African Journal of Advanced Pure and Applied Sciences (AJAPAS)

Online ISSN: 2957-644X

Volume 2, Issue 4, October-December 2023, Page No: 261-269

Website: *https://aaasjournals.com/index.php/ajapas/index*

(1.55) :2023 معامل التأثير العربي SJIFactor 2023: 5.689 ISI 2022-2023: 0.557

The therapeutic effects of Sidr honey in healing the testicular and sperm damage of rats exposed to cigarette smoke

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Received: September 29, 2023	Accepted: November 18, 2023	Published: November 26, 2023

Abstract:

This study was designed to investigate the therapeutic effects of Sidr honey on testes and sperm damage by exposure to cigarette smoke in rats. Adult male rats were divided into four groups: (G1) control group, (G2) rats received Sidr honey orally (100 mg/kg b.w./d.) for 4 weeks, (G3) rats received 5 lit of Karelia red cigarettes (5 times/d.) for 4 weeks, and (G4) rats received Sidr honey orally (100 mg/kg b.w./d.) for 2 weeks then the animals were treated by Karelia red cigarettes generated by a machine smoking with taking the Sidr honey for 4 weeks. Rats in CS group showed a significant decrease of sperm count and an increase of the percentage of motile sperm, and testis weights as well as a higher percentage of abnormal sperm, and many abnormalities of sperm morphology as compared to control and TP groups. The testis tissues from the CS group showed atrophy and degeneration of some seminiferous tubules with an absence of spermatozoa, the presence of cell debris, and loss of the Sertoli cells in some seminiferous tubules. While the testis tissues from TP group had less damage to the tubules and germ cells as compared to the CS group. This study indicates that Sidr honey has therapeutic effects on testes and sperms damaged by exposure to cigarette smoke in rats.

Keywords: Cigarette, Testis, Sidr Honey, Sperm, Rats.

Cite this article as: E. M. A. Alshailabi, N. I. Al-Zail, R. A. Abraheem "The therapeutic effects of Sidr honey in healing the testes and sperms damages of rats exposed to cigarette smoke.," *African Journal of Advanced Pure and Applied Sciences (AJAPAS)*, vol. 2, no. 4, pp. 261–269, October-December 2023.

التأثيرات العلاجية لعسل السدر في شفاء تلف الخصية والحيوانات المنوية لدى الجرذان المعرضة لدخان السجائر

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الملخص

صممت هذه الدراسة لمعرفة التأثيرات العلاجية لعسل السدر في شفاء تلف الخصية والحيوانات المنوية لدى الجرذان المعرضة لدخان السجائر. تم تقسيم ذكور الجرذان البالغة إلى أربع مجاميع: المجموعة الأولى هي المجموعة الضابطة، والمجموعة الثانية تلقت فيها الجرذان عسل السدر عن طريق الفم (100 ملغم/كغم من وزن الجسم/اليوم) لمدة 4 أسابيع، والمجموعة الثالثة تلقت فيها الجرذان 5 من سجائر كاريليا الحمراء المشتعلة (5 مرات/يوم) لمدة 4 أسابيع، أما المجموعة الرابعة تلقت فيها الجرذان عسل السدر عن طريق الفم (100 ملغم/كغم من وزن الجسم/يوم) لمدة أسبوعين ثم عرضت الحيوانات لدخان سجائرالكاريليا الحمراء الناتجة عن آلة التدخين مع أخذ عسل السدر لمدة 4 أسابيع. أظهرت الجرذان في

مجموعة دخان السجائر انخفاضا معنويا في عدد الحيوانات المنوية، وزيادة في النسبة المئوية للحيوانات المنوية المتحركة، وأوزان الخصية وكذلك نسبة أعلى من الحيوانات المنوية غير الطبيعية، والعديد من التشوهات في شكل الحيوانات المنوية مقارنة بالمجموعة الضابطة والمجموعة العلاجية. كما وأظهرت أنسجة الخصية من مجموعة دخان السجائر ضمور وتشوه بعض الأنابيب المنوية مع غياب الحيوانات المنوية، ووجود بقايا الخلايا، وفقدان خلايا سيرتولي في بعض الأنابيب المنوية. في حين أن أنسجة الخصية في المجموعة العلاجية كانت أقل ضررا على الأنابيب والخلايا الجرثومية مقارنة بمجموعة دخان السجائر. تشير هذه الدراسة إلى أن لعسل السدر تأثير علاجي ضد تلف الخصية والحيوانات المنوية الناجم عن دخان السجائر في ذكور الجرذان البالغة.

الكلمات المفتاحية: السجائر، الخصية، عسل السدر، الحيوانات المنوية، الجرذان.

Introduction

The toxicity of cigarette smoke (CS) products is through the huge production of reactive oxygen species (ROS) in humans [1], and may produce inflammatory mediators [2]. Besides that, the major effect of CS that affect health is nicotine, tar, and carbon monoxide [3, 4]. Moreover, CS exposure results in secretor deficiency of Leydig and Sertoli cells leading to impaired sperm maturation process and decrease in the semen volume, sperm concentration, sperm motility, and total sperm count [5, 6, 7]. Exposure to CS causes the release of many dangerous materials in the body that have the direct of free radicals and activating inflammatory cells, which produce ROS [8], where, ROS reduce male sex hormone levels and disrupt the hormonal balance for male reproductive functions, and thus causes infertility [9]. CS is the smoke that rises from the tip of the burning cigarette and constitutes approximately 85% of passive smoke [10].

Honey (H) has been used as food, drug, and raw materials [11]. As a medicinal material, its antioxidant ability and supersaturated sugar solution with high osmotic pressure build up the immunity level of consumers [12]. H was reported to reduce the toxic effects of CS on spermatogenesis and improve serum testosterone levels in rats [13, 14].

The present study examined the therapeutic effects of Sidr honey against testicular and sperms damages caused by exposure to cigarette smoke in male rats.

Material and methods

Chemicals:

•Sidr honey was obtained from local market and was analyzed by the Omar Al-Mokhtar University Center, Libya.100mg/kg of honey was given orally to the rats [15, 16]. Honey at the dose of 1.0 g/kg body weight was freshly diluted with distilled water to prepare 0.5 mL of diluted honey for each rat. Then, 0.5 mL of the diluted honey was immediately administered to each rat by oral gavage.

•Karelia red cigarettes were obtained from the local supermarkets.

Experimental animals:

Twenty-four adult albino male rats (Rattus norvegicus) weighing 180-200 g were used. They were obtained from the animal house of the Zoology Department, Faculty Science, University of Omar Al-Mokhtar, El-Beyda, Libya. They were acclimatized for a period of 20 days and were fed standard rat chow and water ad libitum. Animals were putted in cages at standard laboratory conditions of room temperature (22 ± 2ºC). The procedures and animal protocols were followed in this study in accordance with the guide for the care and use of laboratory animals.

Experimental design:

Rats were randomly assigned into four groups of 6 animals as follows:

1-The normal control group (C), rats were kept under standard laboratory conditions with ventilation and were not exposed to smoke.

2-The honey group (H), rats were given Sidr honey (100 mg/kg b.w./d.) orally by gavage for 4 weeks.

3-The cigarette smoke group (CS). Cigarette smoke exposure was conducted by cigarette smoke generated by a machine (bee smoker) device and a hole was connected to a smoking machine by the connection pipe to the glass box which was designed locally in Libya (Figure 1). The inhalation was made in the closed glass box for condensation of the CS then the box cover was removed to deliver an unforced exchange of fresh air. The glass box is in a cube shape (aquarium shape) with the size of 80 × 30 × 40 cm for keeping the rats [14, 16].

The cigarette smoke was used by the 5 lit Karelia red cigarettes. Each smoking was procedure for 15 minutes including making the smoke and exposing the rats to the smoke for 5 minutes and then, 10 minutes of rest and

ventilation by removing the box cover. This operation was repeated 5 times a day for 4 weeks [16]. Each week, the rats were exposed to the smoke for 6 days [17, 18].
4-The therapeutic group (TP), rats were given Sidr honey (100mg/kg b.w./d.) orally by gavage for 2 weeks then animals treated by Karelia red cigarette generated by a machine smoking (same group 3) with taking the Sidr honey for 4 weeks.
At the end of the treatment, the rats were anesthetized by diethyl ether, then sacrificed, and their testes and epididymis were removed.

Figure 1 The smoking machine.

Determination of the testis weight and relative testis weight:
To obtain a precise measure of the change in organ weights, fresh testis weight was calculated relative to the total body weight according to [19].

Determination of the quantitative semen analysis:
•Sperm samples preparation:
After sacrificing rats, the cauda epididymis was dissected out and placed in 2 ml of 0.9% physiological saline. It was cut into small pieces to release the mature sperms in solution [20]. A small drop of the cell suspension was put on the slide and the spread slides were air-dried without fixation for about 24 hours. Slides were stained with haematoxylin and eosin. Three slides were equipped for each rat.
•Sperm count:
The count of sperm cells was determined using the haematocytometer according to the technique adopted by [21].
•Sperm motility:
Sperm motility was examined according to the method reported by [22].
•Sperm morphology:
Sperm smears were examined by light microscopy. For each rat, 500 sperms were examined and morphological abnormalities involving the head and tail were recorded according to the criteria of [22].
Histopathological examination:
Testis tissue specimens from all groups were fixed in formalin (10%), then dehydrated in graded alcohol and embedded in paraffin and stained with hematoxylin and eosin using standard procedures [23].
Statistics analysis:
The parameters were analyzed using significance by one-way ANOVA. Statistical significance of the differences between the treatment groups was using the Tukey's test at $P < 0.05$ by using (Minitab version 17). The data are expressed as mean ± SE rat within each row, means with different superscripts (A, B & C) were significantly different $P < 0.05$, were means superscripts with the same letters, mean that there is no significant difference ($P < 0.05$). in the first few sentences.

Results and discussion
Determination of the testis weights relative testis weight:
The mean values of the testis weights of control and experimental groups were presented in the table (1). Statistically, a significant increase ($P < 0.05$) occurred in the mean value of the testis weights in the CS group (2.849±0.129) as compared to TP group (2.327±0.184).
However, the data recorded for the relative testis weights were presented in the table (1), showed, a significant increase ($P < 0.05$) was observed on the mean value of CS animals (1.839±0.162) and TP animals (1.320±0.11) when compared with C animals (0.851±0.140). Although, a significant decrease ($P < 0.05$) in the mean value of the TP animals (1.320±0.11) when compared with CS animals (1.839±0.162). Moreover, no remarkable changes in the mean value of the relative testis weights between the H and TP groups. This results agreement with [24],

who suggested that CS exposure significantly had a toxic effect on rat testis, resulting in smaller organs as shown by lower absolute testicular weight compared to the control group. Also, the CS caused testicular damage such as oedema [25, 19]. and decreased spermatogenesis [26]. Although administration of Sidr honey to rats exposed to CS caused significantly improved the testicular weight. These findings might have been due to the role of antioxidants in honey that prevents the increased production of free radicals induced by oxidative damage [27, 16], and due to increased testosterone levels [28].

Table 1: Averages of the mean values of testes weights and relative testes weight in control and experimental groups.

Parameter	C	H	CS	TP
Testis weighs (gm)	2.376 ± 0.09^{AB}	2.4557 ± 0.064^{AB}	2.849 ± 0.129^{A}	2.327 ± 0.184^{B}
Relative of testes weight (%)	0.851 ± 0.140^{C}	0.983 ± 0.0725^{BC}	1.839 ± 0.162^{A}	1.320 ± 0.11^{B}

*C = control group. H= Sidr honey group. CS = Cigarette smoke treated group. P = Therapeutic group.

Determination of the quantitative semen analysis:

The mean values of the sperm count, sperm motility, and abnormal sperm morphology on the control and experimental groups were presented in the table (2), showed a significant decreased ($P < 0.05$) in the mean value of the sperm count in the CS group (35.70±2.08) when compared with the C group (87.19±2.07). Moreover, the mean value of sperm count in the TP group showed a slight increase (43.93±2.62) than the CS group. Moreover, a significant reduction ($P < 0.05$) in the mean value of the sperm motility in the CS group (44.51±1.74) as compared to the C group (80.18±1.70). Besides, there was a significantly increase in the mean value of the sperm motility in the TP group (54.50±2.62) when compared with CS group. Furthermore, a highly significant increase ($P < 0.05$) in the mean value of the abnormal sperm morphology in the CS rats (27.486±0.70) as compared with the C rats (11.934±0.63). Moreover, there was a significantly decrease in the mean value of the abnormal sperm morphology in the TP group (21.24±1.23) as compared to the CS group, similarly to animals treated with CS [30, 19]. Exposed to CS showed histopathological changes in testis and epididymis of rats and resulting impaired spermatogenesis [30]. Besides that, constituents of CS caused decrease in the quality and motility of sperm [19], as well as reduced sperm fertilizing [24]. Moreover, several sperm parameters were positively affected by Sidr honey in the therapeutic group. These findings might suggest by [13, 31], who said that the honey would induce spermatogenesis in rats by increasing epididymal sperm count and increasing sorbitol dehydrogenase activity. Administration of honey might enhance the stages of spermatogenesis [29, 16], and testicular functions in rats [24].

Table 2: Averages of mean values of the sperm count, sperm motility, and abnormal sperm morphology in the control and experimental groups.

Parameter	C	H	CS	TP
Sperm count (million/ajaculate)	87.19 ± 2.07^{A}	87.94 ± 3.18^{A}	35.70 ± 2.08^{B}	43.93 ± 2.62^{B}
Sperm motility (%)	80.18 ± 1.70^{A}	76.44 ± 1.99^{A}	44.51 ± 1.74^{C}	54.50 ± 2.62^{B}
Abnormal sperm morphology (%)	11.934 ± 0.63^{C}	12.166 ± 0.441^{C}	27.486 ± 0.70^{A}	21.24 ± 1.23^{B}

*C = control group. H= Sidr honey group. CS = Cigarette smoke treated group. P = Therapeutic group.

Sperm morphological assessment:

The normal morphological of sperms in the semen smears of the normal control and the Sidr honey rats including, the normal tail is long and attached to head at the base (Figure 2; A and B).

After the rat's exposure to CS for 4 weeks showed, many abnormalities of sperms morphology were seen in the form of the sperm with the curved tail, bent tail, small curved tail sperm, coiled tail, free head, looped tail, banana head, short tail with a protoplasmic droplet. Also, sperm with a protoplasmic droplet, kinked tail, microcephalic head with a curved tail, hookless head, banana head with a curved tail, triangle with kinked tail, triangle, flattened head, microcephalic head, detached head vacuolated head and abaxial attachment of the neck and middle piece to the head were recorded in (Figures 3, 4, 5 & 6; A and B) as compared with the normal control rats. CS increased free radicals in the form of ROS such as superoxide, hydroxyl radicals, and peroxyl radicals, where ROS could cause lipid peroxidation and disrupted the integrity of the spermatozoa plasma membrane [32], and DNA damage [33]. CS caused metabolic disorders that produce ATP which interfered with spermatozoa motility [34]. CS also induces apoptosis in the testis of adult rats [35], and ultrastructural anomalies in the spermatids [36]. Ahmadnia et al. [37] suggested these results may be related to the presence of

various toxic substances in cigarette or histological reactions due to hypoxemia caused by CS. Whereas, the TP rats showed fewer abnormalities of sperms and many normal morphological sperms as revealed in Figure (7; A and B) when compared with the cigarette smoke rats. This finding suggests that honey attenuated the toxic effects of CS on spermatogenesis by restoring the testosterone levels, and may also be involved in spermatogenesis [24]. Honey contains several metals, amongst which is zinc that accumulates in the testis during early spermatogenesis. So, it may be important in DNA synthesis and regulation of spermatogonial proliferation [38].

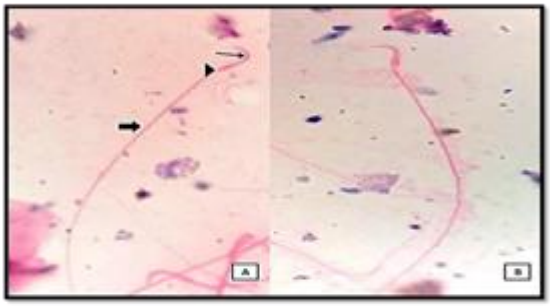

Figure 2 Photomicrographs of the semen smears of the control rats (A), and H rats (B). Showing normal morphological of sperms. Note; normal head (arrow), middle piece (head arrow), and normal tail (thick arrow). (Giemsa satin, X 1000).

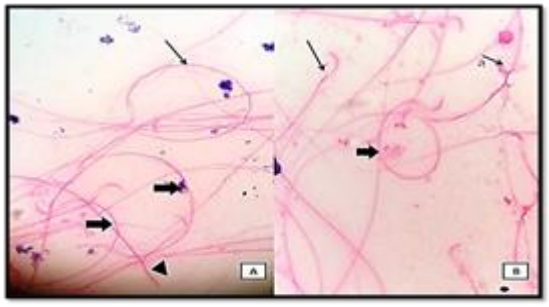

Figure 3 Photomicrographs of the semen smears of the CS rats showing, some abnormal sperms including, curved tail (thick arrows), bent tail (headless sperm) (head arrow), coiled tail (long arrow) (A); free head (detached tail) (short arrow), looped tail (thick arrow), and banana head (long arrow) (B). (Giemsa satin, X 1000).

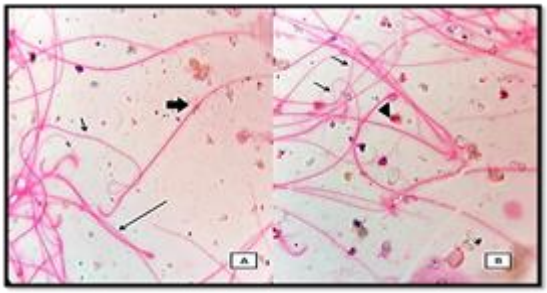

Figure 4 Photomicrographs of the semen smears of the CS rats showing, some abnormal sperms including, short tail with a protoplasmic droplet (long arrows), sperm with a protoplasmic droplet (thick arrow), kinked tail (short arrow) (A); microcephalic head with a curved tail, (head arrow), and kinked tail (short arrow) (B). (Giemsa satin, X 1000).

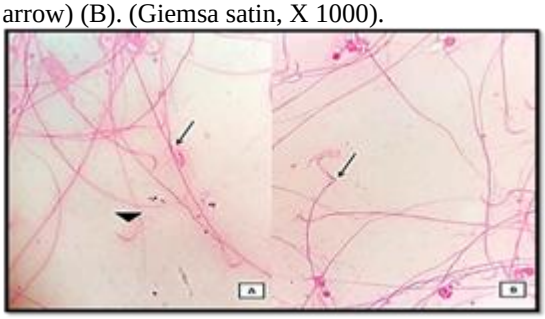

Figure 5 Photomicrographs of the semen smears of the CS rats showing, some abnormal sperms including, microcephalic head (arrow), free head (detached tail) (head arrow) (A); and detached head (arrow) (B). (Giemsa satin, X 1000).

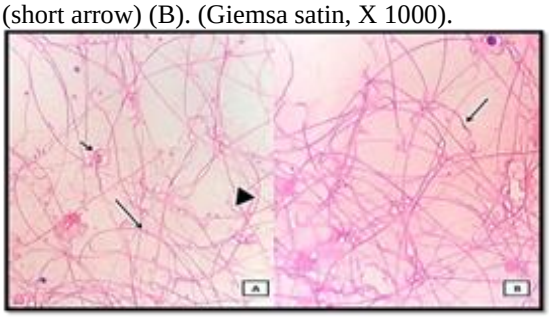

Figure 6 Photomicrographs of the semen smears of the CS rats showing, some abnormal sperms including, banana head (head arrow), curved tail (long arrow), vacuolated head (short arrow) (A); and Abaxial attachment of the neck and middle piece to the head (arrow) (B). (Giemsa satin, X 1000).

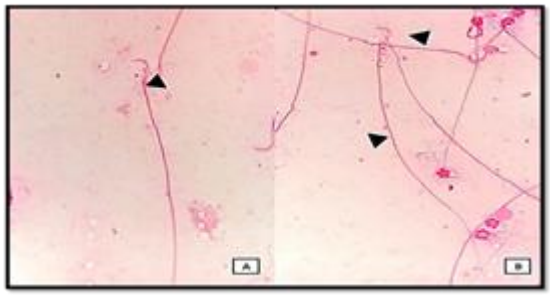

Figure 7 Photomicrographs of the semen smears of the TP rats (A & B). Showing normal morphological of sperms (head arrows). Note; normal head, middle piece, and normal tail. (Giemsa satin, X 1000).

Histopathological examination:

Microscopically, the testis sections of the control group and Sidr honey alone showed a normal seminiferous tubule. The seminiferous tubules containing normal spermatogonia, spermatocytes, spermatids, sperms, and normal basement membrane (Figures 8 & 9), normal interstitial spaces containing interstitial cells of Leydig, and normal seminiferous tubules containing normal Sertoli cells (Figures 10 & 11). Furthermore, histopathological examinations of the testes in rats after exposure to CS alone for 4 weeks showed, different histopathological changes when compared with the control group such as testicular degeneration appeared in most seminiferous tubules with focal atrophied tubule with debris of damaged spermatogenic cells and absence of the spermatozoa in the lumen, necrotic changes in different layers of seminiferous tubule with clumping of spermatids in distorted seminiferous tubule, absent of Sertoli cells, irregular of basement membrane and spermatid giant cells. On the other hand, CS treated rats showed sloughing of germinal epithelium with distorted germ cells and few sperm flagella in their lumen (Figure 12). As displayed in figure (13), irregular basement membrane, thin basement membrane surrounding a seminiferous tubule, fewer elongated spermatids with degenerated spermatogonia, necrotic interstitial Leydig cells, and some infiltration inflammatory cells. This is in agreement with other studies [14, 15, 39], who found that the receiving of CS reduced the number of spermatocytes, spermatids, and Sertoli cells accompanied with thickening of the tunica propria. Moreover, [40, 39], stated that CS was related to immature spermatozoa, sperm head defect, and disturbances in spermatozoa chromatin and DNA integrities in the idiopathic infertile subjects. However, [41] observed that CS-induced reductions of sperm production, maturation, and fertilizing potential in rats were accompanied by a decrease in testosterone and oestradiol levels. Furthermore, [15] reported that CS increased the production of ROS by increasing generations of testicular H_2O_2 and hydroxyl radicals in experimental rats. Moreover, ROS causes damage to the genetic material of spermatozoa by CS [42], inhibits testosterone biosynthesis in Leydig cells [41], and germ cell apoptosis [19].

The testis sections of animals that treated with Sidr honey for two weeks then the animals were exposure to cigarette smoke by a machine smoking with taking the Sidr honey for 4 weeks (TP) manifested minimal histopathological changes when compared with CS group showed, improve testicular arrangement with normal spermatozoa in their lumen, seminiferous epithelium with spermatogonia, reorganization of germinal cell layer that indicator repair of some seminiferous tubules with intraepithelial empty spaces, normal Sertoli cells are seen with attached sperms, interstitial spaces were within normal limit and Leydig cells these were apparent in the figures (14 & 15). These results were agreement with [14, 15], who reported the administration of honey before CS showed a marked reduction in the histopathological changes in the testis tissue. These results might suggest that honey might have protective effects on the oxidative stress in rat testis exposed to CS where they said honey has some vitamins and antioxidants such as vitamins A, C, and E, flavonoids, and phenols. Also, they suggested that honey had an enhancement effect on the hormonal levels, anti-radical and antioxidant properties. Moreover, [15] have also reported that honey could enhance testicular function by possibly healing the testicular injury and decreasing oxidative stress. This might suggest that honey has the potential healing properties against the toxic effects of CS to reduce testis damage.

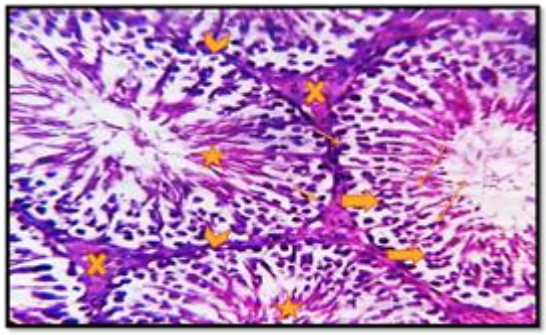

Figure 8 Photomicrograph of the testis section of control rats showing, normal seminiferous tubules containing spermatogonia (small arrows), primary spermatocytes (thick arrows), spermatids (long arrows), and sperms (stars). Normal interstitial spaces (x) and normal basement membrane (head arrows) (H & E stain, X400).

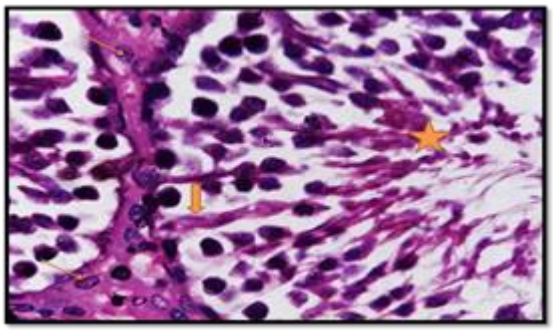

Figure 9 Photomicrograph of the testis section of control rats showing, normal interstitial spaces containing Interstitial cells of Leydig (long arrow), normal seminiferous tubules containing Sertoli cells (thick arrow), and many elongated spermatids (star). (H & E stain, X1000).

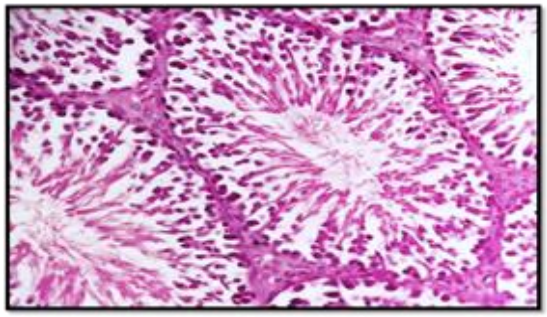

Figure 10 Photomicrograph of the testis section of H rats showing, normal seminiferous tubules containing spermatogonia, primary spermatocytes, spermatids, and sperms. Normal interstitial spaces and normal basement membrane (H & E stain, X400).

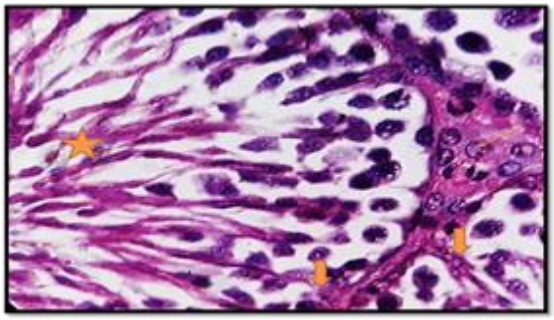

Figure 11 Photomicrograph of the testis section of Sidr honey rats showing, normal interstitial spaces containing Interstitial cells of Leydig (long arrow) and normal seminiferous tubules containing Sertoli cells (thick arrows). Note, many elongated spermatids (star). (H & E stain, X1000).

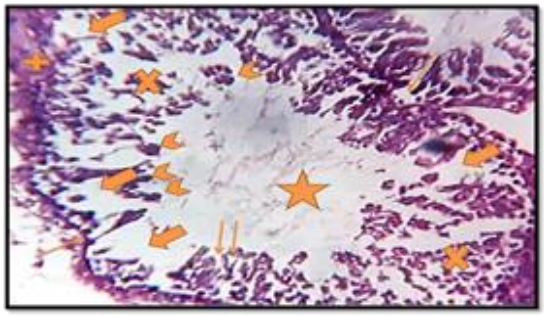

Figure 12 Photomicrograph of the testis section of (CS) rats showing, focal atrophied tubule with debris of damaged spermatogenic cells (X) and absence of the spermatozoa in the lumen (star), necrotic changes in different layers of seminiferous tubule (thick arrows) with clumping of spermatids in distorted seminiferous tubule (double arrow). Absent of Sertoli cells, irregular of basement membrane (long arrows), interstitial oedema (+), and spermatid giant cells (head arrows). (H & E, X400).

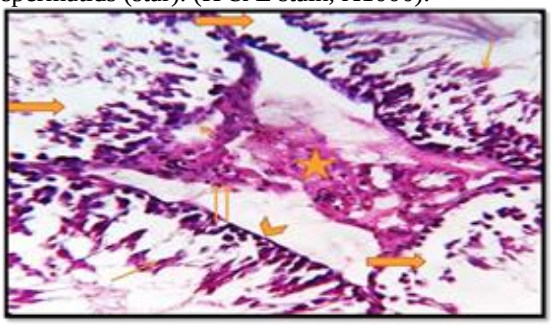

Figure 13 Photomicrograph of the testis section of (CS) rats showing, focal tubular necrosis with architectural distortion of seminiferous tubules (thick arrows), and irregular basement membrane (short arrow), thin basement membrane surrounding a seminiferous tubule (head arrow), fewer elongated spermatids with degenerated of spermatogonia (long arrows), and necrotic interstitial Leydig cells (star). Infiltration inflammatory cells is also seen in the intertubular space (double arrow). (H & E, X400).

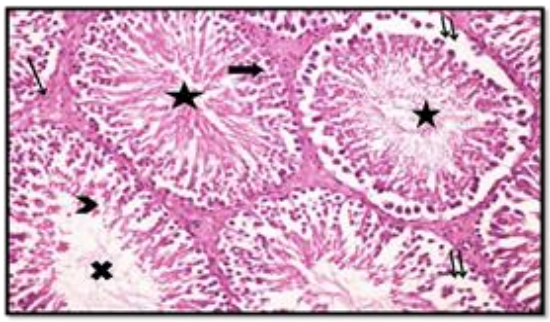

Figure 14 Photomicrograph of the testis section of TP rats showing, improved testicular arrangement with normal spermatozoa in their lumen (star), seminiferous epithelium with spermatogonia, (thick arrow), reorganization of germinal cell layer that indicator repair of some seminiferous tubules with intraepithelial empty spaces (double arrows), interstitial spaces were within normal limit and Leydig cells (arrow), elongated spermatids (head arrow), and few sperms in the lumen (X). (H & E, X400).

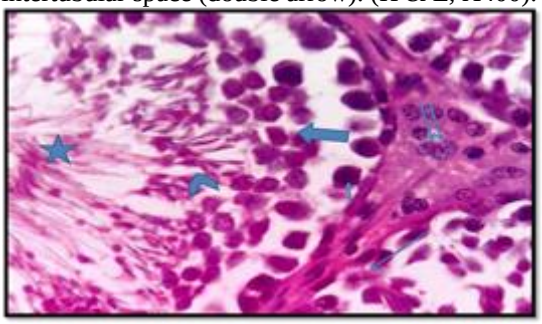

Figure 15 Photomicrograph of the testis section of TP rats showing, regular seminiferous tubules lined by germinal epithelium; spermatogonia (short arrow), spermatocytes (thick arrow), spermatids (head arrow), and sperms (star). Note, Sertoli cells are seen with attached sperms (long arrow), and interstitial spaces were within normal limit and Leydig cells (double arrow). (H & E, X1000).

Conclusion

The present findings clearly demonstrate that exposure to CS is capable of inducing histopathological changes in the testis tissues and sperms damages of the experimental rats, so exposure to CS reduces fertility in adult male albino rats as a reproductive toxicant. Besides, Sidr honey has therapeutic effects on testes and sperm damage by exposed to cigarette smoke in male albino rats the protective effects of Sidr honey as a natural antioxidant to help the damage tissues. However, further investigation is encouraged to confirm the role and mechanisms of action that Sidr honey works on other organ injuries.

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