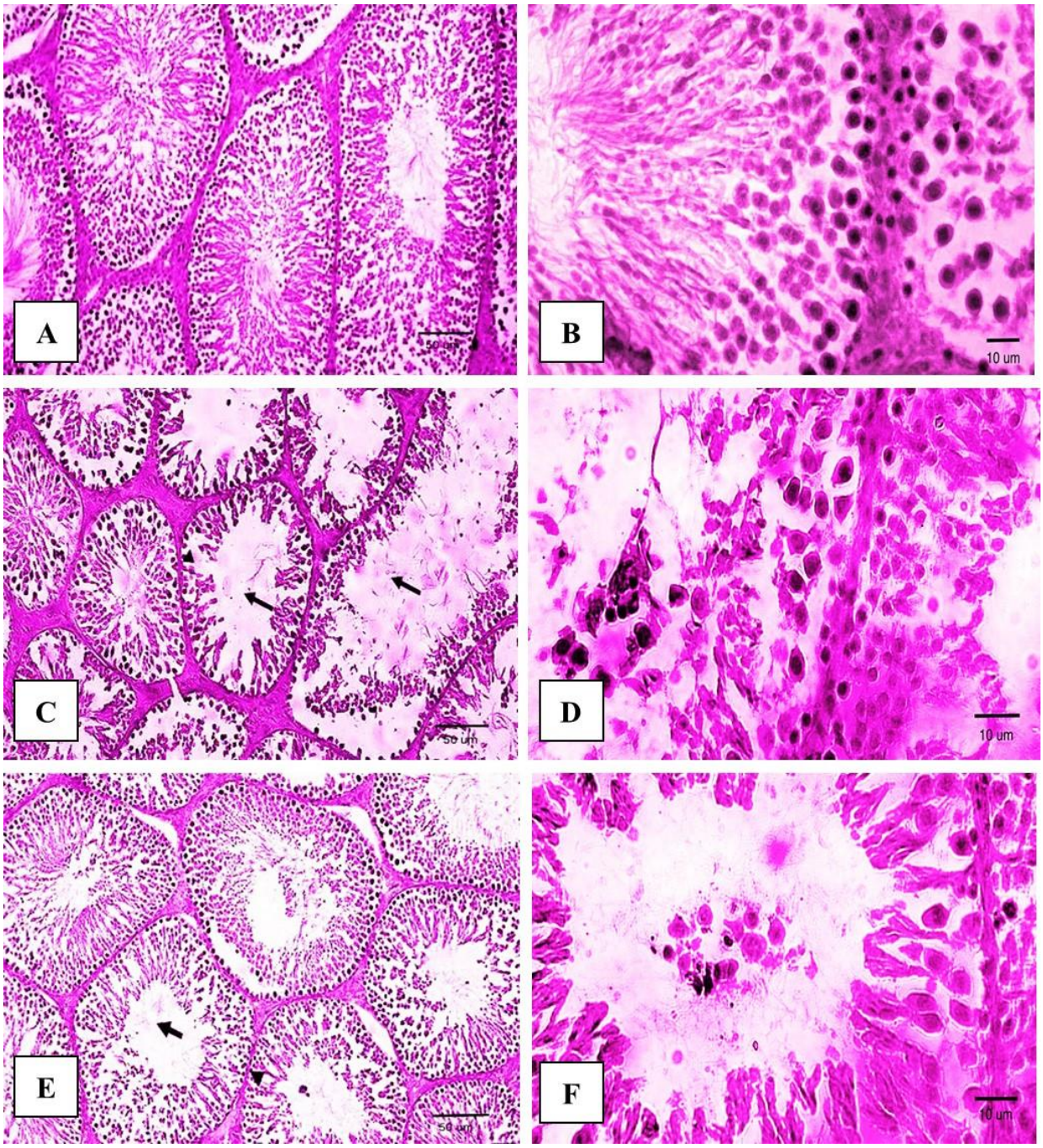

Figure (2): Photomicrographs showing control and the effect of flaxseed and turmeric oils on testis histopathology stained by H&E and photographed at bar 50 mμ & bar 10 mμ. A & B represent the testes of the control rats showed normal histological arrangements of spermatogonia in the seminiferous tubules with Sertoli cells resting on an intact basement membrane. C & D represent the testes s of the flaxseed oil group showed degenerated seminiferous tubules, with a diminished number of mature spermatozoa in the tubular lumen (arrow), reduced diameter of seminiferous tubules and intact basement membrane (arrows head). E &F represent the testes of the turmeric oil group showing degenerated seminiferous tubules, with a diminished number of mature spermatozoa in the tubular lumen (arrow), reduced diameter of seminiferous tubules and intact basement membrane (arrows head) and the presence of exfoliated cells in the tubular lumen.

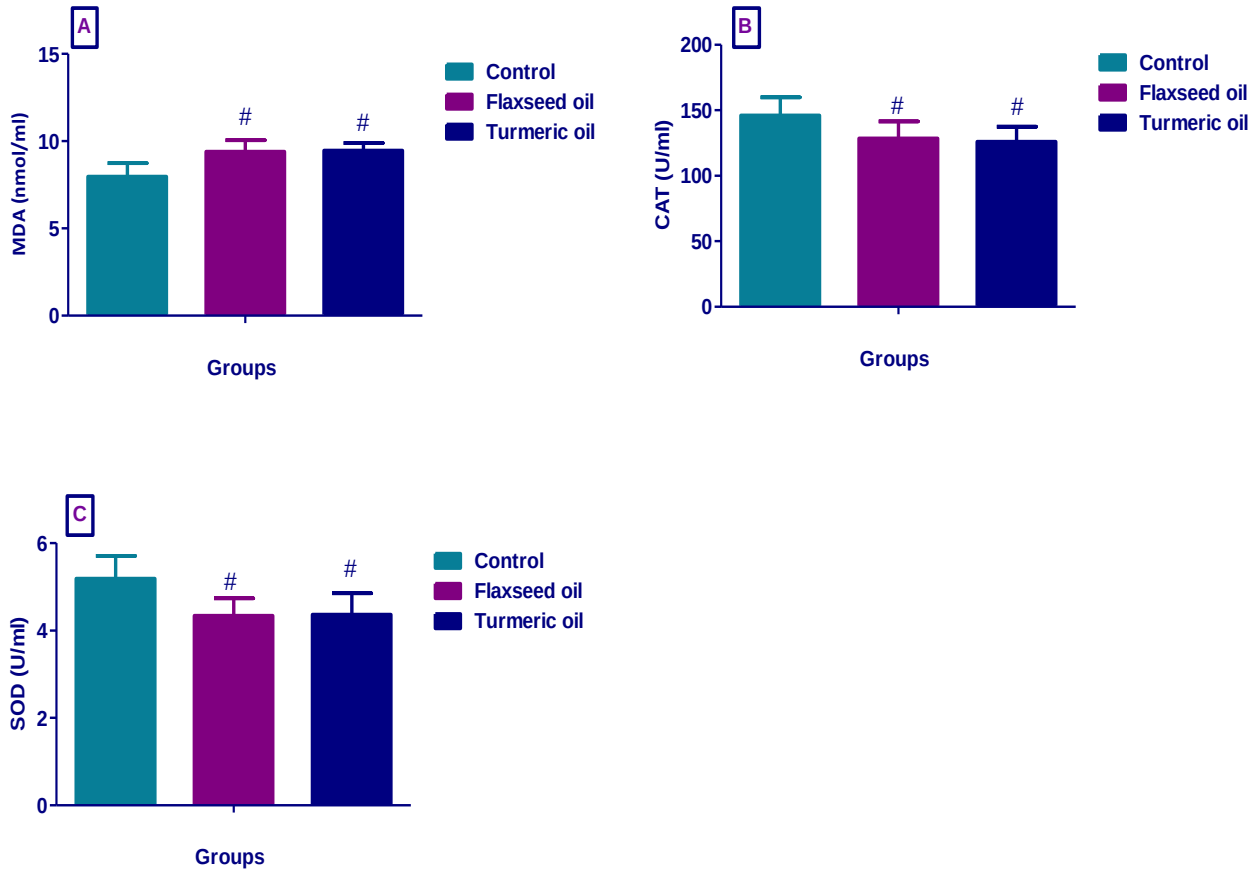

Figure (3): Effect of flaxseed and turmeric oils on the concentration of plasma antioxidant markers (MDA, CAT, and SOD) of male rats. Significance was considered at $p \leq 0.05$. Data were presented as mean ± SD (n=6). #Significant versus control.

DISCUSSION

Phytoestrogens are estrogen analogues present in plants, such as flaxseed and turmeric oils, which affect and interfere with the male reproductive system's function [15]. This study investigated the effects of flaxseed and turmeric oils on the reproductive system of male rats.

The results of this study revealed that flaxseed and turmeric oils caused a significant decrease in weight gain. Ingestion of these oils induced significant oxidative stress. It decreased serum testosterone level, sperm motility, concentration, and viability, with significant increases in serum prolactin, FSH, and LH levels relative to the control rats. The oils consumption also induced testicular histopathological alterations like degenerated seminiferous tubules, with diminished mature spermatozoa in the tubular lumen, reduced diameter of seminiferous tubules and intact basement membrane.

Previous studies reported that flaxseed oil reduced animal weights, which antioxidant constituents, especially alpha-linolenic acid, may explain, the decline of adipocyte hypertrophy in adipose tissue. Moreover, the oil decreased fat absorption by increasing fecal excretion [16,17]. The same trends of results were reported by **Al-Harbi *et al.*** [18] and **Jasem and Tawfeek** [19].

Testosterone is essential for sperm production; thus, a testosterone deficiency results in low sperm counts, reduced sperm motility and viability and increased sperm abnormalities. It also caused degenerated seminiferous tubules. All these factors caused a decrease in gonadosomatic weight. The obtained results could be explained by flaxseed showing increased concentrations of 17b-estradiol [20]. Estrogen plays an inhibitory role in the proliferation of Leydig cells. It prevents their regeneration and works to inhibit the Leydig cell enzymes necessary for testosterone biosynthesis, leading to testosterone deficiency [21,22]. Testosterone deficiency in the blood produces negative action on the pituitary gland, increasing LH secretion. Prolactin has a main role in testosterone synthesis via LH receptors upregulation on Leydig cells; therefore, testosterone reduction induces an increase in prolactin secretion [23-24]. In addition, estrogen-like substances induce inhibition in Sertoli cell proliferation and defects in the spermatogenesis process; they also stimulate the pituitary gland, increasing FSH secretion [25].

Sharma and Singh [26]. reported that curcumin (turmeric) administration caused testes weight and cauda epididymis reductions, with a significant decline in sperm count and motility and increased abnormal sperm tail and head.

Moreover, turmeric extracts decreased the epididymis and testes weights, ventral prostate, and seminal vesicle. Also, it decreased sperm motility and density in cauda epididymis. Seminiferous tubules of testes sizes, and spermatogonia, spermatocytes, and spermatids number were decreased. It significantly reduces the seminiferous tubules and Leydig cell nuclei diameters in male rats [27, 28].

The findings of this study showed an increase in MDA and a decrease in CAT and SOD levels in the groups ingested flaxseed and turmeric oils. The obtained results could be explained *via* testosterone suppression by exogenous steroids, including estrogens. Similarly, it resulted in the suppression of antioxidant enzyme expression, an increase in peroxidative damage, the disruption of spermatogenesis and an increase in germ cell apoptosis [29,30]. In addition, the suppression of antioxidant activity in response to exogenous steroid treatment [30-32].

CONCLUSION

The findings of this study suggested that flaxseed and turmeric oils harm male fertility where they cause a testosterone deficiency and reduced sperm counts, motility and viability with an increase in sperm abnormalities. In addition, they cause the suppression of antioxidant status. Therefore, this study showed that flaxseed and turmeric oils may negatively influence male fertility.

REFERENCES

1. **Halligudi N (2012):** Pharmacological properties of flax seeds: A Review. Hygeia. JD Med, 4(2):70-7.
2. **Goyal A, Sharma V, Upadhyay N *et al.* (2014):** Flax and flaxseed oil: An ancient medicine & modern functional food. J Food Sci Technol, 51(9):1633–53.
3. **Touré A, Xueming X (2010):** Flaxseed lignans: source, biosynthesis, metabolism, antioxidant activity, bio-active components, and health benefits. Comprehensive Rev in Food Sci & Food Safety, 9(3): 261–9.
4. **Tanideh R, Delavari S, Farshad O *et al.*, (2021):** Effect of flaxseed oil on biochemical parameters, hormonal indexes, and stereological changes in ovariectomized rats. Vet Med Sci, 7 (2):521–33.
5. **Kristensen M, Jensen M, Aarestrup J *et al.*, (2012):** Flaxseed dietary fibers lower cholesterol and increase fecal fat excretion, but magnitude of effect depends on food type. Nutri & Metabol 9(1):1–8.
6. **Saxena S, Katare C (2014):** Evaluation of flaxseed formulation as a potential therapeutic agent in mitigation of dyslipidemia. Biomed J, 37(6):386–90.
7. **Ramchandran R, Bhide S, Chaisas S *et al.*, (2000):** Anti-inflammatory activity of turmeric oil in arthritis model in rat. Proceeding of International congress on "Ayurveda-2000", Chennai, 2000, pp:199–200.
8. **Hewlings S, Kalman D (2017):** Curcumin: A Review of Its' Effects on Human Health. Foods 6:6.
9. **Darka H (1999):** Hypolipidemic effect of Curcuma longa in rabbit. Hamdard Med,42: 26–9.
10. **Kim M, Kang H, Moon A (2001):** Inhibition of invasion and induction of apoptosis by curcumin in H-rat-transformed MCF-10A human breast epithelial cells. Arch Pharm Res, 24: 349–54.
11. **Bhagat M, Purohit A (2001):** Antifertility effect of various extract of Curcuma longa in male albino rats. Indian Drugs,38: 78–81.
12. **Karaca S, Eraslan G (2013):** The effects of flaxseed oil on cadmium-induced oxidative stress in rats. Biol Trace Elem Res.,155:423–30.
13. **Helal E, Abd-El-Aziz M, Ahmed S (2019):** Effect of Anise (*Pimpinella Anisum* L.) as phytoestrogen on some sex hormones and biochemical parameters. Egyptian J of Hospital Med, 75 (1):1918–22.
14. **Narayana K, D'Souza U, Rao K (2002):** Effect of ribavirin on epididymal sperm count in rats. Indian J Physiol Pharmacol, 46: 97–101.
15. **Giwercman A (2011):** Estrogens and phytoestrogens in male infertility. Current Opinion in Urology, 21(6): 519–26.
16. **Baranowski M, Enns J, Blewett H *et al.*, (2012):** Dietary flaxseed oil reduces adipocyte size, adipose monocyte chemoattractant protein-1 levels and T-cell infiltration in obese, insulin-resistant rats. Cytokine, 59: 382–91.
17. **Kajla P, Sharma A, Sood D (2015):** Flaxseed-A potential functional food source. J. Food Sci. Technol, 52:1857–71.
18. **Al-Harbi N, Hadi M, Abbas F (2017):** Effect of flax seeds (*Linum usitatissimum* L.) extract in male reproductive system of albino rat treated with cyproterone acetate. J Global Pharma Technol, 10(9): 221–31.
19. **Jasem H, Tawfeek F (2020):** Effect of aromatase inhibitor, sage and flaxseed on spermatogenesis in rat. J Education and Sci, 29 (1):149–62.
20. **Cardozo L, Boaventura G, Brant L *et al.*, (2012):** Prolonged consumption of flaxseed flour increases the 17b-estradiol hormone without causing adverse effects on the histomorphology of Wistar rats' penis. Food ChemToxicol, (50): 4092–6.
21. **Abney T (1999):** The potential roles of estrogens in regulating Leydig cell development and function: a review. Steroids, 64: 610–7.
22. **Abney T, Myers R (1991):** 17b-estradiol inhibition of Leydig cell regeneration in the ethane dimethylsulfonate-treated mature rat. J Androl ,12:295–304
23. **Purvis K, Clausen O, Olsen A *et al.*, (1979)**: Prolactin and Leydig cell responsiveness to LH/hCG in the rat. Archives of Androl, 3 (3): 219–30.
24. **Mathur U, Bartke A, Weisz J (1975):** Effects of prolactin and LH on the activity of Δ5-3β hydroxy-steroid dehydrogenase, dihydro orotic dehydrogenase, β-hydroxybutyrate dehydrogenase and glucose 6 phosphate dehydrogenase in the testis of the dwarf mice. Indian J Physiol Pharmacol, 19(2): 58–64.
25. **Sharpe R, Atanassova N, McKinnell C *et al.*, (1998):**Abnormalities in functional development of the Sertoli cells in rats treated neonatally with diethylstilbestrol: a possible role for estrogens in Sertoli cell development. Biol Reprod, 59:1084–94.
26. **Sharma P, Singh R (2010):** Protective role of curcumin on lindane induced reproductive toxicity in male Wistar rats. Bull Environ Contam Toxicol , 84: 378–84.

27. **Ashok P, Meenakshi B (2004):** Contraceptive effect of *Curcuma longa* (L.) in male albino rat. Asia J Androl 6: 71– 4.
28. **Mishra R, Singh S (2009):** Reversible antifertility effect of aqueous rhizome extract of *Curcuma longa* L. in male laboratory mice. Contraception, 79: 479–87.
29. **Ghosh D, Das U, Ghosh S *et al*., (2002):** Testicular gametogenic and steroidogenic activities in cyclophosphamide treated rat: A correlative study with testicular oxidative stress. Drug Chem Toxicol, 25:281–92.
30. **Peltola V, Huhtaniemi I, Metsa-Ketela T *et al*., (1996):** Induction of lipid peroxidation during steroidogenesis in the rat testis. Endocrinol,137:105–12.
31. **Chainy G, Samantaray S, Samanta L (1997):** Testosterone-induced changes in testicular antioxidant system. Andrologia,29:343–9.
32. **Chaki S, Misro M, Gautam D *et al*., (2006):** Estradiol treatment induces testicular oxidative stress and germ cell apoptosis in rats. Apoptosis,11:1427–37.