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Effect of canola oil on ultrastructure of testis in adult male albino rat

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ABSTRACT

The incidence of infertility is increasing world wild. Among the diet factors, are the quality and quantity of the fat that contributes to spermatogenesis. Canola oil is known to contain oleic acid which is an omega-9 monounsaturated fatty acid, linoleic acid which is an essential omega-6 polyunsaturated fatty acid and alpha-linolenic acid, a plant-based omega-3. Canola oil has the lowest saturated fatty acid content among cooking oil. This study has been designed to investigate the possible effect of Canola oil, the recently used cooking oil, on the testicular structure and ultra-structure.

Twelve adult male albino rats (200-250g) were obtained from the animal house in Faculty of Pharmacy, Mansoura University. The animals were housed two in a cage at a constant temperature of 18°C and humidity 45%. Rats were divided into control and treated; treated rats received a diet contains 7ml/100gm canola oil for 39 days. Six rats in each group were weighed and sacrificed at the end of the experiment; testis was dissected and weighed. The specimens were used for paraffin sections and electron microscope examination.

The Canola oil-treated group showed irregular seminiferous tubules, germ cells with vacuolated cytoplasm and deeply stained nuclei compared with the control group. The treated testis ultrastructure showed degenerated Sertoli cell with an irregular nucleus, degenerated spermatocytes with the cytoplasm showing phagosomes, lysosomes and increased autophagic vacuoles. Interstitial cells appeared degenerated with vacuolated cytoplasm an.

Canola oil is low in saturated fat and high in monounsaturated fat content, making it an ideal healthy cooking oil. This study revealed that Canola oil usage resulted in distorted seminiferous tubules, degenerated germ cells and ultrastructural changes in the treated testis. Canola oil should be used with caution, especially in males, to avoid its hazardous effect on the testis.

Keywords: Canola oil, testis, Autophagic vacuoles

INTRODUCTION

The incidence of infertility is increasing world wild. Some reports consider that diet may affect fertility **(**Sharpe, 2010). Among the diet factors,are the quality and quantity of the fat that contributes to spermatogenesis. The essential fatty acids, like linoleic acid (LA) and alpha-linolenic acid (ALA), are located in Sertoli and germ cells. So, the diet-lipid relation of those fatty acids could alter fertility (Wathes *et al*., 2007).

It was documented that overweight might reduce spermatozoa (Hammoud *et al*., 2008). Besides, increased adipose tissue in the scrotum is associated with increased temperature and oxidative stress; this change in the testicular micro-environment may alter the spermatogenesis (Kasturi *et al*., 2008). Furthermore, it has been reported that obese persons expressed hyperactivity of the aromatase enzyme and testosterone to estrogen, which may cause spermatic changes (de Boer *et al*., 2005).

Canola oil is a novel dietary oil known to contain saturated fatty acid, oleic acid which is an omega-9 monounsaturated fatty acid, linoleic acid which is an essential omega-6 polyunsaturated fatty acid and alpha-linolenic acid, a plant-based omega-3. Canola oil has the lowest saturated fatty acid content among cooking oil, making it an excellent choice for cooking as a healthy oil (Sacks *et al*., 2017).

Based on the previous knowledge, this study was designed to investigate the possible effect of Canola oil, the recently used cooking oil, on the testicular structure and its ultra-structure to find out the possible hazardous or beneficial effects.

MATERIALS AND METHODS

Experimental animals:

Twelve adult male albino rats (200-250gm) were obtained from theanimal house in Faculty of Pharmacy, Mansoura University. The animals were housed two in a cage at a constant temperature 18°C and humidity 45% on a 12-h light/dark cycle. They had free access to standard diet and drinking water. All the experiments were carried out according to the rules and regulations laid down by the committee on animals' experimentation of Mansoura University.

Experimental protocol:

Animals were weighed and divided randomly into two groups, six rats each.

Group 1: control rats received vehicle only.

Group 2: Canola treated rats; received a diet contains 7ml/100gm canola oil for 39 days **(**Furriel *et al*., 2012). The diets consisted of the casein (20 g), cornstarch (53 g), sucrose (10 g), fibre (5 g), mineral (3.5 g) and vitamin mix (1 g), L-cystine (0.3 g) and choline bitartrate (0.25 g), per 100 g diet (Reeves, 1997).

The sacrifice of rats, specimens' collection and preparation:

Six rats in each group were weighed and sacrificed at the end of the experiment under general anesthesia; testis was dissected and weighed. The specimens used for paraffin sections. The slides were then stained with hematoxylin-eosin and were prepared for electron microscope examination.

For ultrastructure study, small pieces were obtained from the testis. Specimens were fixed in 3.5% glutaraldehyde and washed in phosphate buffer. After that, they have fixed in osmium tetra-oxide solution. Semithin sections prepared by the ultra-tome. Ultrathin sections were then prepared (Hayat, 1989). Finally, cells were examined by JEOL-100SX transmission electron microscope.

Quantitative and statistical analysis:

The diameters of seminiferous tubules were measured.Per cent of germ cells with autophagy vacuoles were counted. All measurements were calculated in a fixed field in serial sections using an image analyzer computer.All measurements were through Image J software. One way

ANOVA was used to compare measurements.$P \leq 0.05$ was accepted as a significant level.

RESULTS

Histological results:

Control testis showed rounded or oval seminiferous tubules surrounded by tunica propria and myoid cells. Sertoli cells and spermatogenic cells in different stages of maturations lined the tubules. Spermatogenic cells included spermatogonia, the primary spermatocytes, the secondary spermatocytes, spermatids and many sperms were apparent in the lumen of the seminiferous tubules. The Interstitial cells are grouped between the tubules (Fig. 1A, B & Fig 2A).

The Canola oil-treated group showed irregular seminiferous tubules. The spermatogonia were separated from the basal membrane with vacuolated cytoplasm and deeply stained nuclei. The interstitial cells were few, with deeply stained nuclei. (Fig. 1 C, D and Fig. 2, B).

Ultrastructure study

The control sections showed normal Sertoli cell with triangular nuclei. The cytoplasm contained mitochondria. The primary spermatocytes were rounded, and the cytoplasm showed mitochondria, lysosomes and autophagic vacuoles. The fusiform spermatids appeared with pyriform nuclei and noticeable acrosomal cap on the anterior nuclear part (Fig. 3A, B and Fig. 4A).

The treated testis ultrastructure showed degenerated Sertoli cell with an irregular nucleus, degenerated spermatocytes with the cytoplasm showing lysosomes and autophagic vacuoles (Fig. 3C, D). The per cent of autophagic vacuoles was significantly increased (Table 1). Interstitial cells appeared degenerated with vacuolated cytoplasm (Fig. 4D).

Morphometric results and statistical analysis:

1- **Testicular weight:**

The treated group showed an insignificant difference in the testicular weight compared with the control group (Table 1, Fig.5).

2-**Diameter of STs:**

The treated group showed a significant reduction in seminiferous tubules diameter compared with the control group (Table 1, Fig. 6).

3-**Per cent of germ cells with autophagy vacuoles:**

The treated group showed a significant rise in % of germ cells expressing autophagic vacuoles when compared with the control group (Table 1, Fig. 7).

Table 1. Mean testicular weight, diameter of STs and % germ cells with autophagic vacuoles.

	Control	**Treated**	**P-value**
Mean testis weight (g) ±SD	1.397±.152	1.316±.149	0.1
Diameter of STs± SD	256.17±56	143.98±73	0.001
% germ cells with autophagic vacuoles	23±2.67	37±3.8	0.001

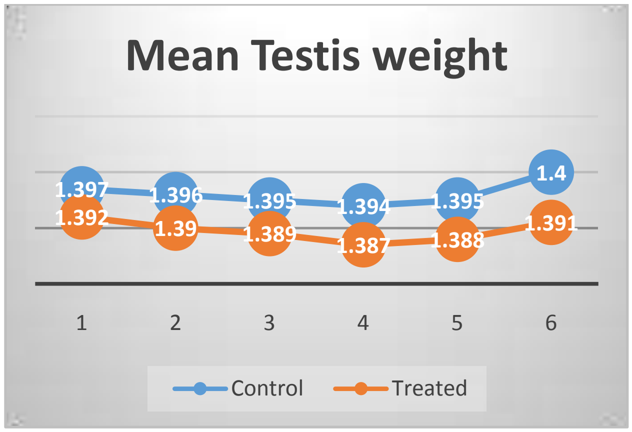

Fig. 5. The treated group shows insignificant deference in the testicular weight when compared with the control group.

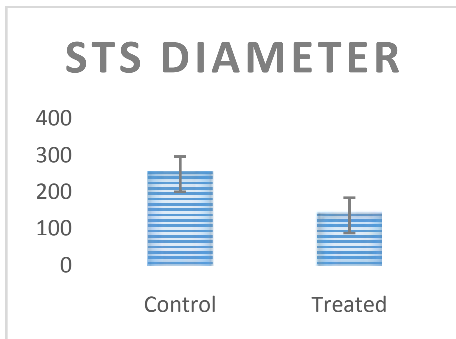

Fig. 6. The treated group shows a significant reduction in seminiferous tubules diameter when compared with the control group.

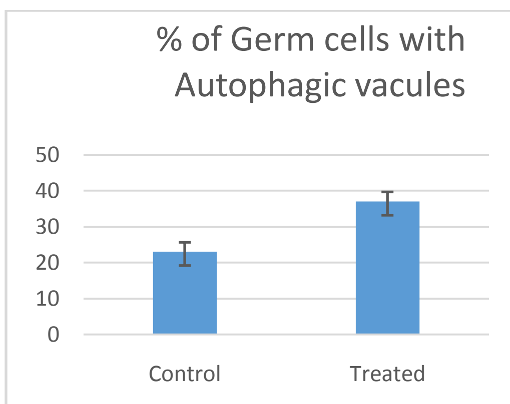

Fig. 7. The treated group shows a significant rise in % of germ cells expressing autophagic vacuoles when compared with the control group.

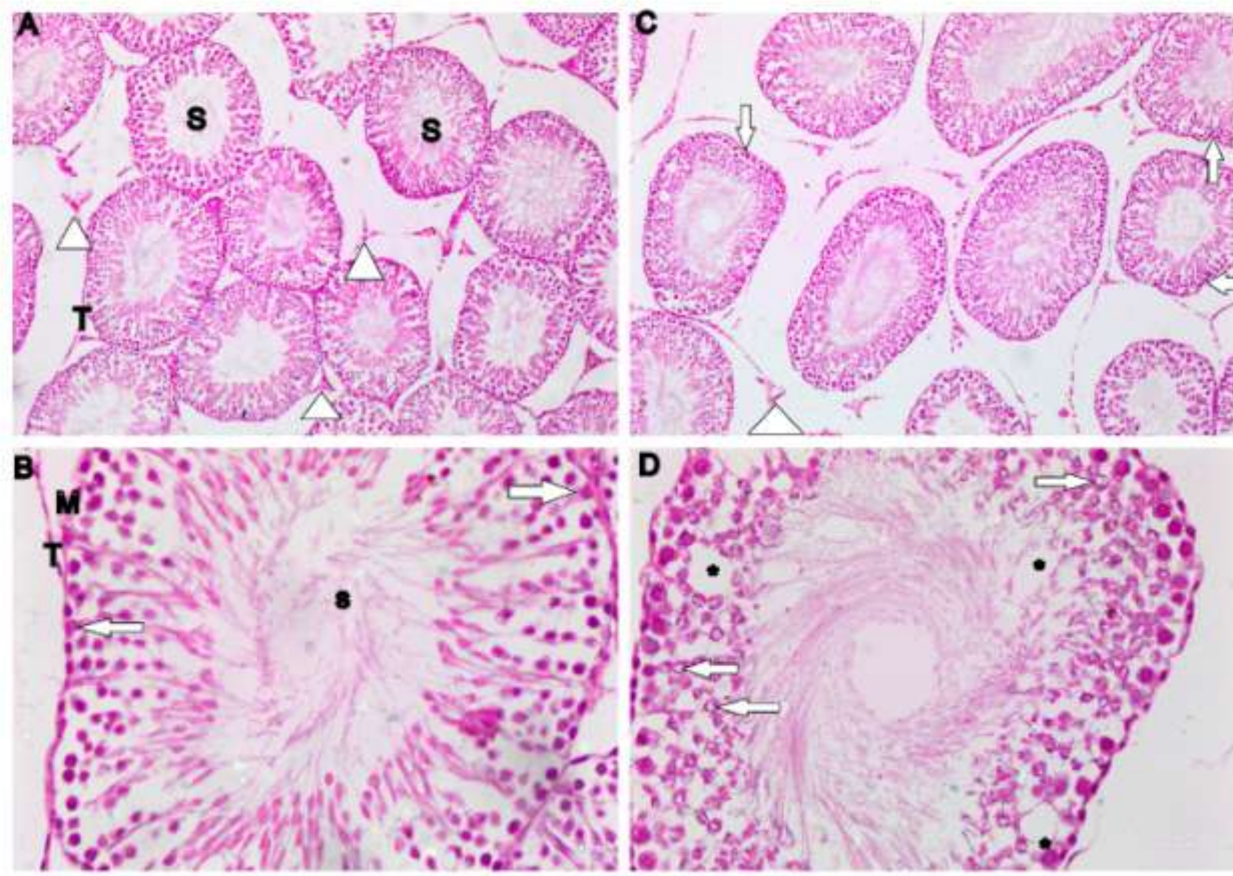

Fig.1. Photomicrograph of testis tissue sections stained with H&E .
A. Control group is showing rounded or oval seminiferous tubules (S) surrounded by tunica propria (T). Germ cells appear with many sperms in the lumen of the seminiferous tubules (S). Interstitial cells are grouped between the tubules (arrowheads) (X 100). **B.** Control group showing Sertoli cells (arrows), spermatids (s), tunica propria (T), and myoid cell (M) (X 400). **C.** The Canola oil-treated group is showing irregular seminiferous tubules (arrows). The interstitial cells appears vacuolated (arrowheads) (X 100). **D.** The spermatogenic cells are separated from the basal membrane with vacuolated cytoplasm deeply stained nuclei (arrows) and vacuoles in between cells (*) (X 400).

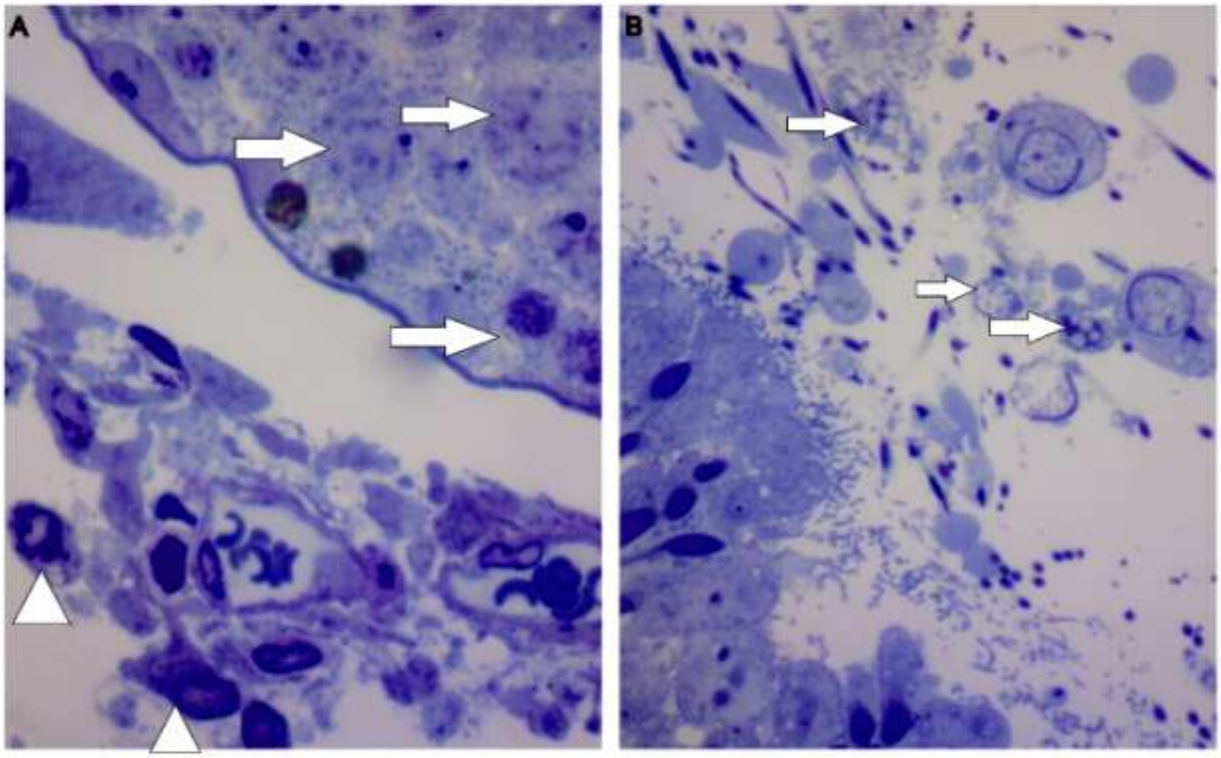

Fig.2: Photomicrograph of semithin sections of the testis. A. The control group is showing spermatogonia (s), primary spermatocyte (arrows), and interstitial cells appear normal (arrowheads). **B.** Treated testis shows degenerated germ cells with vacuolated cytoplasm (arrows). (Toluidine blue stain X1000).

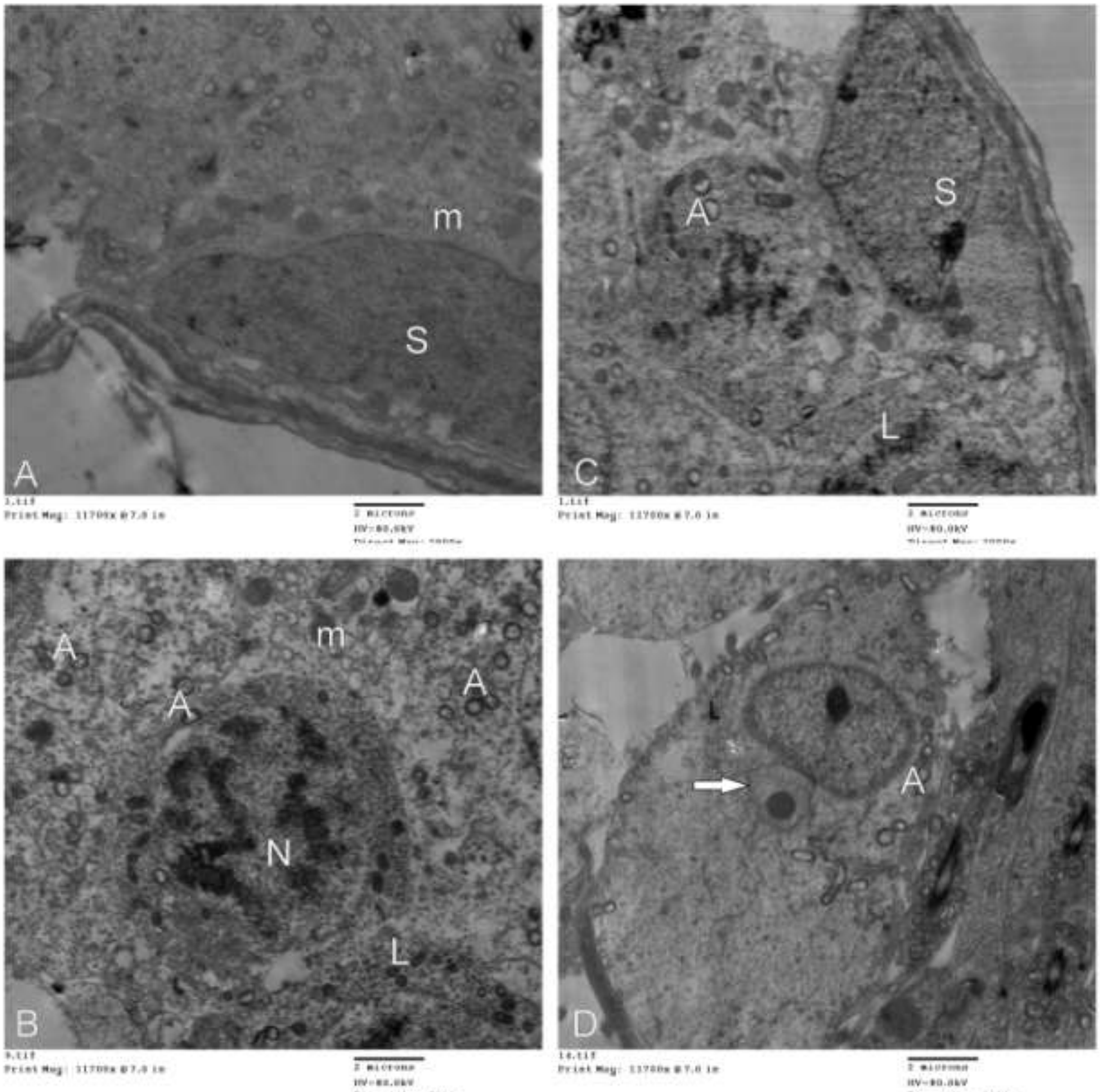

Fig.3.A. Transmission electron micrograph ofthe control testis shows Sertoli cell (S), the cytoplasm contains mitochondria (m). **B**. The primary spermatocytes with rounded nucleus (N). The cytoplasm shows mitochondria (m), lysosmoes (L) and autophagic vacuoles (A). **C.** The treated testis shows degenerated Sertoli cell with irregular nucleus (S), **D**. degenerated spermatocytes with the cytoplasm showing lysosomes (L), autophagic vacuoles (A) and irregular acrosomal cap (arrow).

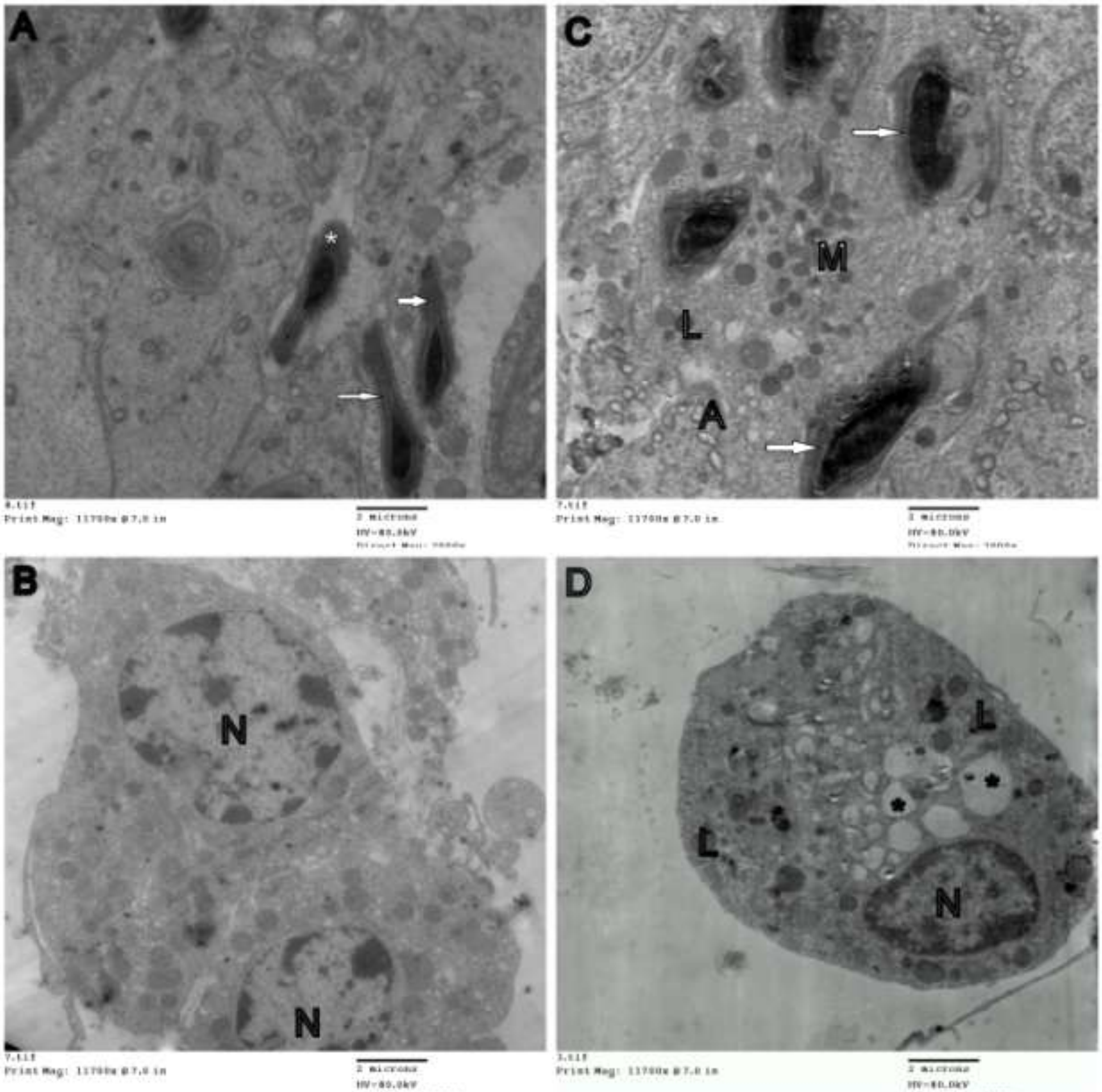

Fig.4.A. Ultrathin section of the control testis shows the fusiform spermatids (arrows) appear with pyriform nuclei and noticeable acrosomal cap (*) on the anterior nuclear part. **B.** Normal interstitial cells appar with normal nuclei (N). **C.** The treated testis shows abnormal forms of spermatids (arrows) with lysosomes (L), mitochondria (M) and autophaghic vacuoles (A). **D.** Interstitial cells appear degenerated, vacuolated (*) with lysosomes (L) .

DISCUSSION

Various studies reported that increased dietary fat intake causes obesity. Obesity is associated with metabolic disorders like type 2 diabetes and dyslipidemia (Fernandez *et al*., 2011). Some studies showed an inverse relation between hyperlipidemia and male reproductive functions. Leptin in obese men might reduce testosterone level, and result in hypogonadism (Michalakis *et al*., 2013). In addition, fatty diet could affect the gonadotropin receptors, alter testosterone secretion from Leydig cells and disturb spermatogenesis in seminiferous tubules (Mah *et al*., 2010).

The beneficial or hazardous effect of any oil is related to its content of fat. However, the linolenic and oleic acids are essential for the synthesis of acetyl CoA, which is implicated in spermatogenesis (McLennan and Dallimare, 1995). In the same time, the activity of the spermatogenic cells is controlled by other factors such as the level of testosterone hormone secreted from interstitial cells (Stevens and Lowe, 1996) and the luteinizing hormone (anterior pituitary) (Payne and youngblood, 1995).

The present study revealed that~~,~~ the testicular weight did not show significant change after administration of Canola oil. These results are in agreement with previous reports for rats fed on hydrogenated fats (Zevenbergen *et al*., 1988), rats fed on a high-fat diet for 12 weeks (Mejia *et al*., 2015) and rats fed with *Eruca stavia* seed oil (Salem and Moustafa, 2001).

In this study, the seminiferous tubule diameter expressed a significant reduction after administration of Canola oil. A similar finding was reported after treatment of rat diet with a high dose of *Eruca stavia* seed oil in large quantity (Salem and Moustafa, 2001). This could be explained by the degenerated spermatogenic cells and reduction of their activity.

In parallel to our finding, Ravet *et al*. (1985) reported degeneration of spermatogenic cells in rats animals fed a diet rich in erucic acid. Moreover, according to Purohit and Daradka, (1999), the rats fed with high fat diet showed arrested spermatogenesis at the primary spermatocyte, and few numbers of secondary spermatocytes could be observed. In addition, high dose of *Eruca sativa* oil was found to cause Leydig cell degeneration and in turn, reduced the testosterone hormone level (Salem and Moustafa, 2001). This could be due to erucic acid content of ES oil which affects Leydig cell and testosterone secretion (Blesbois *et al*., 1997). Rotkiewicz *et al*. (1997) found rats fed on rapeseed oil (ALA-rich oil; omega-three fatty acids) exhibited necrotic seminiferous tubules.

Okuyana *et al*. (2010) reported a reduction in testosterone level in rats fed on 12% Canola oil for 84 days. An additional explanation might be the aromatase enzyme activity in metabolic syndrome, which result in more conversion of testosterone to estrogen (Kasturi *et al*., 2008).

Commonly, autophagy in the human spermatogenic cells is involved in cell vitality and motility (Aparicio *et al*., 2016). According to Mu *et al*. (2017), autophagy was over-activated in the testis of high fat diet-fed mice, indicating that it may play a role in fatty induced spermatogenic deficiency. The increased per cent of autophagic vacuoles in the canola treated rats in this study could be explained by the inhibited phosphorylation of adenosine monophosphate-activated protein kinase (Kuwabara *et al*., 2015), leading to inhibition of mTOR, which is an antagonist of autophagy (Ma *et al*., 2016). However, other reports showed decreased autophagy in the high-fat diet-fed mice (Liu *et al*., 2015). It was documented that starvation could induce autophagy in the spermatogenic cells in the testis of Prawns. This observation might support the hypothesis that autophagy is involved in the testicular maturation and sperm production (Kankuan *et al*., 2019).

From the present study, it could be concluded that Canola oil is low in saturated fat and high in monounsaturated fat content, making it an ideal healthy cooking oil. However, this study revealed that Canola oil usage resulted in distorted seminiferous tubules, degenerated germ cells and ultrastructural changes in the treated testis. It could be concluded that Canola oil should be used with caution, especially in males, to avoid its hazardous effect on the testis.

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تأثير زيت الكانولا على التركيب الدقيق للخصية فى ذكر الفأر الأبيض البالغ

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المستخلص

يتزايد انتشارالعقم في العديد من البلدان. بعض الأدلة تعتبرأن محتوى الغذاء قد يؤثرعلى الخصوبة. وقد أثبت علميا أن زيادة الوزن ترتبط بانخفاض عدد الحيوانات المنوية. يحتوي زيت الكانولا على حمض الأوليك. أوميغا 9 الأحماض الدهنية الأحادية غيرالمشبعة، حمض اللينوليك. الأحماض الدهنية الأساسية غيرالمشبعة أوميغا 6 ،حمض ألفا لينولينيك.يحتوي زيت الكانولا على أدنى محتوى من الأحماض الدهنية المشبعة مقارنة بأى زيت أخر.

الهدف من هذا البحث دراسة التغيرات المحتملة فى التركيب الدقيق للخصية فى ذكر الجرذ الابيض البالغ. وقد تم استخدام 12من ذكورالجرذان البيضاء من بيت الحيوان بكلية الصيدلة، جامعة المنصورة. تم تقسيم الحيوانات عشوائياً إلى مجموعتين 6 فئران في كل منهما؛ الضابطة والمعالجة. فئران الكانولا المعالجة؛ حصلت على نظام غذائي يحتوي على زيت الكانولا (100g/7ml)لمدة 39 يوما. تم وزن الفئران في كل مجموعة في نهاية التجربة والتضحية بها، وتم تشريح الخصية ووزنها. تم أعداد قطاعات لدراسة التركيب الهيستولوجى للخصية بالميكروسكوب الضوئى والمجهرالإلكتروني

أظهرت المجموعة المعالجة بزيت الكانولا وجود انابيب منوية متقلصة مشوهة غيرمنتظمة وخلايا منوية متحللة ونواة داكنة.كما أظهر التركيب الدقيق للخلايا تغيرات على مستوى النواة والليسوسومات.

وخلصت الدراسة الى ان زيت الكانولا يحتوي على نسبة منخفضة من الدهون المشبعة ونسبة عالية من الدهون الأحادية غيرالمشبعة، مما يجعله زيتًا صحيًا مثاليًا للطبخ. أظهرت هذه الدراسة أن استخدام زيت الكانولا أدى إلى تشوها لأنابيب المنوية وخلايا منوية متحللة ونواة داكنة في الخصية المعالجة. لذلك يجب استخدام زيت الكانولا بحذر خاصة مع الذكور لتجنب تأثيره الخطيرعلى الخصية.