

Pumpkin Seed Oil and Vitamin E Improve Reproductive Function of Male Rats Inflicted by Testicular Injury



Prof.

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فَلَوْلَا أَنَّهُ كَانَ مِنَ الْمُسَبِّحِينَ (143) لَلَبِثَ فِي بَطْنِهِ إِلَى يَوْمِ يُبْعَثُونَ (144) ﴿٦﴾ فَنَبَذْنَاهُ بِالْعَرَاءِ وَهُوَ سَقِيمٌ (145) وَأَنْبَتْنَا عَلَيْهِ شَجَرَةً مِّنْ يَقْطِينٍ (146). سورة الصافات

وفي صحيح البخاري عن أنس بن مالك رضي الله عنه قال: إِنَّ خَيْطًا دَعَا رَسُولَ اللَّهِ صَلَّى اللَّهُ عَلَيْهِ وَسَلَّمَ لِبَطْنِهِ، قَالَ أَنَسٌ: فَذَهَبْتُ مَعَ رَسُولِ اللَّهِ صَلَّى اللَّهُ عَلَيْهِ وَسَلَّمَ إِلَى ذَلِكَ الطَّعَامِ، فَقَرَّبَ إِلَيَّ رَسُولُ اللَّهِ صَلَّى اللَّهُ عَلَيْهِ وَسَلَّمَ خُبْزًا مِنْ شَعِيرٍ، وَمَرَقًا فِيهِ دُبَّاءٌ وَقَدِيدٌ، قَالَ أَنَسٌ: فَرَأَيْتُ رَسُولَ اللَّهِ صَلَّى اللَّهُ عَلَيْهِ وَسَلَّمَ يَتَّبَعُ الدُّبَّاءَ مِنْ حَوْلِ الصَّخْفَةِ، فَلَمْ أَزَلْ أُحِبُّ الدُّبَّاءَ مِنْ يَوْمِئِذٍ، وَقَالَ ثُمَامَةُ: عَنْ أَنَسٍ: فَجَعَلْتُ أُجْمَعُ الدُّبَّاءَ بَيْنَ يَدَيْهِ.

➤ INTRODUCTION

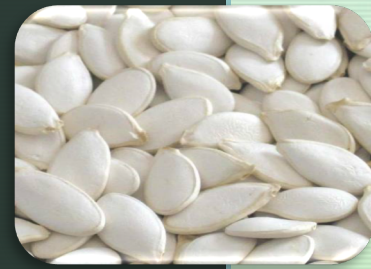
- Infertility is one of the major health problems in life and approximately about 30 % of this problem is due to male factors
- Several factors (as smoking, stress, drinking alcohol and increasing age) and diseases decrease male fertility
- Insufficient intake of vitamins has been reported to produce harmful effects on the process of spermatogenesis and production of normal sperms.



- dietary intake of antioxidants
- ▶ from natural products with vitamins E and C can protect sperm DNA from oxidative stress in the rat testis .
- Pumpkin seed oil (PSO) is rich in many antioxidants and beneficial nutritional components such as essential fatty acids, amino acids, phytosterols, B- carotenes, lutein and selenium .



- ▶ ■ Studies showed that vitamin E could normalize the damaging effect of oxidative stress induced by free radicals in rat testis and improve male fertility .
- The present study was designed to investigate the protective effect of pumpkin seed oil and vitamin E against sodium valproate -induced testicular injury in male rats. Tissue lipid peroxidation and antioxidant capacity as well as histopathology of testes were also carried out.



➤ MATERIALS AND METHODS

- **Pumpkin Seed Oil and Chemicals:**
 - **Pumpkin seed oil (PSO)** was purchased from Arab Company for vegetable oils extraction and refining (ARECO), Egypt.
 - The human therapeutic dose of PSO is 320 mg /day. equivalent rat dose of this oil was calculated using conversion table of Paget and Barnes .
 - **Vitamin E (α-tocopherol)** was obtained from Pharco Company for Pharmaceuticals, Alexandria, Egypt.



➤ MATERIALS AND METHODS

- **Hormonal Assay:**
 - **Serum testosterone** concentration was determined using radioimmunoassay (RIA).
 - **Serum levels of FSH and LH** were determined by enzyme linked immunosorbent assay (ELISA).



➤ **MATERIALS AND METHODS**

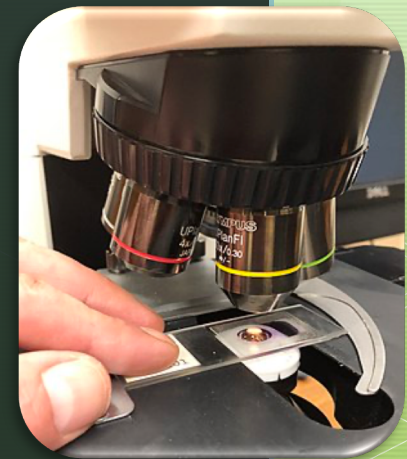
▪ **Semen Analysis:**

- The obtained semen content was diluted 10 times with 2.9% sodium citrate solution and rapidly examined to estimate the percentage of sperm progressive motility and sperm cell count .
- one drop of semen suspension was withdrawn, and examined microscopically to determine sperm vitality (alive/dead ratio).
- Other seminal smears were prepared and stained with Sperm Blue dye for examining sperm morphology .

➤ **MATERIALS AND METHODS**

■ **Histological Procedure:**

- The preserved specimens of left testes were trimmed, washed and dehydrated in ascending grades of alcohol.
- These specimens were cleared in xylene, embedded in paraffin, sectioned at 4-6 microns thickness using microtome and stained with Hematoxylin and Eosin (H&E) then examined microscopically.



➤ RESULTS:

- **oral administration of sodium valproate (SVP) in a dose of 500 mg/kg/day for 7 consecutive days to male rats**
 - induced a significant ($P < 0.05$) decrease in the weight of testes when compared with the negative control group.
- **Oral pretreatments with pumpkin seed oil (PSO), vitamin E (Vit E) and their combination**
 - significantly ($P < 0.05$) increased the weight of testes when compared with the positive control group.
 - Non significant changes were found in weights of seminal vesicles and prostate glands between the pretreated groups and the control groups .

Effect of pumpkin seed oil (PSO), vitamin E (Vit E) and their combination on weights of male sex organs of rats with testicular damage induced by sodium valproate (SVP)

Groups	Relative weight of sex organs (g/100 g b.wt.)		
	Testes	Seminal vesicles	Prostate glands
Group (1):Negative control	2.80 ± 0.03 _a	0.88 ± 0.02 _a	0.58 ± 0.01 _a
Group (2): Positive (SVP) control	1.24 ± 0.02 _b	0.82 ± 0.03 _a	0.56 ± 0.02 _a
Group (3):PSO (40 mg/kg)	1.48 ± 0.05 _b	0.83 ± 0.01 _a	0.55 ± 0.02 _a
Group (4):Vit E (200 mg/kg)	1.57 ± 0.03 _b	0.85 ± 0.03 _a	0.57 ± 0.01 _a
Group (5):PSO (40 mg/kg)+Vit E (200 mg/kg)	2.25 ± 0.04 _b	0.81 ± 0.02 _a	0.56 ± 0.02 _a

Means ± SEM with different superscript letters in the same column are significant at $P < 0.05$ using one-way ANOVA test and those with the same superscript letters are not significant. n = 9 rats.

The positive control group was compared with the negative control.

The pretreated groups were compared with the positive control group.

Effect of pumpkin seed oil (PSO), vitamin E (Vit E) and their combination on weights of male sex organs of rats with testicular damage induced by sodium valproate (SVP)

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The positive control group was compared with the negative control.

The pretreated groups were compared with the positive control group.

➤ RESULTS:

- **oral administration of sodium valproate (SVP) in a dose of 500 mg/kg/day for 7 consecutive days to male rats**
 - caused significant ($P < 0.05$) decreases in serum levels of testosterone, FSH and LH when compared with the negative control group.
- **Oral pretreatments with pumpkin seed oil (PSO), vitamin E (Vit E) and their combination**
 - significantly ($P < 0.05$) normalized serum levels of testosterone, FSH and LH when compared with the positive control group.

Effect of pumpkin seed oil (PSO), vitamin E (Vit E) and their combination on serum levels of total testosterone (TT), FSH and LH in rats with testicular damage induced by sodium valproate (SVP)

Groups	TT(ng/ml)	FSH(ng/ml)	LH(ng/ml)
Group (1):Negative control	4.30 ± 0.03 _a	9.7 ± 0.3 _a	1.8 ± 0.02 _a
Group (2): Positive (SVP) control	4.30 ± 0.03 _a	5.5 ± 0.1 _a	0.9 ± 0.02 _a
Group (3):PSO (40 mg/kg)	1.32 ± 0.04 _d	6.3 ± 0.4 _c	1.2 ± 0.04 _c
Group (4):Vit E (200 mg/kg)	2.96 ± 0.02 _b	7.7 ± 0.3 _c	1.4 ± 0.03 _c
Group (5):PSO (40 mg/kg)+Vit E (200 mg/kg)	3.28 ± 0.02 _b	8.4 ± 0.2 _b	1.6 ± 0.01 _b

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The positive control group was compared with the negative control.

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The positive control group was compared with the negative control.

The pretreated groups were compared with the positive control group.

➤ RESULTS:

- **oral administration of sodium valproate (SVP) in a dose of 500 mg/kg/day for 7 consecutive days to male rats**
 - significant decreases in sperm count, progressive motility and vitality and an increase in sperm morphological abnormality when compared with the negative control group.
- **Oral pretreatments with pumpkin seed oil (PSO), vitamin E (Vit E) and their combination**
 - significantly ($P < 0.05$) increased sperm count, progressive motility and vitality and decreased sperm cell abnormality when compared with the positive control group .

Effect of pumpkin seed oil (PSO), vitamin E (Vit E) and their combination on sperm parameters of rats with testicular damage induced by sodium valproate (SVP)

Abnormality	Sperm parameters			
	Count (10 ⁶ /ml)	Motility (%)	Vitality (%)	%
Group (1):Negative control	76.54 ± 1.4 _a	90.0 ± 0.1 _a	90.0 ± 3.2 _a	1.5 ± 0.2 _a
Group (2): Positive (SVP) control	54.30 ± 1.2 _c	65.2 ± 2.1 _c	45.6 ± 3.6 _c	18.6 ± 0.3 _c
Group (3):PSO (40 mg/kg)	60.35 ± 1.4 _b	73.3 ± 2.2 _c	55.6 ± 2.6 _c	10.5 ± 0.3 _c
Group (4):Vit E (200 mg/kg)	66.25 ± 3.3 _b	75.5 ± 2.1 _c	60.6 ± 2.8 _c	6.5 ± 0.1 _c
Group (5):PSO (40 mg/kg)+Vit E (200 mg/kg)	72.24 ± 3.1 _b	80.0 ± 4.2 _b	75.6 ± 3.8 _b	4.4 ± 0.3 _c

Means ± SEM with different superscripts in the same column are significant at $P < 0.05$ using one-way ANOVA test. n= 9 rats.

The positive control group was compared with the negative control.

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The positive control group was compared with the negative control.

The pretreated groups were compared with the positive control group.

➤ RESULTS:

- **Oral pretreatments with PSO, Vit E and their combination** decreased percents of sperm morphological abnormality .
- **The most frequently seen sperm morphological abnormalities in positive control rats were:**
 - **double head (3.4%)**



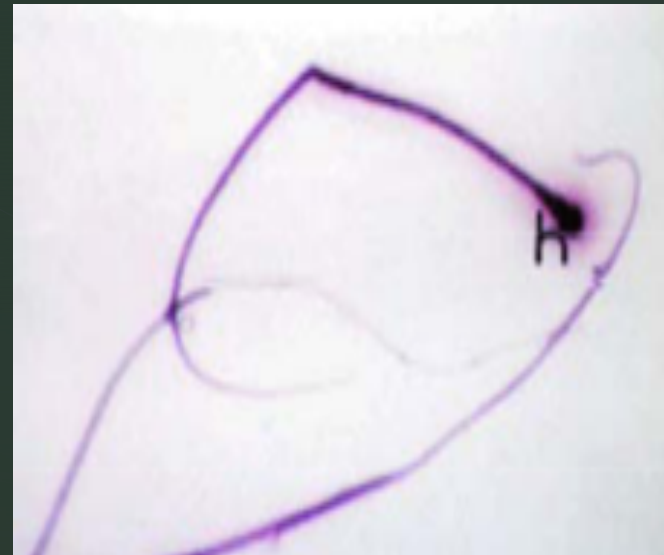
➤ RESULTS:

- **Oral pretreatments with PSO, Vit E and their combination** decreased percents of sperm morphological abnormality to **10.5, 6.5 and 4.4 %, respectively**, versus to **18.6%** in the positive control group.
- **The most frequently seen sperm morphological abnormalities in positive control rats were:**
 - double head (3.4%)
 - detached head (7.2%)



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 - double head (3.4%)
 - detached head (7.2%)
 - large oval head (3.0 %)



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 - coiled tail (5.0 %)



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- **The most frequently seen sperm morphological abnormalities in positive control rats were:**
 - double head (3.4%)
 - detached head (7.2%)
 - large oval head (3.0 %)
 - coiled tail (5.0 %)
 - as compared to the normal sperm in the negative control group .



➤ RESULTS:

- **oral administration of sodium valproate (SVP) in a dose of 500 mg/kg/day for 7 consecutive days to male rats**
 - significantly ($P < 0.05$) decreased tissue reduced glutathione (GSH) and increased malondialdehyde (MDA) contents in testes when compared with the negative control group.
- **Oral pretreatments with pumpkin seed oil (PSO), vitamin E (Vit E) and their combination**
 - significantly ($P < 0.05$) increased tissue GSH and decreased MDA when compared with the positive control group.

Effect of pumpkin seed oil (PSO), vitamin E (Vit E) and their combination on testes reduced glutathione (GSH) and malondialdehyde (MDA) in rats with testicular damage induced by sodium valproate (SVP)

Groups	GSH (nmol/min/mg protein)	MDA (nmol/min/mg protein)
Group (1):Negative control	27.4 ± 1.3 _a	0.35 ± 0.05 _a
Group (2): Positive (SVP) control	12.5 ± 2.2 _a	0.95 ± 0.03 _a
Group (3):PSO (40 mg/kg)	18.7 ± 2.4 _b	0.65 ± 0.04 _b
Group (4):Vit E (200 mg/kg)	20.9 ± 1.4 _c	0.55 ± 0.07 _b
Group (5):PSO (40 mg/kg)+Vit E (200 mg/kg)	24.2 ± 2.3 _c	0.50 ± 0.02 _b

Means ± SEM with different superscripts in the same column are significant at $P < 0.05$ using one-way ANOVA test. n= 9 rats.

The positive control group was compared to the normal control.

The pretreated groups were compared to the positive control group.

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➤ RESULTS:

- **oral administration of sodium valproate (SVP) in a dose of 500 mg/kg/day for 7 consecutive days to male rats**
 - significantly ($P < 0.05$) decreased the activity of superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) enzymes in testes when compared with the negative control group.
- **Oral pretreatments with pumpkin seed oil (PSO), vitamin E (Vit E) and their combination**
 - significantly ($P < 0.05$) increased the activity of tissue SOD, GPx and CAT enzymes when compared with positive control group.

Effect of pumpkin seed oil (PSO), vitamin E (Vit E) and their combination on activities of testicular superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) in rats with testicular damage induced by sodium valproate (SVP)

Groups	SOD (U/mg protein)	GPx (nmol/min/mg protein)	CAT (nmol/min/mg protein)
Group (1):Negative control	25.10 ± 0.3 _a	241.4 ± 5.2 _a	369.9 ± 6.4 _a
Group (2): Positive (SVP) control	14.20 ± 0.4 _a	133.5 ± 3.8 _a	285.6 ± 3.8 _a
Group (3):PSO (40 mg/kg)	16.15 ± 0.8 _a	145.6 ± 4.6 _a	295.2 ± 6.2 _a
Group (4):Vit E (200 mg/kg)	17.25 ± 0.7 _a	150.2 ± 5.6 _a	300.5 ± 8.2 _a
Group (5):PSO (40 mg/kg)+Vit E (200 mg/kg)	22.25 ± 0.2 _a	197.5 ± 4.2 _a	325.2 ± 7.5 _a

Means ± SEM with different superscripts in the same column are significant at $P < 0.05$ using one-way ANOVA test. n= 9 rats.

GPx unit = nmol of GSH utilized/min/mg protein.

CAT unit = nmol of H₂O₂ utilized/min/mg protein.

The positive control group was compared to the normal control.

The pretreated groups were compared to the positive control group.

Effect of pumpkin seed oil (PSO), vitamin E (Vit E) and their combination on activities of testicular superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) in rats with testicular damage induced by sodium valproate (SVP)

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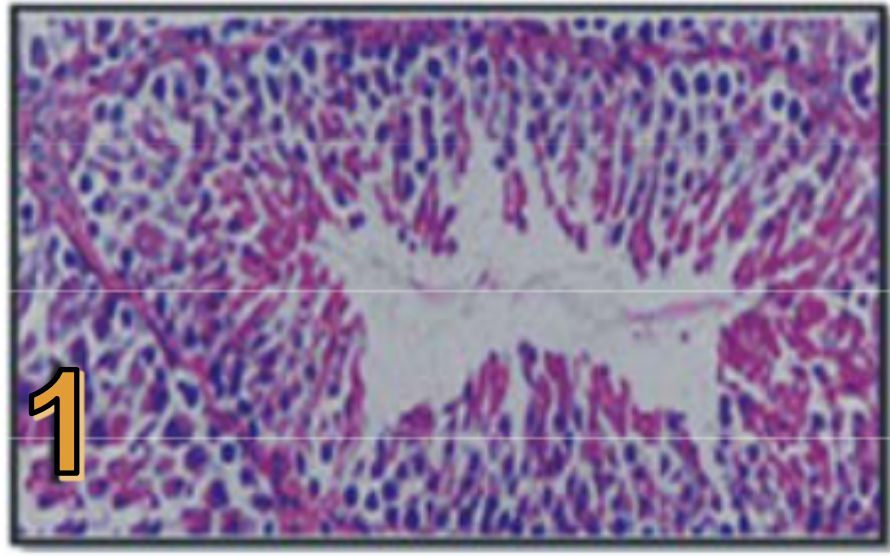
The pretreated groups were compared to the positive control group.

➤ RESULTS:

- **Histopathological examination of the testes showed that rats pretreated with**

vitamin E (Vit E) alone

- **had intact and functioning seminiferous tubules containing mature spermatozoa in the lumen and complete spermatogenic germ cell series (1)**



➤ RESULTS:

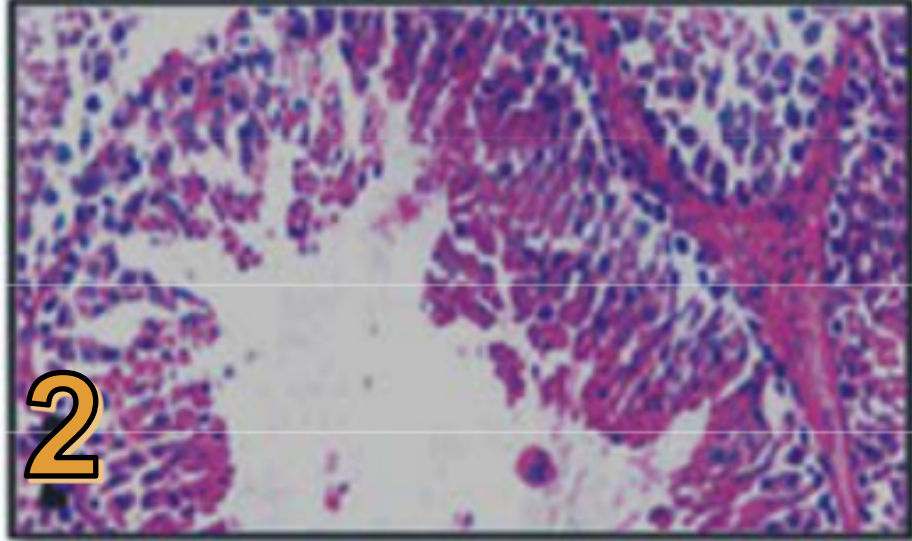
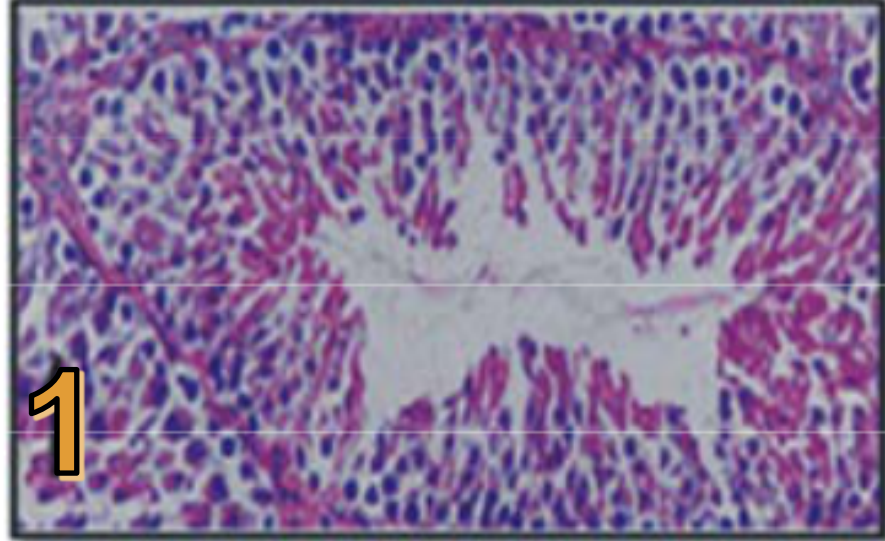
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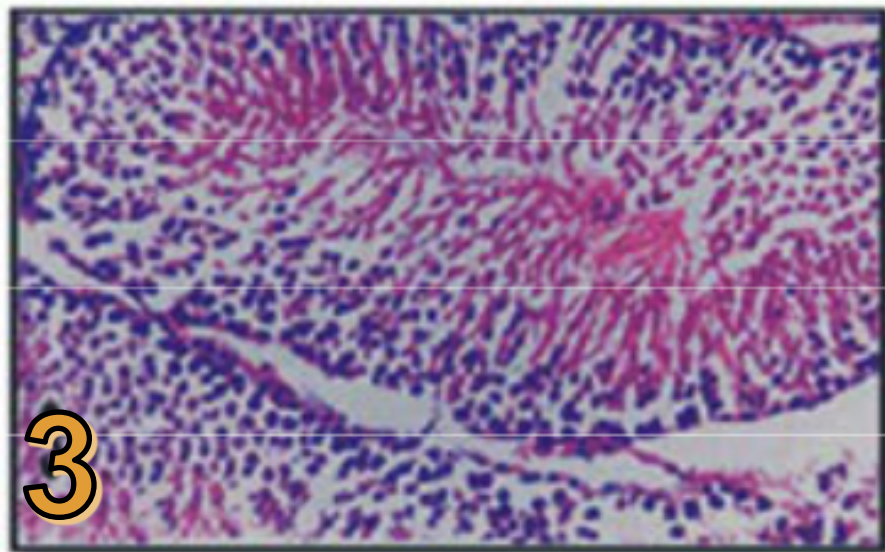
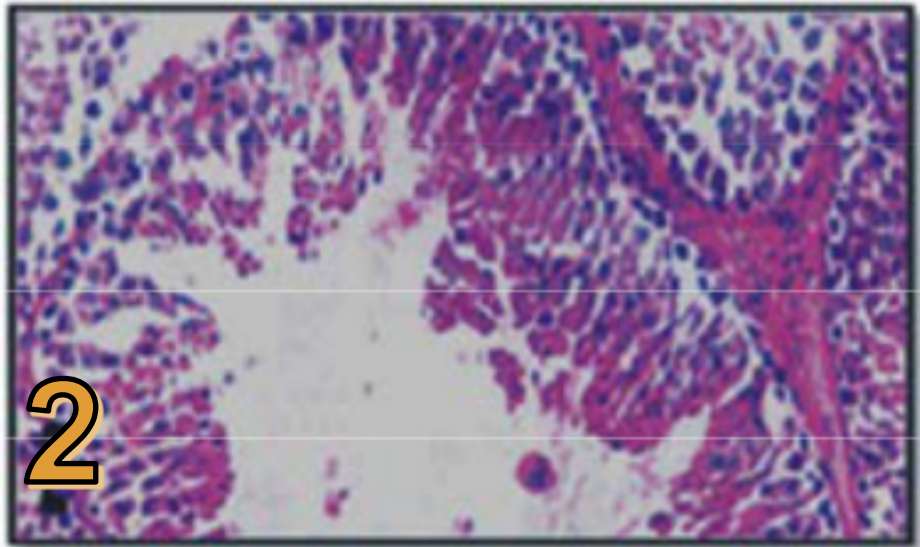
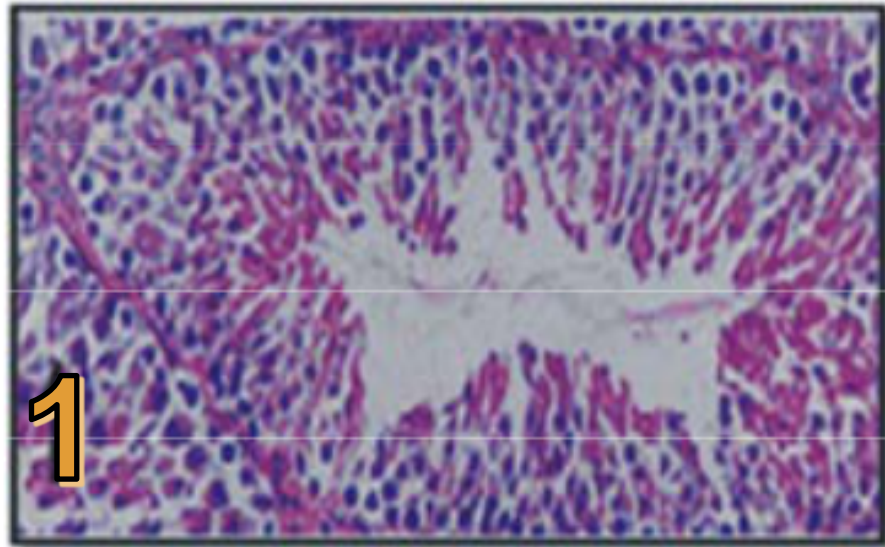
pumpkin seed oil (PSO) alone

- **the examination of testes revealed relatively intact seminiferous tubules with few spermatozoa in the lumen, mild interstitial edema and leukocytes infiltration as demonstrated in (2)**



➤ RESULTS:

- **Histopathological examination of the testes showed that rats pretreated with combination of PSO and Vit E**
- **showed normal intact seminiferous tubules completely filled with mature spermatozoa and complete spermatogenic germ cell series (3).**



➤ RESULTS:

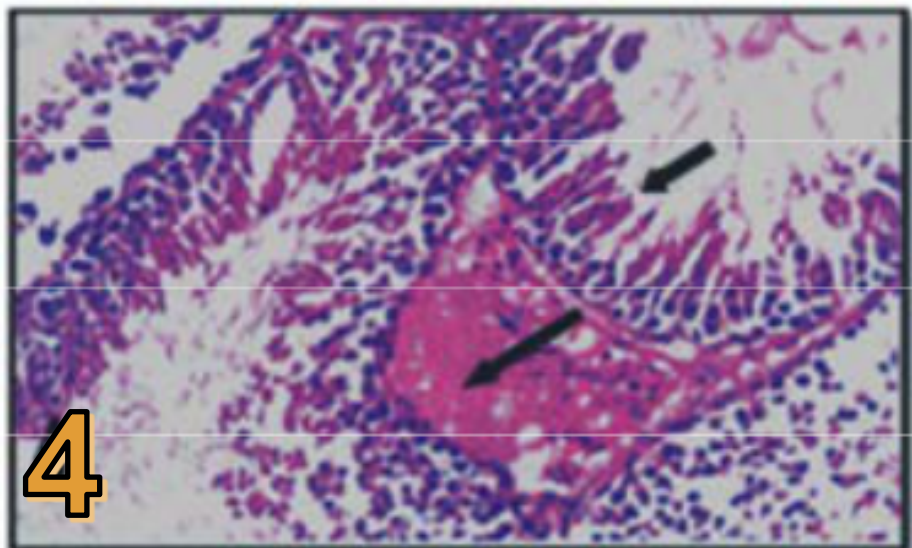
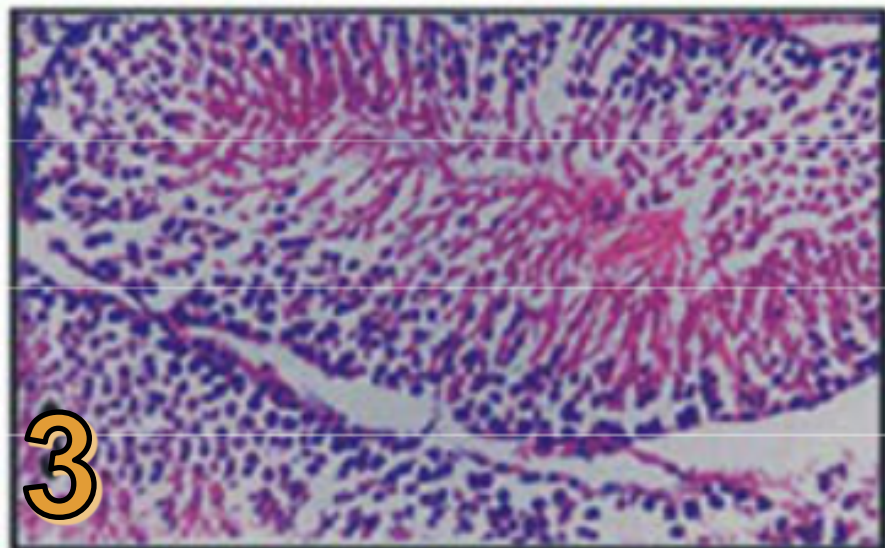
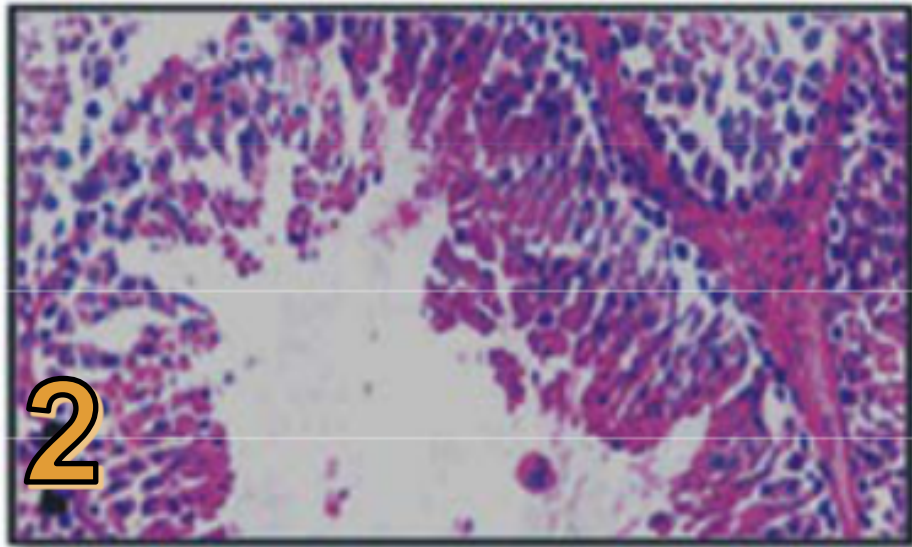
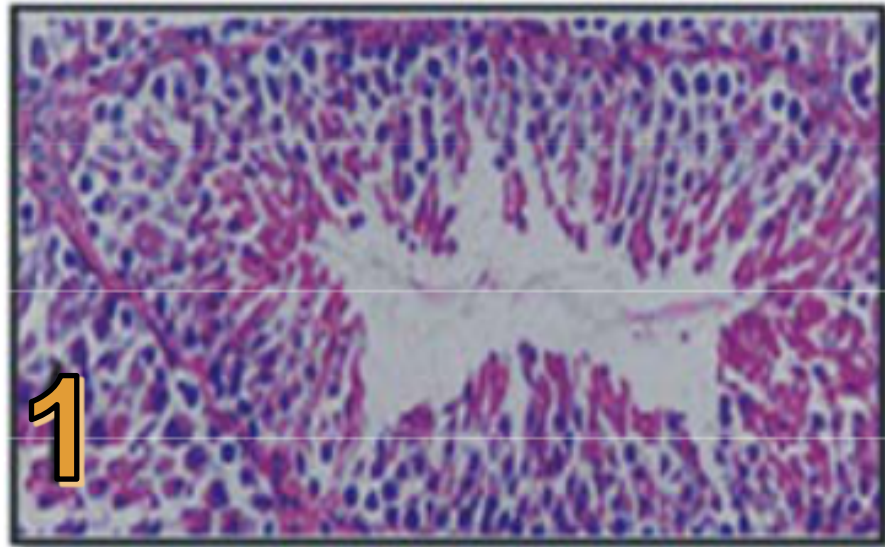
- **Histopathological examination of the testes showed that rats pretreated with**

combination of PSO and Vit E

- **showed normal intact seminiferous tubules completely filled with mature spermatozoa and complete spermatogenic germ cell series (3).**

orally given SVP (control positive group)

- **showed testicular degenerative changes of spermatogenic germ cell series with diffuse interstitial edema and inflammatory leukocytes infiltration (4).**



➤ CONCLUSION

- Combination of **PSO and Vit E** exhibits protective and antioxidant activities against SVP-induced testicular damage in rats.
- The improvement of male reproductive efficiency by **PSO and Vit E** could be due to their antioxidant activity and to the increase in serum testosterone, FSH and LH which are necessary hormones for normal spermatogenesis and production of normal sperms.
- The study suggests that **dietary intake of PSO and Vit E** may be beneficial for patients who suffer from male infertility due to oxidative stress.

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