**Journal of Endocrinology and Reproduction,** *Vol 20(2), 118-125, 2016, DOI: 10.18311/jer/2016/15471*
*ISSN (Print) : 0971-913X*
*ISSN (Online) : 2455–1244*

# Effect of *Avena sativa* (Oats) on Spermatogenesis and Reproductive Health

**Vara Prasad Saka*[1], Siva Reddy Challa[2] and Akondi Butchi Raju[3]**

[1]*Department of Pharmacology, Vignan Pharmacy College, Vadlamudi, Guntur District, Andhra Pradesh, India.
[2]Department of pharmacology, K.V.S.R.Siddhartha College of Pharmaceutical sciences, Vijayawada, Andhra Pradesh, India.
[3]Department of Clinical Pharmacy and Pharmacology, Ibn Sina National College for Medical Studies; drraju2020@gmail.com

## Abstract

Infertility is a major problem of the day amongst men and women and marked by no pregnancy even after one year of unprotected intercourse. Almost 30% of infertility has been related with male factors, concerning sperm-low concentration, poor motility, decreased viability, and deformities. Factors like pollution, drugs, stress, life style changes, toxicants and nutritional deficiencies inflict deleterious effects on reproductive health, especially spermatogenesis. Fluoride is one such potent toxicant to which humans are exposed. The problem of fluorosis is known for long in India, especially in Andhra Pradesh. It was reported in several studies that fluoride interferes with the structural and functional integrity of the male reproductive system resulting in male factor infertility. Oat, *Avena sativa,* has a wide range of chemical and mineral constituents. The present research has been undertaken to evaluate the efficacy of oats as a nutrient and food supplement to lessen the fluoride-induced infertility in male rats. In this study fluoride, at a dose of 0.01 g/kg body weight, administered through oral route, induced infertility by causing damage to histoarchitecture of the testis and decrease in the levels of plasma testosterone, FSH, and LH. Treatment with hydroalcoholic extract of oats resulted in decreased damage to the reproductive organs and lesser impact on sperm parameters- sperm count, viability, morphology, motility, etc. Form the present study it is concluded that oats has the ability to decrease the toxic effect of fluoride.

**Keywords:** *Avena sativa*(oats), Male Infertility, Spermatogeresis, Fluoride

## 1. Introduction

Infertility can be defined as a lack of pregnancy after a year of unprotected regular intercourse[1]. The number of infertility cases around the world is increasing and, currently, between 15% and 20% of couples of reproductive age are infertile. Recent reports suggest that approximately 30% of infertility is due to a male factor[2] wherein low sperm count is the main cause. The total sperm count, motility and normal morphological characteristics are generally measured in men and, in most cases, these indices are clearly defined[4]. A couple of conditions can interfere with spermatogenesis and cause decline in sperm counts and quality. Factors such as therapeutics, in particular anti-cancer and antimicrobial drugs, toxins, polluted water or air, and malnutrition, can all produce deleterious effects on sperm production and fertility[1]. Many environmental toxins and irradiation are well studied worldwide in relation to deterioration of semen quality[5,6].

Fluoride is one of the vulnerable toxicants to which human beings are exposed[7]. The problem of fluorosis has been quite well known in India. When the fluoride concentration exceeds 1.5 mg per liter of water, it affects the development of teeth during the stage of calcification in children. Fluoride toxicity can damage the lungs and fetus. Latest reports affirm the relationship between fluoride lev-

*Author for correspondence

els in drinking water and increasing infertility[8]. Fluoride is believed to produce abnormalities in the structural and functional integrity of the testis, epididymis and vas deferens. Animal studies have shown the effect of fluoride on morphology and metabolism of spermatozoa. High fluoride intake can lead to Zn deficiency in the testes and male reproduction[9,10] leading to reduction in testosterone levels that is critically necessary for testicular development and maintenance[9,11]. High fluoride content is also reported to increase oxidative stress in the testes affecting spermatogenesis[9,11,12].

The grass/Poaceae family of oats (*Avena sativa* L.), is an annual green grass[3,14,15]. *A. sativa* originated in England, France, Poland, Germany and Russia and is now grown all around the world. The word Avena is derived from the Sanskrit word "avi", which means "sheep" or "avasa" meaning "food". In traditional medicine, oat is used to treat nervous debility, insomnia and nerve weakness. It is considered as a good antispasmodic, antitumoral, cyanogenic, demulcan, diuretic, neurotonic, stimulant, and tonic. Several studies have reported that oat and its constituents have various pharmacological effects such as reduction of blood cholesterol and sugar levels, immunomodulation, anti-cancer, antioxidant, antiatherogenic, and anti-inflammatory besides control of childhood asthma, body weight, etc. Oatmeal is rich in protein, has many beneficial minerals like iron (Fe), calcium (Ca), potassium (K), magnesium (Mg), copper (Cu), zinc (Zn), and silicon (Si), fatty acids, selenium (Se) and a number of vitamins such as Vitamin B1, B2, B6, B12, Niacin, Vitamin C, Vitamin A, and Vitamin E.

Selenium works with Vitamin E in various essential cancer prevention agent frameworks all through the body[16]. Likewise, it also works with glutathione peroxide (GSH-PX), a protein that ensures the internal structures of cells against free radicals. It is a cancer prevention agent by acting at the level of lipids of the cell membrane[17].

Zn plays a key role in spermatogenesis[18–23] and reverses fluoride toxicity[9,10]. Also, vitamin E has been proved to reverse fluoride toxicity in mice[7]. As oats are rich in selenium, vitamin E and zinc, the present study focused on finding if fluoride induced infertility in male albino rats could be reversed by oats.

# 2. Materials and Methods

## 2.1 Plant Material and Preparation

*Avena sativa* (oat) whole grains were purchased from local grains shop and dried under shade.Oat grains were milled and passed through #10 mesh screen. The oat powder was mixed with the solvent in 1:4 ratio and then added to 50% v/v aqueous ethanol solution at 40$^0$C. The resultant blend was then mixed by swirling for 60 minutes, then cooled to room temperature. This blend was centrifuged for 7 min to separate the supernatant. The pellet was resuspended in solvent and centrifuged. The supernatant was then gathered, dried and pulverized[24].

## 2.2 Chemicals

Sodium fluoride from Loba Chemie (Mumbai, India) was used as infertility inducing agent. Testosterone propionate from Sigma Aldrich was used as standard medication. Physiological saline was utilized as vehicle for oat extract and sodium fluoride, and arachis oil was used as vehicle for testosterone propionate.

## 2.3 Animals, Maintenance and Experimentation

The protocol for the experimentation was approved by IAEC (approval no: 007/IAEC/NCPA/M. Pharm/2012-13). Male Wistar albino rats were obtained from Gosh and Gosh Enterprises, Kolkata, and maintained in animal house at 25±2 °C temperature with 12 hr dark and light cycle with standard pellet diet and water *ad libitum*.

Male albino rats were divided into 6 groups of 6 each. Rats in group I served as control and received normal saline. Rats in group II were administered with sodium fluoride, dissolved in normal saline, at a daily dose of 10 mg/kg body weight through oral route for 30 days. The dose was mainly based on the $LD_{50}$ for rat, 250 mg/kg body weight, and the present dose is 1/25th of the $LD_{50}$[25]. Rats in group III were administered with oat extract, dissolved in normal saline, at a daily dose of 400 mg/kg body weight dose, through oral route. Rats in groups IV andV were administered with oats extract at 200 and 400 mg/kg body weight along with NaF at 10 mg/kg body weight through oral route. Rats in group VI were administered with testosterone propionate at a dose of 0.5 mg/kg body weight through subcutaneous route[26]. All the groups were treated for 28 days[1,27] and sacrificed 24 hr after the last dose.

## 2.4 Analyses

At the time of sacrifice the rats were first weighed and then subjected to cervical dislocation[28,29]. The abdominal cavity was dissected to expose the reproductive organs.

The testes were removed and cleared free of mucus and surrounding tissue[28].

The testes were weighed in an electronic balance, while the testis volumes were measured using immersion method[28].

The content of cauda epididymal duct was released in 0.5 ml of normal saline taken in a petri dish and the temperature was maintained at 37 °C[1,30]. The sperm count was carried out using a haemocytometer[29,31]. The sperm in four corner squares were counted[29,31]. A drop of sperm suspension was placed on a clear glass slide and then covered with a cover slip. The slide was then examined in a microscope at 400x, and the motility was scored in the different fields of view. Spermatozoa showing any degree of movement were considered to be motile. The spermatozoa (motile as well as immotile, separately) were counted.

Sperm viability was assessed using a supravital staining technique based on the principle that cells with damaged plasma membrane take up the stain, whereas viable ones do not. All glassware as well as the eosin–Nigrosine stain were maintained at 37 °C. Spermatozoa appearing pinkish (stained) were considered as dead, whereas those appearing colorless (unstained) were counted as viable. Using the same preparation, sperm morphology was determined. Abnormalities were classified as headless sperm, banana head, bent neck and bent tail[5]. The sperm were classified into normal and abnormal. The total sperm abnormality was expressed as percentage incidence[33].

Blood was collected from rats by cardiac puncture, centrifuged plasma was separated and stored for hormonal assays. Plasma testosterone, luteinizing hormone, and follicle stimulating hormone levels were determined by chemilumin escence-immunoassay using immunoassay kits and ADVIA Centaur immuno-analyzer.

The testis was fixed in Bouin's fluid for 24 hr for histological analysis. The tissues were dehydrated in an ascending series of alcohol, treated with xylene, and embedded in paraffin wax. Sections of 6-µm thickness were cut in a rotary microtome and stained with hematoxylin and eosin. The permanently mounted sections were viewed in a microscope, and images were captured ina digital camera[1].

### 2.5 Statistical Analysis

Data were analyzed by one-way ANOVA with Tukey's post hoc test using graph pad prism software, and expressed as mean ± SEM. P≤0.01 was considered as statistically significant.

## 3. Results

### 3.1 Body and Organ Weights

As seen in Table 1, in the fluoride treated rats body as well as organ weights decreased significantly. In the rats treated fluoride accompanied by oats extract at 200 mg/kg body weight there was no significant difference when compared with disease control group. But when fluoride treated rats were also treatedwith oat extract at 400 mg/kg body weight there was significant increase in the body weight as well as organ weight. This indicates that oat

**Table 1:** Effect of oat extract on fluoride induced changes in weight parameters on male rats.

| Group/parameter | Body weight (gm.) | | Testis weight (mg) |
|---|---|---|---|
| | Initial | Final | |
| Normal control | 200.2±4.672 | 211.3±3.293 | 0.933±0.0123 |
| NaF (10 mg/kg) treated | 200.7±2.753 | 181.0±6.506[c] | 0.826±0.0203[b] |
| Oat extract (400 mg/kg) only | 203.3±4.410 | 217.3±2.871 | 0.986±0.00232 |
| NaF - oat extract (200 mg/kg) | 202.2±3.609 | 194.8±2.482 | 0.833±0.0195 |
| NaF + oat extract (400 mg/kg) | 201.3±2.186 | 202.3±0.9545[#] | 0.970±0.00404[$] |
| Testosterone (0.5 mg/kg) | 202.2±3.167 | 221.0±2.840[c] | 1.07±0.0319[b] |

Values are expressed as mean ±SEM of each group (n=6) and are significant when done ONE WAY ANOVA with Tukey's post hoc test. 'b' P<0.01; 'c' p<0.001 when compared with normal control; '#'p<0.01; $p<0.001 when compared with disease control.

**Table: 2** Effect of oat extract on fluoride induced changes in semen parameters of male rats.

| Group/parameter | Sperm count (millions/ml) | Sperm motility (%) | Sperm viability (%) | Sperm morphology (%) |
|---|---|---|---|---|
| Normal control | 29.17±0.4773 | 30.50±0.6708 | 61.83±1.67 | 29.50±1.088 |
| NaF (10 mg/kg) treated | 22.67±1.145[a] | 11.83±1.721[c] | 32.67±1.453[c] | 62.33±1.054[c] |
| Oat extract (400 mg/kg) only | 40.83±1.302[c] | 32.83±1.302[c] | 64.33±2.261[c] | 29.33±0.8028[c] |
| NaF + oat extract (200 mg/kg) | 31.83±1.108[$] | 21.17±1.302[$] | 49.83±2.638[$] | 51.83±1.167[$] |
| NaF + oat extract (400 mg/kg) | 38.50±1.478[$] | 32.67±1.145[$] | 61.33±1.358[$] | 43.00±1.528[$] |
| Testosterone (0.5 mg/kg) | 50.83±1.641[c] | 70.83±1.641[c] | 71.00±1.414[c] | 20.50±1.310[c] |

Values are expressed as mean ±SEM of each group (n=6) and are significant when done ONE WAY ANOVA with Tukey's post hoc test. 'a' P<0.05; 'c'<0.001 when compared with normal control; $p<0.001 when compared with disease control.

extract at 400 mg/kg treatment obviated the weight loss caused by fluoride treatment.

## 3.2 Semen Parameters

In the fluoride treated animals sperm count, viability and motility were significantly reduced at p<0.001 when compared with the respective controls (Table 2). However, co-administration of oat extract with fluoride attenuated the decrease in sperm count, viability and motility. There was a significant increase (P < 0.001) in sperm with abnormal morphologies in fluoride treated rats; however, co-administration of oat extract reduced (p<0.001) the percentage abnormal morphologies, although the number did not reach the saline control value.

## 3.3 Hormone Levels

Levels of FSH, LH and testosterone decreased in the fluoride treated rats (Table 3). Co-administration of oat extract to fluoride-treated rats increased the levels of FSH and testosterone above the control, whereas the LH level also was significantly increased but slightly lesser than control value.

## 3.4 Histopathological Changes

Sections of testis of healthy control rats revealed normal histoarchitecture. There were uniform, well-organized seminiferous tubules with all stages of spermatogenesis and normal interstitial tissue (Figure 1A). Testicular tissue of rats that received only fluoride showed degenerative changes in the majority of the seminiferous tubules (Figure 1B). These changes were characterized by shrunken, disorganized seminiferous tubules with irregular, buckled basement membrane and incomplete spermatogenesis. Moreover, the seminiferous tubules were almost devoid of spermatids and spermatozoa. Vacuolar degeneration of spermatogonia and Sertoli cells was evident. Degenerated germinal epithelial cells were sloughed into the lumina of most seminiferous tubules. The histological findings of the testicular sections of rats that received only oat extract (Figure 1C) showed normal and healthy histoarchitecture in which well-developed seminiferous tubules with complete spermaotgenesis and intact interstitial tissue were seen along with the uniformly distributed Leydig cells which were absent in fluoride treated animals. Testicular sections of the rats treated oat extract (200 mg/kg body weight) along with fluoride (Figure 1D) showed well-organized seminiferous tubules with incomplete spermatogenesis, and the seminiferous epithelium was sloughed into the lumen of the seminiferous tubules. Testicular sections of rats that received oat extract (400 mg/kg) (Figure 1E) along with fluoride showed the well-organized seminiferous tubules with complete spermatogenesis. Well-developed spermatids and uniform connective tissue were seen in the interstitial space and resembled the histoarchitecture of the normal and testosterone-treated rats. Well organized and completely developed seminiferous tubules with spermatids, spermatozoa and complete spermaotgenesis and also with uniformly distributed Leydig cells in the con-

nective tissue filling the interstitial space were observed in the testicular sections of the rats treated with testosterone (0.5 mg/kg) as reference standard (Figure 1F). The histological findings showed that oat extract at a dose of 400 mg/kg significantly reversed the fluoride toxicity and maintained the histoarchitecture of the reproductive organ of the rats by marked improvement in spermatogenesis evidenced by the presence of well-organized seminiferous tubules and well developed Leydig cells.

### *3.4.1 Histological Examination*

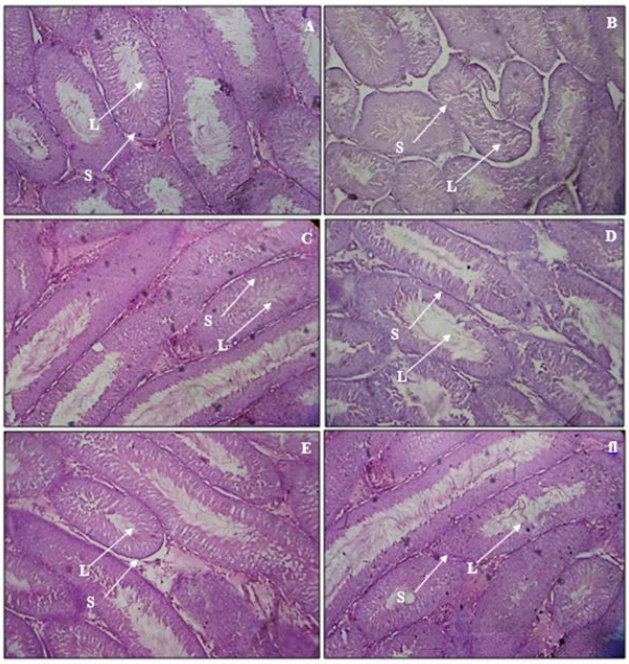


**Figure 1.** Histopathological observation of the effect of hydro-alcoholic extract of *Avena sativa* on fluoride induced testicular toxicity; L indicates eumen of seminiferous tubule; ST indicates seminiferous tubule. A: Normal; B: Fluoride treated; C: Oats alone; D: Fluoride+Oats 200 mg/kg; E: Fluoride + Oats 400 mg/kg; F: Fluoride+Testosterone treated.

## 4. Discussion

In the present study, the role of *Avena sativa* in preventing the infertility induced by sodium fluoride was studied. The model employed in this work has been used previously by several investigators to assess the toxicity of fluoride on fertility and reproduction in laboratory animals[7–9,25,34,35,37]. The concentration of NaF used in this study was chosen according to previous studies[25]. The drinking water route of exposure was chosen to mimic human exposure and to reflect the impact on fertility of the sustained blood levels of fluoride that would occur from water consumption throughout the day[35].

The results of present study show that sodium fluoride treatment would cause a significant decrease in the body weight. Similar results were reported by other workers in rats and mice fed different concentrations of fluoride[78] and it is due to oxidative damage[38] to the body tissues. However, the extract of *Avena sativa* when administered along with sodium fluoride maintainedthe body and organ weights at normal significantly and it might be the result of antioxidant activity of *Avena sativa*[13,15,16].

In the present study the count, motility and viability of epididymal sperm in NaF treated group of rats declined significantly and increased in abnormal morphologies. The decrease could be correlated with the testicular spermatogenic arrest and decline in fertility following fluoride ingestion in mice, rats and rabbits[7,8,9,25,39]. The hormones, like testosterone, FSH and LH were decreased significantly due to fluoride intoxication.

Fluoride toxicity led to the decline in sperm count via hormonal imbalance[9], structural and functional defects in spermatozoa[40,41] and alterations in epididymis and accessory reproductive glands[40]. Fluoride toxicity resulted in sloughing off the spermatogenic cells in seminiferous tubules of testes leading to disorganization of their epithelium[42], and hampered steriodo genesis and spermatogenesis. Fluoride intoxication causes oxidative damage[38], and zinc deficiency, disturbs signal transduction[9] and suppresses testosterone level[43–46]. Fluoride directly interferes with spermatogenesis by depressing EGF and EGFR[47], modifying G-protein signaling[48], diminishing the levels of Androgen Receptor (AR), disturbing the levels of estradiol and increasing the levels of FSH and LH[9,44,45]. Though decrease in estrogen levels is known to increase FSH acting in the negative feedback loop, an F-induced decrease in estradiol level may reduce FSH, leading to decreased spermatogenesis[9].

In previous studies, it has been reported that high fluoride intake can lead to zinc deficiency in testes and the accessory male reproductive organs[9,49,50]. Zinc deficiency may suppress the testosterone levels critically necessary for testis development[9,46] and, more importantly, it increases oxidative stress in testes leading to poorer quality spermatozoa[9,12,46,51].

The mechanism by which fluoride affects sperm motility has not been clearly elucidated. However, it has been postulated that fluoride could decrease the level of

**Table: 3** Effect of oat extract on fluoride induced changes in hormonal parameters of male rats.

| Group/parameter | Serum testosterone (ng/dl) | FSH (mIU/ml) | LH (mIU/ml) |
|---|---|---|---|
| Normal control | 57.51±1.570 | 0.1900±0.005774 | 0.2350±0.01176 |
| NaF (10 mg/kg) treated | 51.51±0.8232[b] | 0.1450±0.009916[a] | 0.1650±0.01668[c] |
| Oat extract (400 mg/kg) only | 64.47±1.133[c] | 0.2400±0.01065[b] | 0.2370±0.01269[c] |
| NaF + oat extract (200 mg/kg) | 58.86±1.378[$] | 0.1950±0.009916[#] | 0.2050±0.009916 |
| NaF + oat extract (400 mg/kg) | 63.32±0.9058[$] | 0.2250±0.007638[$] | 0.2150±0.009916 |
| Testosterone (0.5 mg/kg) | 70.39±0.534[c] | 0.3100±0.007303[c] | 0.3983±0.01662[c] |

Values are expressed as mean ±SEM of each group (n=6) and are significant when done ONE WAY ANOVA with Tukey's post hoc test. 'a' P<0.05; 'b' P<0.01; 'c'<0.001 when compared with normal control; #P<0.01;$p<0.001 when compared with disease control.

fructose, the sugar, which provides energy to sperm for motility[7], by inhibiting many enzymes like enolases, and acid and alkaline phosphatases by binding with cofactors like Mg, Ca, Zn, and Se[52] and, thus, inhibit glycolysis, respiration and motility of sperm[9]. The testicular oxidative stress caused by fluoride has been shown to result in damage of sperm cell membrane which might accountable for inhibition of spermatogenesis and impairment of sperm activity[53].

This study reveals the therapeutic effect of *Avena sativa* extract in significantly preventing these changes from occurring. This might be due to the presence of zinc, vitamin E, selenium, and antioxidants like aventhramides in *Avena sativa*[15]. Zinc[23] is involved in initiation of spermatogenesis by ribonuclease activity[54], by participating in spermatozoa maturation[54] and preserving seminiferous tubules and the germinal epithelium[55], and also by enhancing sperm motility[54]. Vitamin E is also important in maintaining the male reproductive health. The role of Vitamin E in the reproductive system is elaborately studied and it has been demonstrated that Vitamin E also facilitates reproductive health by supporting testicular and epididymal structure and function[15,23,56–58].

This study leads to the conclusion that *Avena sativa,* which is in vitamin E, aventhramides rich like antioxidants and several minerals like zinc, selenium etc., has the potential to be protective and preventive in the context of fluoride-induced impairment of male reproductive structure and function.

## 5. References


1. Sharma V, Boonen J, Spiegeleer BD, Dixit VK. Androgenic and spermatogenic activity of alkylamide-rich ethanol solution extract of *Anacyclus pyrethrum* DC. Phytother Res. 2013; 27(1):99–106. https://doi.org/10.1002/ptr.4697 PMid:22473789
2. Agarwal A, Allamaneni SSR. Oxidants and antioxidants in human fertility. Middle East Fertil Soc J. 2004; 9(3):187–97.
3. Yang WM, Kim HY, Park SY, Kim HM, Chang MS, Park SK. *Cynomorium songaricum* induces spermatogenesis with Glial Cell-Derived Neurotrophic Factor (GDNF) enhancement in rat testes. J Ethnopharmacol. 2010; 128(3):693–6. https://doi.org/10.1016/j.jep.2010.02.020 PMid:20219665
4. Mahdi AA, Shukla KK, Ahmad MK, Rajender S, Shankhwar SN, Singh V, Dalela D. *Withania somnifera* improves semen quality in stress-related male fertility. Evidence-Based Complement Altern Med. 2011; 9:1-8. https://doi.org/10.1093/ecam/nep138 PMid:19789214 PMCid:PMC3136684
5. Morakinyo AO, Achema PU, Adegoke O A. Effect of *Zingiber officinale* (Ginger) on sodium arsenite-induced reproductive toxicity in male rats. Afr J Biomed Res. 2010; 13:39–45.
6. Sarkar R, Mohanakumar KP, Chowdhury M. Effects of an organophosphate pesticide, quinalphos, on the hypothalamo-pituitary-gonadal axis in adult male rats. J Reprod Fertil. 2000; 118:29–38. https://doi.org/10.1530/jrf.0.1180029 PMid:10793623
7. Chinoy N, Arti S. Amelioration of fluoride toxicity by vitamins E and D in reproductive functions of male mice. Fluoride. 1998; 31(4):203–16.

8. Sharma JD, Solanki D, Mamta S. Amelioration of fluoride toxicity in rats through vitamins (C, D) and calcium. Toxicol Int. 2013; 15(2):111–6.
9. Hu L, Ying J, Mu L, Yu S, Liang Z, Carole C. Fluoride toxicity in the male reproductive system. Fluoride. 2009; 42(4):260–76.
10. Krasowska A, Wlostowski T, Bonda E. Zinc protection from fluoride-induced testicular injury in the bank vole (*Clethrionomys glareolus*). Toxicol Lett. 2004; 147(3):229–35. https://doi.org/10.1016/j.toxlet.2003.11.012 PMid:15104114
11. El-Seweidy MM, Hashem RM, Abo-Elmathy DM, Mahamed RH. Frequent inadequate supply of micronutrients in fast food induces oxidative stress and inflammation in testicular tissues of weanling rats. J Pharm Pharmacol; 2008; 60(9):1237–43. https://doi.org/10.1211/jpp.60.9.0017 PMid:18718129
12. Nair N, Bedwal S, Prasad S, Saini MR, Bedwal RS. Short-term zinc deficiency in diet induces increased oxidative stress in testes and epididymis of rats. Indian J Exp Biol. 2005; 43(9):786–94. PMid:16187529
13. Chatuevedi N, Sachdev Y, Shukla K. Diversified therapeutic potential of *Avena sativa*: An exhaustive review. Asian J Plant Sci Res. 2011; 1:103–14.
14. Ola SM, Mostafa MS, Zeinab YA, Hanan AA, Heba SM. Improving effect of dietary oat bran supplementation on oxidative stress induced by hyperlipidemic diet. Researcher. 2011; 3:1–10.
15. Rajinder S, Subrata De, Asma B. *Avena sativa* (Oat), a potential neutraceutical and therapeutic agent: An overview. Crit Rev Food Sci Nutr. 2013; 53:126–44. https://doi.org/10.1080/10408398.2010.526725 PMid:23072529
16. Emmons CL, David MP, Gregory LP. Antioxidant capacity of oat (*Avena sativa* L.) extracts. 2. In vitro antioxidant aetiv ity and contents of phenolic and tocol antioxidants. J Agric Food Chem. 1999; 47:4894–8. https://doi.org/10.1021/jf990530i PMid:10606549
17. Kamran D, Sayed MA, Aram M. Effects of in vitro selenium addition to the semen extender on the spermatozoa characteristics before and after freezing in water buffaloes (*Bubalus bubalis*). Vet Res Forum. 2012; 3:263–8.
18. Abdella AM, Elabed BH, Bakhiet AO, Gadir WSA, Adam SEI. In vivo study on lead, cadmium and zinc supplementations on spermatogenesis in albino rats. J Pharmacol Technol. 201; 6(2):141–8.
19. Deepa K, Neena N, Ranveer SB. Effect of dietary zinc deficiency on testes of Wistar rats: Morphometric and cell quantification studies. J Trace Elem Med Biol. 2011; 25:47–53. https://doi.org/10.1016/j.jtemb.2010.11.002 PMid:21145718
20. Sørensen MB, Bergdahl IA, Hjøllund NHI, Bonde JPE, Stoltenberg M, Ernst E. Zinc, magnesium and calcium in human seminal fluid: Relations to other semen parameters and fertility. Mol Hum Reprod. 1999; 5:331–7. https://doi.org/10.1093/molehr/5.4.331 PMid:10321804
21. Croxford TP, McCormick NH, Kelleher SL. Moderate zinc deficiency reduces testicular Zip 6 and Zip 10 abundance and impairs spermatogenesis in mice. J Nutr. 2011 Mar; 141(3):359–65. https://doi.org/10.3945/jn.110.131318 PMid:21248196 PMCid:PMC3040901
22. Tuncer I, Sunar F, Toy H, Baltaci AK, Mogulkoc R. Histological effects of zinc and melatonin on rat testes. Bratisl Lek Listy. 2011; 112:425–7.
23. Yunsang C, Yang W. Functions of essential nutrition for high quality spermatogenesis. Adv Biosci Biotechnol. 2011; 2:182–97. https://doi.org/10.4236/abb.2011.24029
24. Ronald GT. Method for producing oat extract. United States Patent; 1995. 5468491.
25. Chinoy NJ, Shruti S, Amita SW, Bhattacharya S. Fluoride toxicity on rat testis and cauda epididymal tissue components and its reversal. Fluoride. 1997; 30:41–50.
26. Chauhan NS, Dixit VK. Spermatogenic activity of rhizomes of *Orchioides gaertin* male rats. Int J Appl Res Nat Prod. 2008; 1:26–31.
27. Nassiri M, Khaki A, Ahmadi-Ashtiani HR, Rezazadeh SH, Rastgar H, Gharachurlu SH. Effects of ginger on spermatogenesis in streptozotocin-induced diabetic rat. J Med Plants. 2009; 8:1–7.
28. Godson GA, Oluwaseyi SO, Chia LS, Babatunde O, Ayomide JB, Emmanuel OS. Ameliorative effect of *Moringa oleifera* (drumstick) leaf extracts on chromium-induced testicular toxicity in rat testes. World J Life Sci Med Res. 2012; 2:20–26.
29. Rahul BP, Shreya RV, Meena MP. Protective effect of spermatogenic activity of *Withania somnifera* (Ashwagandha) in galactose-stressed mice. Ann Biol Res. 2012; 3:4159–65.
30. Sethi S, Chaturvedi CM. Temporal synergism of neurotransmitters (serotonin and dopamine) affects testicular development in mice. Zoology. 2009; 112:461–70. https://doi.org/10.1016/j.zool.2009.03.002 PMid:19765962
31. Rahul BP, Shreya RV, Meena MP. Spermatogenic activity of dietary antioxidant in oxidatively stressed mice. J Cell Tissue Res. 2008; 8:1519–24.
32. Ibukun PO, RajiY, Benjamin OE, Adeyombo FB. Effects of nicotine on sperm characteristics and fertility profile in adult male rats: A possible role of cessation. J Reprod Infertil. 2011; 12:201–7.
33. Khaki A, Fathiazad F, Nouri M, Khaki AA, Khamenehi HJ, Hamadeh M. Evaluation of androgenic activity of *Allium cepa* on spermatogenesis in the rat. Folia Morphol. 2009; 68:45–51.

34. Bataineh HN, Nusier MK. Impact of 12-week ingestion of sodium fluoride on aggression, sexual behavior, and fertility in adult male rats. Fluoride. 2006; 39:293–301.
35. Ahmed E, Homa D, Ahmad SAH. Fertility effects of sodium fluoride in male mice. Fluoride. 2000; 33:128–34.
36. Chinoy NJ, Mehta D, Jhala DD. Effects of fluoride ingestion with protein-deficient or protein-enriched diets on sperm function of mice. Fluoride. 2006; 39:11–16.
37. Messer HH, Armstrong WD, Singer L. Influence of fluoride uptake on reproduction in mice. J Nutr. 1976; 106:1115–22.
38. De Lamirande E, Gagnon C. Impact of reactive oxygen species on spermatozoa: A balancing act between beneficial and detrimental effects. Hum Reprod. 1995; 1:15–21. https://doi.org/10.1093/humrep/10.suppl_1.15
39. Chinoy NJ, Mehta D, Jhala DD. Effects of fluoride on physiology of some animals and human beings. Indian J Environ Toxicol. 1991; 1:17–32.
40. Chinoy NF, Narayana MV, Dalal V, Rawat M, Patel D. Amelioration of fluoride toxicity in some accessory reproductive glands and spermatozoa of rat. Fluoride. 1995; 28:75–86.
41. Kumar A, Susheela AK. Ultrastructural studies of spermiogenesis in rabbit exposed to chronic fluoride toxicity. Int J Fertil Monopausal Stud. 1994; 39:164–71. PMid:7920753
42. Shashi A. Histopathological changes in rabbit testis during experimental fluorosis. Folia Morphol. 1990; 38:63–5.
43. Chinoy NJ, Narayana MV, Sequeira E, Joshi SM, Barot JM, Purohit RM. Studies on effects of fluoride in 36 villages of Mehsana District, North Gujarat. Fluoride. 1992; 25:101–10.
44. Ortiz-Perez D, Rodriguez-Martinez M, Martinez F, Borja-Aburto VH, Castelo J, Grimaldo JI. Fluoride-induced disruption of reproductive hormones in men. Environ Res. 2003; 93:20–30. https://doi.org/10.1016/S0013-9351(03)00059-8
45. Tokar VI, Savchenko ON. Effect of inorganic fluorine compounds on the functional state of the pituitary-testis system. Probl Endokrinol (Mosk). 1977; 23:104–7.
46. El-Seweidy MM, Hashem RM, Abo-Elmathy DM, Mahamed RH. Frequent inadequate supply of micronutrients in fast food induces oxidative stress and inflammation in testicular tissues of weanling rats. J Pharm Pharmacol. 2008; 60:1237–43. https://doi.org/10.1211/jpp.60.9.0017 PMid:18718129
47. Wan S, Zhang J, Wang J. Effect of high fluoride on sperm quality and testicular histology in male rats. Fluoride. 2006; 39:17–21.
48. Chabre M, Aluminofluoride and beryllium fluoride complexes: A new phosphate analog in enzymology. Trends Biochem Sci. 1990; 15:17–21. https://doi.org/10.1016/0968-0004(90)90117-T
49. Krasowska A, Wlostowski T. Photoperiodic elevation of testicular zinc protects seminiferous tubules against fluoride toxicity in the bank vole (*Clethrionomys glareolus*). Comp Biochem Physiol. C Pharmacol Toxicol Endocrinol. 1996; 113:81–4. https://doi.org/10.1016/0742-8413(95)02049-7
50. Krasowska A, Wlostowski T, Bonda E. Zinc protection from fluoride-induced testicular injury in the bank vole (*Clethrionomys glareolus*). Toxicol Lett. 2004; 147:229–35. https://doi.org/10.1016/j.toxlet.2003.11.012 PMid:15104114
51. Oteiza PL, Olin KL, Fraga CG, Keen CL. Oxidant defense systems in testes from zinc deficient rats. Proc Soc Exp Biol Med. 1996; 213:85–91. https://doi.org/10.3181/00379727-213-44040 PMid:8820828
52. Zakrzewska H, Udala J, Blaszczyk B. In vitro influence of sodium fluoride on ram semen quality and enzyme activities. Fluoride. 2002; 35:153–60.
53. Singh PK, Feroz AD, Sheeba H, Khalil A, Samir AM. Beneficial effect of *Tamarindus indica* on the testes of albino rat after fluoride intoxication. Int J Pharmacol Bio Sci. 2012; 3:487–93.
54. Hidiroglou M, Knipfel JE. Zinc in mammalian sperm. J Dairy Sci. 1984; 67:1147–56. https://doi.org/10.3168/jds.S0022-0302(84)81416-2
55. Mason KE, Burns WA, Smith Jr. Testicular damage associated with zinc deficiency in pre- and post-pubertal rats: Response to zinc repletion. J Nutr. 1982; 112:1019–28. https://doi.org/10.1093/jn/112.5.1019 PMid:7077414
56. Wang S, Wang G, Barton BE, Murphy TF, Huang HF. Beneficial effects of vitamin E in sperm functions in the rat after spinal cord injury. J Androl. 2007; 28(2):334–41. https://doi.org/10.2164/jandrol.106.001164 PMid:17079740
57. Bratt K, Sunnerheim K, Bryngelsson S, Fagerlund A, Engman L, Andersson RE, Dimberg LH. Avenanthramides in oats (*Avena sativa* L.) and structure-antioxidant activity relationship. J Agric Food Chem. 2003; 51(3):594–600. https://doi.org/10.1021/jf020544f PMid:12537428
58. Xu JG, Tian CR, Hu QP, Luo JY, Wang XD, Tian XD. Dynamic changes in phenolic compounds and antioxidant activity in oats (*Avena nuda* L.) during steeping and germination. J Agric Food Chem. 2009; 57(21):10392–8. https://doi.org/10.1021/jf902778j PMid:19827789