



Effect of Libyan Sidr honey on thyroid gland damage induced by cigarette smoke in male rats.

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ABSTRACT

The toxicity of cigarette smoke (CS) products is through vast production of reactive oxygen species. So, this study aimed to evaluation the effect of Libyan Sidr honey on thyroid gland damage induced cigarette smoke in male rats. 28 adult male rats were divided into four groups; Group 1: Control group (NC); group 2: rats were received the Libyan Sidr honey (LSH) orally (100 mg/kg b.w./d.) for 4 w.; group 3: rats were exposed to 5 lit of the Karelia red cigarette smoke (KRC) (5 times/d.) by a machine smoking for 4 w.; and group 4: (LSH+KRC) rats were received the LSH orally (100 mg/kg b.w./d.) for 2 w., then the rats were exposed to the KRC generated by a machine smoking for 4 w. with the continuation of LSH doses. The result revealed that T4 showed, non-significant decrease in the KRC group compared to the NC group. While, it was significant declining in T3 with a significant increase in TSH levels as compared to NC group. Moreover, the (LSH+KRC) group showed a noticeable improvement in T4 & T3 as compared with the KRC group. Furthermore, the (LSH + KRC) group showed a significantly positive change in TSH as compared with KRC group. The histopathological examination of the thyroid of rats after exposure to KRC alone showed different histopathological changes when compared with control group. Whereas, the (LSH + KRC) group showed, improve thyroid arrangement with normal thyroid follicles when compared with KRC group. Conclusion, results indicate that the Libyan Sidr honey antioxidant activity against thyroid tissues damage induced cigarette smoke in adult male albino rats.

تأثير عسل السدر الليبي على تلف الغدة الدرقية الناجم عن دخان السجائر في ذكور الجرذان.

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الكلمات المفتاحية:

دخان السجائر
عسل
أمراض أنسجة
هرمونات
الغدة الدرقية
الجرذان

الملخص

تحدث سمية منتجات دخان السجائر من خلال الإنتاج الواسع لأنواع الأوكسجين التفاعلية. هدفت هذه الدراسة إلى تقييم تأثير عسل السدر الليبي على تلف الغدة الدرقية الناجم عن دخان السجائر في ذكور الجرذان. تم تقسيم 28 ذكراً من الجرذان البالغة إلى أربع مجموعات: المجموعة 1: مجموعة التحكم؛ المجموعة الثانية: تم اعطاء عسل السدر الليبي للجرذان عن طريق الفم (100 مجم/كجم من وزن الجسم) لمدة 4 أسابيع. المجموعة 3: عرضت الجرذان لدخان خمسة سجائر مشتعلة (5 مرات / يوم) الناتج عن آلة التدخين لمدة 4 أسابيع. المجموعة 4: تم اعطاؤها عسل السدر عن طريق الفم (100 مجم/كجم من وزن الجسم) لمدة أسبوعين ثم عرضت الجرذان لدخان سجائر الكريلا الحمراء الناتج عن آلة التدخين بالاستمرار في إعطائها عسل السدر لمدة 4 أسابيع. بينت النتائج أن الثيروكسين أظهر وجود انخفاض غير معنوي في مجموعة دخان السجائر مقارنة بمجموعة التحكم. بينما كان الانخفاض معنوياً في ثلاثي يودوثيرونين مع زيادة معنوية في مستويات الهرمون المحفز للغدة الدرقية مقارنة بمجموعة التحكم. علاوة على ذلك، أظهرت مجموعة الحماية تحسن طفيف في الثيروكسين و ثلاثي يودوثيرونين مقارنة بمجموعة دخان السجائر. علاوة على ذلك، أظهرت مجموعة الحماية تغير إيجابي بشكل معنوي في الهرمون المحفز للغدة الدرقية مقارنة بمجموعة دخان السجائر. أظهر الفحص المرضي للنسيج في مجموعة دخان السجائر تغيرات نسيجية مختلفة مقارنة بمجموعة التحكم. حيث أظهرت مجموعة الحماية تحسن في نسيج الغدة الدرقية بالمقارنة مع مجموعة دخان السجائر. الخلاصة: تشير النتائج إلى أن عسل السدر

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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, exposure to cigarette smoke causes the release of many harmful substances in the body that have the direct potential of forming free radicals and activating inflammatory cells, which produce ROS [5].

Thyroid gland is the largest single endocrine gland in the body, it is located at a midline anlage in the pharyngeal floor [6]. It is also regulates many body functions by secreting triiodothyronine (T3), thyroxine (T4), and calcitonin hormones [7]. These hormones play a role in the development and function of the cardiovascular, nervous, immune and reproductive system [8]. Some studies have found that cigarette smoke causes changes in the serum concentration of thyroid stimulating hormone (TSH), T4, and T3 [9], [10].

Honey has been used as food, drug, and raw materials [11]. Also, it is used as medicinal substantial because its antioxidant ability and high osmotic pressure build up the immunity level of users [12]. Furthermore, It has specific physicochemical properties that healing effect, anti-inflammatory potency, and free radical scavenging ability [13]. Therefore, the present study aimed to study the antioxidant activity of bee honey on thyroid gland damage induced cigarette smoke in male rats.

Materials and Methods:

1. Chemicals:

- Libyan Sidr honey (LSH) used was obtained from local agricultural market and was analyzed by the Centre Lab of Omar Al-Mokhtar University, El-Beida, Libya. 100mg/kg of honey was administered to the rats orally by gavage [14].
- Karelia red cigarettes (KRC) were obtained from the local supermarkets. Rats were exposed to 5 lit KRC by a machine smoking [15].

2. Animals:

28 adult male albino rats (*Rattus norvegicus*), 10 weeks old weighing 180-200 g were used. Rats 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 3 weeks and were housed in cages at standard laboratory conditions of room temperature ($22 \pm 2^\circ\text{C}$). Animals were fed standard rat chow and water ad libitum. The animal protocols were followed in this study in accordance with the guide for the care and use of laboratory animals.

3. Experimental design:

28 Adult male rats were randomly assigned into four groups of 6 animals as follows:

Group 1: The control group (NC), rats were kept under standard laboratory conditions with ventilation and were not exposed to smoke.
Group 2: The Libyan Sidr honey group (LSH), rats were given the Sidr honey (100 mg/kg b.w./d.) [14], orally by gavage for 4 weeks.

Group 3: The Karelia red cigarettes group (KRC). Cigarette smoke exposure was conducted by KRC 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 the Zoology Department, Faculty Science, University of Omar Al-Mokhtar, El-Beyda, Libya (Fig. 1). The glass box is in a cube shape (aquarium shape) with the size of $80 \times 30 \times 40$ cm for keeping the rats [15], [16]. The inhalation was performed in the closed glass box for condensation of the smoke a cover was removed to provide an unforced exchange of fresh air.

The cigarette smoke was used five lit of the KRC by using a smoking machine for 15 minutes and exposing the rats to the cigarette smoke for five minutes, then the rats were rested to 10 minutes and ventilation by removing the box cover. This operation was repeated five times a

day for 4 weeks, where the rats were exposed to the KRC for 6 days in a week [17], [18].

Group 4: The protective group (LSH + KRC), rats were given the LSH (100mg/kg b.w./d.) orally by gavage for 2 weeks then animals treated with the KRC generated by a machine smoking (same group 3) for 4 weeks with the continuation of LSH doses.



Fig. 1: The glass box and smoking machine.

4. Hormonal assay:

At the end of the treatment, the blood samples were collected then centrifuged at 25°C for 10 minutes with 4000 rpm to obtain the serum. The serum samples kept in deep freezer (-18°C). The blood hormones will analyze by using radioimmunoassay (RIA) (TESTO-CTK, DiaSorin, P3093) kit. The serum samples obtained analyzed to determine the concentration of T3, T4 and TSH of the control group and the experimental groups were performed in the Al-Beida Laboratory for Medical Analysis, El-Beida City. Principle of methods were described by [19], [20].

5. Histopathological examination:

After the completion of the treatment period, all rats were anesthetized with diethyl ether, then sacrificed, and their thyroid gland samples from all groups were fixed in formalin (10%), then dehydrated in graded alcohol and embedded in paraffin. Sections of $5\mu\text{m}$ thickness were stained with hematoxylin and eosin using standard procedures. The sections were examined under a light microscope [21].

6. Statistical analysis:

Results were expressed as mean \pm standard error (SE). The parameters were analyzed using significance by one way ANOVA. Means were separated using Turkey's test at $P < 0.05$. The T test also using for compared between two means. All statistical procedures were performed with the Minitab statistical analysis package program (Minitab version 17). The percentage of change was calculated according to the following:

Percentage of change (%) = $\frac{[(\text{Mean of unknown} - \text{Mean of control}) / (\text{Mean of control})] \times 100}{}$

Results:

1. Determination of the hormones activities of thyroid glands:

1.1. Determination of the thyroxin hormone (T4) :

The mean values of the T4 level of control and experimental groups were presented in table (1). The mean values of T4 showed, non-significant decrease ($P < 0.05$) in the KRC group (2.087 ± 0.246) compared to the NC group (2.73 ± 0.135) with a percentage of decrease (-23.6%). While, (LSH + KRC) groups showed noticeable improvement in this hormone with percentage of increase (3.68%) when compared with KRC group.

1.2. Determination of the triiodothyronine hormone (T3):

Averages of the T3 level of rats belonging to the control and experimental groups are given in table (1). The mean values of T3 showed, a significant decrease ($P < 0.05$) in the KRC group (0.199 ± 0.036) as compared with NC group (0.661 ± 0.064). Whereas, slight improvement was observed on the mean value of (LSH + KRC) group (0.36 ± 0.053) compared to KRC group (0.199 ± 0.036). Where the

improvement was recorded in (LSH + KRC) group by the percentage of increase (80.90%) as compared to KRC group.

1.3. Determination of the thyroid-stimulating hormone (TSH):

From results recorded in the table (1). The mean values of TSH showed, a highly significant increase ($P < 0.05$) in the KRC group (0.024 ± 0.003) as compared with NC group (0.009 ± 0.001). Whilst, the (LSH + KRC) group showed a significant positive decline ($P < 0.05$) in the mean value (0.014 ± 0.001) as compared with KRC group, where it was non-significant ($P < 0.05$) between the (LSH+KRC) group (0.014 ± 0.001) and the NC group (0.009 ± 0.001)

Table 1: Average of mean values of T4, T3, and TSH levels in control and experimental groups.

Parameters	NC	LSH	KRC	LSH+KRC
T4 (mg/ml)	2.731± 0.135 AB	3.207± 0.199 A	2.087 ± 0.246 B	2.164 ± 0.106 B
% of change 1		17.42%	-23.6%	-20.8%
% of change 2				3.68%
T3 (mg/ml)	0.661 ± 00.064 A	0.691± 0.059 A	0.199 ± 00.036 B	0.36 ± 0.053 B
% of change 1		4.53%	-69.9 %	-45.53%
% of change 2				80.90%
TSH (µLu/ml)	0.009 ± 0.001 B	0.008 ± 0.001 B	0.024 ± 0.003 A	0.014 ± 0.001 B
% of change 1		-11.1%	166.7%	55.6%
% of change 2				-41.67%

*Data are expressed as mean ± SE rat within each row, means with different superscript (A & B) were significantly different $p < 0.05$, were means superscripts with the same letters, mean that there is no significant difference ($p < 0.05$).

* NC =Normal control. LSH= Libyan Sidr honey treated group. KRC = Karelia red cigarettes group (LSH + KRC)= Protective group.

* % of change 1= Percentage of change between NC and other groups.

* % of change 2= Percentage of change between (LSH + KRC) and KRC group.

2. Histopathological studies:

2.1. The thyroid sections of the NC group:

Microscopically, the thyroid sections of the control group showed a normal thyroid follicles lined with simple cuboidal follicles epithelial cells, parafollicles cells, and filled with homogenous acidophilic colloid (Figs. 2 & 3).

2.2. The thyroid sections of the LSH group:

Light microscopic examination of the thyroid after administration of LSH alone revealed a normal histological structure: Normal thyroid follicles lined with simple cuboidal follicles epithelial cells, parafollicles cells, and filled with homogenous acidophilic colloid as in the control group (Figs. 4 & 5).

2.3. The thyroid sections of rats exposure to KRC:

Histopathological examination of the thyroid of rats after exposure to KRC alone showed different histopathological changes when compared with control group such as degeneration of the thyroid follicles with reduced or disappeared colloids in thyroid follicles, architecture of thyroid follicles were markedly shrunken and distorted, deformed, deciduous and necrotic epithelial cells of the thyroid follicles, oedema and widening of the interstitial tissue, and congestion of blood vessels between thyroid follicles (Fig. 6). In addition, some follicles showed focal atrophied of thyroid follicles with desquamated follicular cells, and necrosis of the thyroid follicles (Fig. 7). Also, in the figure (8) found interstitial oedema with inflammatory cells, congestion of blood vessels between thyroid follicles, and shattered of basement membrane.

Moreover, figure (9) showed vacuolization in many thyroid follicles, and lymphoid follicle and some pyknotic nuclei. On the other hand, KRC treated rats showed hyperplasia and hypertrophy in the follicle

epithelial cells are seen in the figure (10), severe degenerative change and atrophy of thyroid follicles with follicles appear involuted with minimal amount of colloid, detached and desquamated follicular cells, vacuolization in the lining epithelium of the follicles as well as irregular of basement membrane in Fig. (11).

On the other area, reduced or disappeared colloids in thyroid follicles, and necrosis with architectural distortion of thyroid follicles, and sever hemorrhage between the thyroid follicles (Fig. 12). This was accompanied by the presence of degeneration of the thyroid follicles with reduced colloids in the thyroid follicles and deformed, deciduous and destroyed with the epithelial lining of vacuolated cytoplasm and most follicles with larger nuclei. Architectural distortion of thyroid follicles with discontinuity of their basement membrane, congestion of blood vessels between thyroid follicles, vacuolization in some thyroid follicles were noticed in Fig, (13). In the gross level, the thyroid of male adult rats after exposure to KRC at 4 weeks showed severe damage in the thyroid tissues.

2.4. The thyroid sections of protective rats (LSH + KRC):

The thyroid sections of animals that treated with LSH for two weeks then the animals were exposure to KRC by a machine smoking after taking the LSH for 4 weeks manifested minimal histopathological changes when compared with KRC group. Marked improvement of the thyroid follicles with vacuolization in some thyroid follicles, irregular of basement membrane, and necrosis between the thyroid follicles as well as hemorrhage, and oedema with inflammatory cells (Fig. 14). Improve thyroid arrangement with normal thyroid follicles lined with simple cuboidal follicles epithelial cells and filled with homogenous acidophilic colloid with few larger nuclei and few vacuolization in the lining epithelium of the follicles these were apparent in the figure (15). Finally, in many areas of thyroid tissues in protective rats attained almost normal patterns.

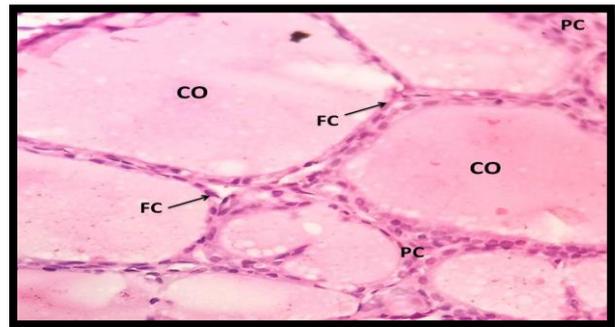


Fig. 2: Photomicrograph of the thyroid section of NC rats showing, normal thyroid follicles lined with simple cuboidal follicles epithelial cells (FC), parafollicles cells (PC), and filled with homogenous acidophilic colloid (CO). (H & E stain, X400).

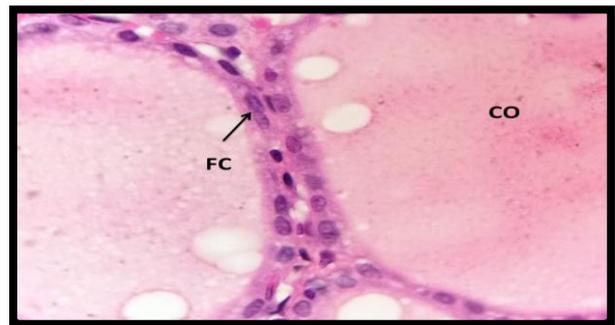


Fig. 3: Photomicrograph of the thyroid section of NC rats showing, normal thyroid follicles lined with simple cuboidal follicles epithelial cells (FC), and filled with homogenous acidophilic colloid (CO) (H & E stain, X1000).

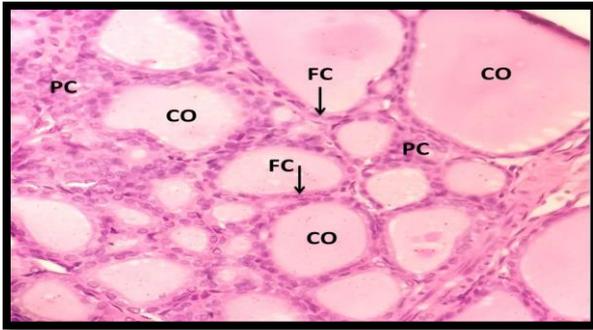


Fig. 4: Photomicrograph of the thyroid section of LSH rats showing, normal thyroid follicles lined with simple cuboidal follicles epithelial cells (FC), parafollicles cells (PC), and filled with homogenous acidophilic colloid (CO). (H & E stain, X400).

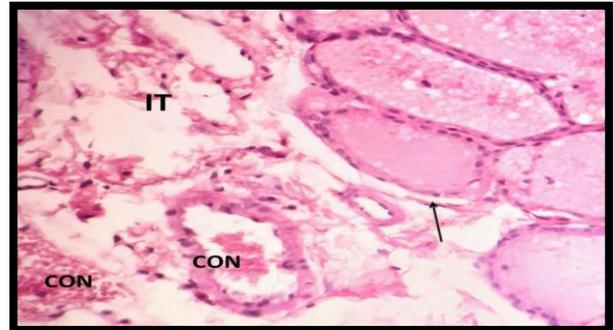


Fig. 8: Photomicrograph of the thyroid section of KRC rats showing, interstitial oedema with inflammatory cells (IT), congestion of blood vessels between thyroid follicles (CON), and shattered of basement membrane (arrow) (H & E, X400).

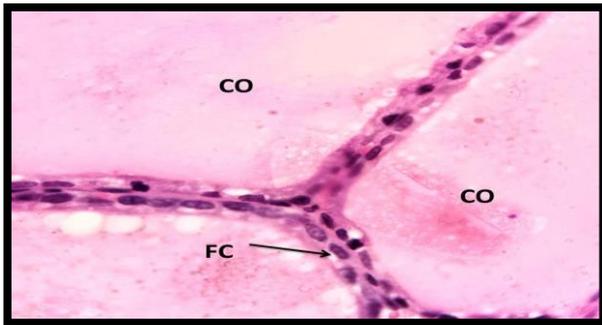


Fig. 5: Photomicrograph of the thyroid section of LSH rats showing, normal thyroid follicles lined with simple cuboidal follicles epithelial cells (FC), and filled with homogenous acidophilic colloid (CO) (H & E stain, X1000).

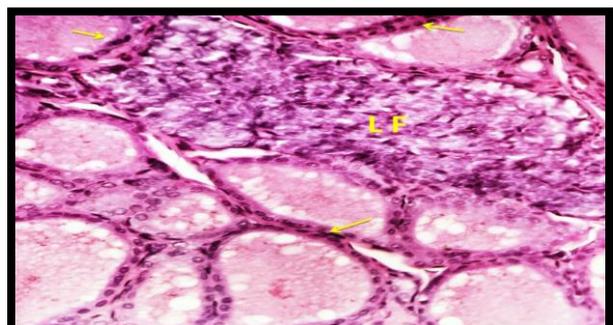


Fig. 9: Photomicrograph of the thyroid section of KRC rats showing, vacuolization in many thyroid follicles, and lymphoid follicle (LF). Note some pyknotic nuclei (arrows) were seen (H & E, X400).

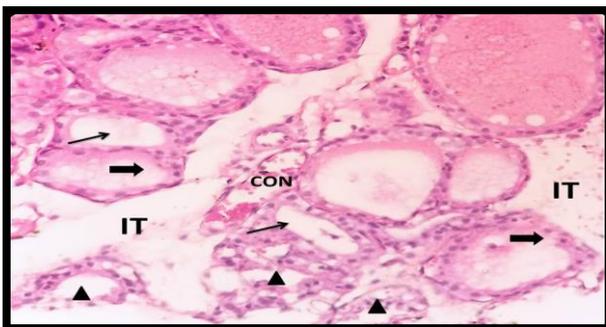


Fig. 6: Photomicrograph of the thyroid section of KRC rats showing, degeneration of the thyroid follicles with reduced or disappeared colloids in thyroid follicles (thin arrows), architecture of thyroid follicles were markedly shrunken and distorted (head arrows), deformed, deciduous and necrotic epithelial cells of the thyroid follicles (thick arrows), oedema and widening of the interstitial tissue (IT), and congestion of blood vessels between thyroid follicles (CON) (H & E, X400).

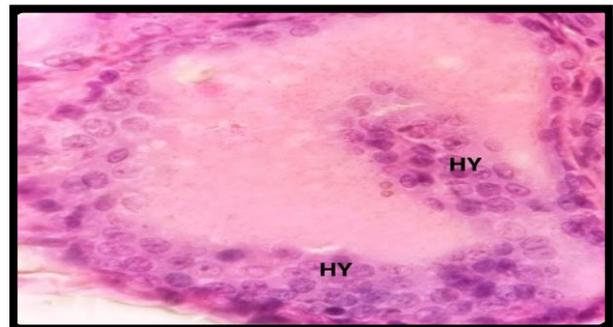


Fig. 10: Photomicrograph of the thyroid section of KRC rats showing, hyperplasia and hypertrophy in the follicle epithelial cells (HY) (H & E, X1000).

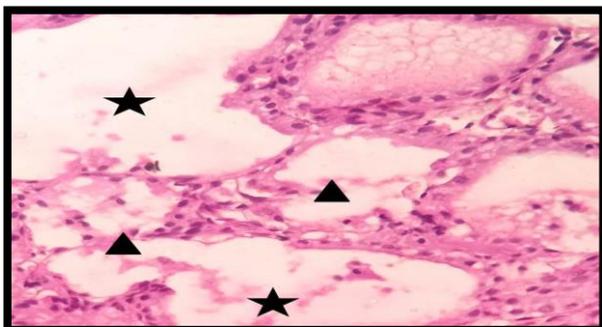


Fig. 7: Photomicrograph of the thyroid section of KRC rats showing, focal atrophied of thyroid follicles with desquamated follicular cells (head arrows), and necrosis of the thyroid follicles (stars) (H & E, X400).

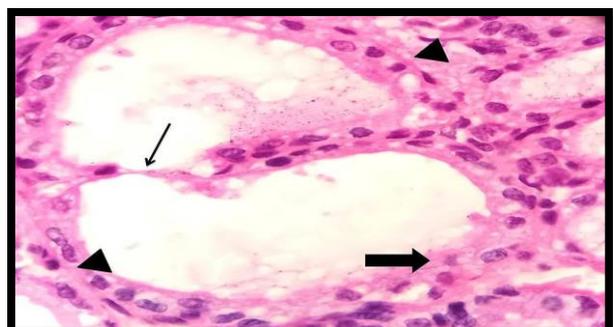


Fig. 11: Photomicrograph of the thyroid section of KRC rats showing, severe degenerative change and atrophy of thyroid follicles with follicles appear involuted with minimal amount of colloid, detached and desquamated follicular cells (thick arrow), vacuolization in the lining epithelium of the follicles (head arrows) as well as irregular of basement membrane (long arrow) (H & E, X1000).

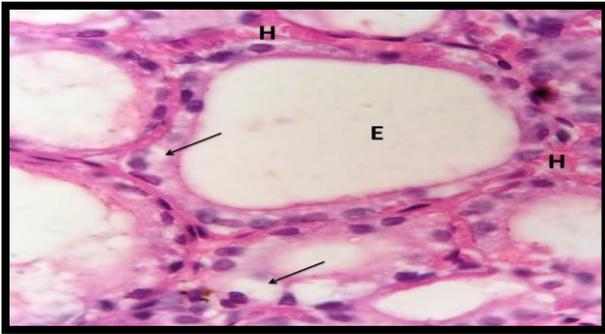


Fig. 12: Photomicrograph of the thyroid section of KRC rats showing, reduced or disappeared colloids in thyroid follicles (E), and necrosis with architectural distortion of thyroid follicles (arrows). Sever hemorrhage between the thyroid follicles (H) (H & E, X1000).

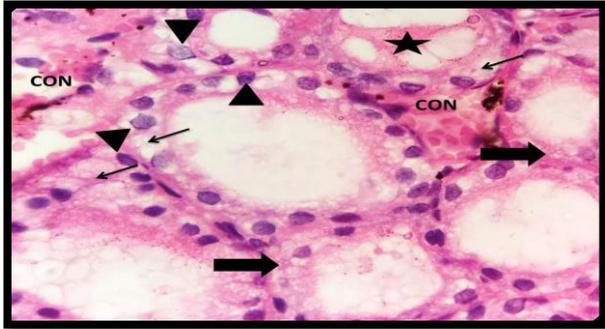


Fig. 13: Photomicrograph of the thyroid section of KRC rats showing degeneration of the thyroid follicles with reduced colloids in the thyroid follicles and deformed, deciduous and destroyed with the epithelial lining of vacuolated cytoplasm and most follicles (thin arrows) with larger nuclei (head arrows). Architectural distortion of thyroid follicles with discontinuity of their basement membrane (thick arrow), congestion of blood vessels between thyroid follicles (CON), vacuolization in some thyroid follicles (star) (H & E, X1000).

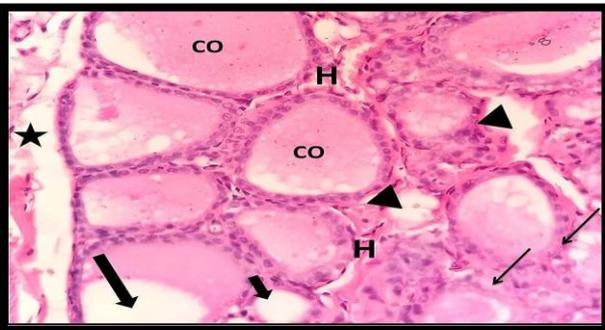


Fig. 14: Photomicrograph of the thyroid section of (LSH+KRC) rats showing, marked improvement of the thyroid follicles with vacuolization in some thyroid follicles (thick arrows), irregular of basement membrane (thin arrows), and necrosis between the thyroid follicles (head arrows) as well as hemorrhage (H), and oedema with inflammatory cells (star) (H & E, X400).

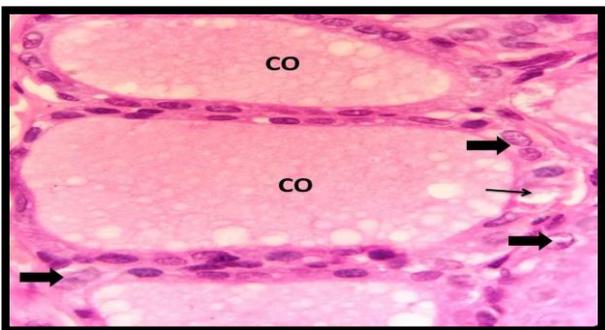


Fig. 15: Photomicrograph of the thyroid section of (LSH+KRC) rats showing, improved thyroid follicles lined with simple cuboidal follicles epithelial cells and filled with homogenous acidophilic

colloid (CO) with few larger nuclei (thick arrow) and few vacuolization in the lining epithelium of the follicles (thin arrow) (H & E, X1000).

Discussion:

Cigarette smoke contains most of the toxic and carcinogenic compounds in mainstream smoke, where it is effects on human health, which caused by free radicals, oxidative stress, the nicotine, the carbon monoxide, and other contains [22]. Exposure to CS has been shown to have variable effects on thyroid function, reflected by changes in serum T4 & T3 concentrations [23]. The result obtained from the present investigation revealed that the mean values of T4 showed, non-significant decrease in the KRC group as compared with NC group. Whereas, the treatment with KRC showed a significant decrease in T3. Besides, the data showed, a significant increase in TSH levels compared to NC group. These results are in line with those observed by many investigators [24], [10].

Wiersinga [24] documented that the effect of the CS on thyroid function may be confounded by body mass index (BMI), where TSH is associated with BMI, and smokers may have lower BMI. Also, they said that the association of BMI with higher TSH and lower T4. Where thyroid hormones play an essential role in metabolism. Moreover, the inhaled cigarette extracts could lead to decreases in serum T4 & T3, which could in turn lead to increases in serum TSH levels [25]. The reduced of the thyroid hormone secretion associated may due to decreased metabolic rate as well as a result of with hypothyroidism and increased glucose storage [26]. In addition [27], [28] found that, chemical components of cigarette such as nicotine, thiocyanate and benzpyrene produced the effect on the thyroid hormone synthesis and the promotion of goiter, which caused directly or indirectly to abnormal thyroid hormone production. On the other hand, the exposure to CS leads to a decline in thyroid hormones' function i.e., T4 & T3 [29]. The reduced secretion of T4 & T3 is accompanied by inhibition of many functions of organs, because these hormones play a role in the development and function of cardiovascular, nervous, immune and reproductive system [8]. Moreover, [30], [31] and [32] reported that the decrease in thyroid hormones levels is due to the effect of chemicals toxin on inhibits 5-deiodinase enzyme, thyroid peroxidase enzymes (TPO) and blocks intrathyroidal and peripheral conversion of T4 & T3. In addition, univariate analysis revealed that TSH levels in people who exposed to cigarette smoke were significantly higher than in healthy people [25]. In this regard, some studies have shown that the cigarette contains monoamine oxidase inhibitors; monoamine oxidase leads to decomposition of certain neurotransmitters such as monoaminergic, dopamine, and norepinephrine, where the dopamine affects the hypothalamus and therefore pituitary, and then influences thyroid [33]. However, the inhibition of serum levels of thyrotropin caused to hypersecretion of pituitary TSH and an amplified increase in serum TSH level [34]. Additionally, the hypothyroidism disease leading to turbulences of thyroid gland, inhibits the synthesis of thyroid hormones, suppression of antioxidants and raised of ROS [35], [36] and [37]. Moreover, [38] who reported that the T4 & T3 are increased and TSH level is decreased by from alteration in the monodeiodination pathway. Besides that, [39] explained that the increase of serum T3 is due to the stimulation in monodiodination of T4 in peripheral tissues.

On the other hand, this study demonstrates the rats in the (LSH+KRC) group showed a slight increase in the mean value of T4 & T3 as compared with the KRC group. Furthermore, the (LSH + KRC) group showed a significant decrease in the mean value of TSH as compared with KRC group which is in agreement with other studies [28] who indicated that the ant-oxidative effect of honey oral administration of honey to the CS group could be due to the presence of antioxidant compounds in honey as well as phenolic compounds are present in honey, where these compounds are to enhance free radical scavenging activity and also reduce lipid peroxidation. Similarly, the protective effects of honey by the phenolics, flavonoids and other antioxidants of honey [40].

The results indicated that, the histopathological examination of thyroid tissues in KRC group showed different changes as compared with NC group such as degeneration of the thyroid follicles with reduced or disappeared colloids in thyroid follicles, architecture of thyroid

follicles were markedly shrunken and distorted, deformed, deciduous and necrotic epithelial cells of the thyroid follicles, oedema and widening of the interstitial tissue, and congestion of blood vessels between thyroid follicles with reduced colloids in the thyroid follicles and vacuolated cytoplasm the epithelial lining of follicles. Similar result was reported by [41].

This was concomitant with the results of other researches [8]. They said the histological alteration could be attributed to low level of T4 that led to increased TSH level, which was responsible for the proliferative activity of follicular. Also, hypothyroidism produce of oxidative stress in the thyroid tissues damage and apoptosis [42], [8], and disturbed thyroid tissues and decreasing thyroid hormones [8]. This could be responsible for the increased activity of superoxide dismutase as protective mechanism against elevated oxidative stress status [25]. Furthermore, CS is a major exogenous source of ROS, which are capable of inducing lipid peroxidation, DNA damage, apoptosis of cells and increased oxidative stress. Also, Morariu [43] said that, the converted of thyroid peroxidase into reactive metabolites that caused localized destruction of the thyroid gland.

On the other hand the (LSH + KRC) group showed, improve thyroid arrangement with normal thyroid follicles lined with simple cuboidal follicles epithelial cells and filled with homogenous acidophilic colloid when compared with KRC group. These findings indicate that the LSH as a natural product have a protective effect on the thyroid gland structure and this may be useful for preventing or delaying the development of hypothyroidism and its complications. These results might suggest that honey might have protective effects on the oxidative stress in rat tissues exposed to CS [15], [14]. Also, they believed the honey has antioxidants such as flavonoids and phenols with some vitamins (A, C, and E). Moreover, [44] confirmed that honey plays a important role in attenuating oxidative stress induced cell death. The authors [45] reported that the honey considerably inhibited oxidation of cell membrane and prevented cellular damage and its extracts reduced oedema and pain in inflammatory tissues by inhibition of paw oedema and the loss of anti-inflammatory effect of honey.

Conclusion:

In conclusion, these observations indicate that the Libyan Sidr honey antioxidant activity of the general toxic effects extracted by CS-induced thyroid tissues damage in adult male albino rats. Therefore, Sidr honey may be a beneficial in treatment the complications of hypothyroidism.

References:

- [1]- Ou, B., Huang, D., (2006), Fluorescent approach to quantitation of reactive oxygen species in mainstream cigarette smoke. *Analytical chem.*, **78**, 3097-3103. DOI: 10.1021/ac051993s.
- [2]- Facchinetti, F., Amadei, F., Geppetti, P., (2007), Alpha, beta unsaturated aldehydes in cigarette smoke release inflammatory mediators from human macrophages. *AJRCMB.*, **37**, 617-623. DOI: 10.1165/rcmb.2007-0130OC.
- [3]- Omotoso, O. G., Hambolu, O. Z. Alabi, A. S., (2017), Cigarette smoke alters testicular and epididymal histology in adult Wistar rats. *JECA.*, **16**, 25-28. DOI: 10.4103/jeca.jeca-31-16.
- [4]- Ufele, A. N., (2017), Effects of nicotine and sodium bicarbonate on blood parameters of albino rats (*Rattus norvegicus*). *Cell Biol.*, **5**, 33-37. DOI: 10.11648/j.cb.20170504.11.
- [5]- Diniz, M. F., Dourado, V. A., Silva, M. E., Pedrosa, M. L., Bezerra, F. S., Lima, W. G., (2013), Cigarette smoke causes changes in liver and spleen of mice newborn exposed during pregnancy. *J Cytol Histol.*, **4**, 1-5. DOI:10.4172/2157-7099.1000168.
- [6]- Nilsson, M., Fagman, H., (2017), Development of the thyroid gland. *Development*, **144**, 2123-2140. doi:10.1242/dev.145615.
- [7]- Diekman, M. J., Anghelescu, N., Endert, E., Bakker, O., Wiersina, W. M., (2000), Changes in plasma low-density lipoprotein (LDL) and high-density lipoprotein cholesterol in hypo- and hyperthyroid patients are related to changes in free thyroxine, not to polymorphisms in LDL receptor or cholesterol ester transfer protein genes. *J Clin Endocrinol Metab.*, **85**, 1857-1862. DOI: 10.1210/jcem.85.5.6595.
- [8]- Amra, E. A., Abd El Rehim, S. A., Lashein, F. M., Shoaeb, H. S., (2022), Effect of a bradykinin potentiating factor separated from honey bee venom on thyroid gland and testis in hypothyroid white rats. *JOBAS.*, **83**, 1-10. Doi:10.1186/s41936-021-00263-6.
- [9]- Belin, R. M., Astor, B. C., Powe, N. R., Ladenson, P. W., (2004), Smoke exposure is associated with a lower prevalence of serum thyroid autoantibodies and thyrotropin concentration elevation and a higher prevalence of mild thyrotropin concentration suppression in the Third National Health and Nutrition Examination Survey (NHANES III). *J Clin Endocrinol Metab.*, **89**:6086-6077. DOI: 10.1210/jc.2004-0431.
- [10]- Sawicka-Gutaj, N., Gutaj, P., Sowiński, J., Wender-Ożegowska, E., Czarnywojtek, A., Brązert, J., Ruchala, M., (2014), Influence of cigarette smoking on thyroid gland - an update. *Endokrynol.*, **65**, 54-62. DOI: 10.5603/EP.2014.0008.
- [11]- Cooper, R. A., Halas, E., Molan, P. C., (2002), The efficacy of honey in inhibiting strains of *Pseudomonas aeruginosa* from infected burns. *J. Burn Care Rehabil.*, **23**, 366-370. DOI: 10.1097/00004630-200211000-00002.
- [12]- Dixon, B., (2003), Bacteria cannot resist honey. *Lancet Infect. Dis.*, **3**, 116. DOI: 10.1016/s1473-3099(03)00524-3.
- [13]- Tashkandi, H., (2021), Honey in wound healing: An updated review. *Open Life Sci.*, **16**, 1091-1100. DOI: 10.1515/biol-2021-0084.
- [14]- Kolawole, T. A., Oyeyemi, W. A., Adigwe, C., Leko, B., Udeh, C., Dapper, D. V., (2015), Honey attenuates the detrimental effects of nicotine on testicular functions in nicotine treated Wistar rats. *Niger. J. Physiol. Sci.*, **30**, 11-16.
- [15]- Mohamed, M., Sulaiman, S. A., Jaafar, H., Sirajudeen, K. N. S., (2011), Antioxidant protective effect of honey in cigarette smoke-induced testicular damage in rats. *Int. J. Mol. Sci.*, **12**, 5508-5521. DOI: 10.3390/ijms12095508.
- [16]- Al-Zail, N. I., Alshailabi, E. M.A., Abraheem, R. A., (2022). Changes in sexual hormone induced by cigarette smoke and the potential protective role of sidr LJBS., **19**, 19-28.
- [17]- Khalaf, G., Mostafa, H. K. K., (2012), Histological and immunohistochemical study on the effect of passive smoking on the skin of adult male albino rats and the possible protective role of *Nigella sativa* oil. *Egypt J. Histol.*, **35**, 87-94. DOI: 10.1097/01.EHX.0000410719.45416.6f.
- [18]- Alshailabi, E. M. A., Abdalally, O. A., Majeed, S. F., (2021), Histopathological study on the protective effect of vitamin C against paracetamol-induced acute hepatic damage in rat. *Global Libyan J.*, **53**,1-15.
- [19]- Ladenson, P. W., (1996), Optimal laboratory testing for diagnosis and monitoring of thyroid nodules, goiter, and thyroid cancer. *Clin chem.*, **42**, 183-187.
- [20]- Shenkman, L., Mitsuma, T., Hollander, SC. S., (1973), Modulation of pituitary responsiveness to thyrotropin-releasing hormone by triiodothyronine. *J Clin Inv.*, **52**, 205-209.
- [21]- R. D. Lillie, *Histopathological techniques and practical histochemistry*. 3rd ed. McGraw-Hill, New York, USA, 1954.
- [22]- Malenica, M., Prnjavorac, B., Bego, T., Dujic, ST., Semiz, A., Skrbo, S., Gusic, A., Hadzic, A., Causevic, A., (2017), Effect of cigarette smoking on haematological parameters in healthy population. *Med Arch.*, **71**, 132-136. DOI: 10.5455/medarh.2017.71.132-136.
- [23]- Soldin, O. P., Goughenour, B. E., Gilbert, S. Z. Landy, H. J., Soldin, S. J., (2009), Thyroid hormone levels associated with active and passive cigarette smoking. *Thyroid*, **19**, 817-823. DOI: 10.1089/thy.2009.0023.
- [24]- Wiersinga, W. M., (2013), Smoking and thyroid. *Clin Endocrinol.*, **79**, 145-151. DOI: 10.1111/cen.12222.
- [25]- Zhang, Y., Shi, L., Zhang, Q., Peng, N., Chen, L., Lian, X., Liu, C., Shan, S., Shi, B., Tong, N., Wang, S., Weng, J., Zhao, J., Teng, W., (2019) The association between cigarette smoking and serum thyroid stimulating hormone, thyroid peroxidase antibodies and thyroglobulin antibodies levels in Chinese residents: A cross-sectional study in 10 cities. *PLoS ONE*, **14**, e0225435. DOI: 10.1371/journal.pone.0225435.
- [26]- Adewoye, E. O., Omolekulo, T. E., (2014), Effect of honey on altered thyroid state in female Wistar rats. *Arch. Bas. App. Med.*, **2**, 65-70.

- [27]- Meral, I., Arslan, A., Him, A., Arslan, H., (2015), Smoking-related alterations in serum levels of thyroid hormones and insulin in female and male students. *alternative therapies in health and medicine. Altern Ther Health Med.*, **21**, 9- 24.
- [28]- Brown, S. J., Bremner, A. P., Hadlow, N. C., Feddema, P., Leedman, P. J., O'Leary, P. C., Walsh, J. P., (2016), The log TSH-free T4 relationship in a community-based cohort is nonlinear and is influenced by age, smoking and thyroid peroxidase antibody status. *Clin Endocrinol.*, **85**,789-96. DOI: 10.1111/cen.13107.
- [29]- Tadayon, S., Dehkordi, Z. R., Jafarzadeh, L., (2018), The effect of secondhand smoke exposure on level of maternal thyroid hormones. *IJPPR*,**8**, 53-58.
- [30]- Moriyama, K., Tagami, T., Usui, T., Naruse, M., Nambu, T., Hataya, Y., Kanamoto, N., Li, Y., Yasoda, A., Arai, H., Nakao, K., (2007), Antithyroid drugs inhibit thyroid hormone receptor-mediated transcription. *J Clin Endocrinol Metab.*, **92**, 1066-1072. DOI:10.1210/jc.2006-1621.
- [31]- Manna, D., Roy, G., Mughesh, G., (2013), Antithyroid drugs and their analogues: synthesis, structure, and mechanism of action. *Acc. Chem. Res.*, **46**, 2706-2715. DOI: 10.1021/ar4001229.
- [32]- Alassaf, F. A., Qazzaz, M. E., Alfahad, M., Abed, M. N., Jasim, M. H. M., Thanoon, I. A., (2021), Effects of bee propolis on thyroid function tests in healthy volunteers. *Tropical Journal of Pharmaceutical Research*, **20**, 859-863. DOI:10.4314/tjpr.v20i4.28.
- [33]- MacDonald, A. A., Herbison, G. P., Showell, M., Farquhar, C. M., (2010), The impact of body mass index on semen parameters and reproductive hormones in human males: A systemic review with meta-analysis. *Hum Reprod Update.*,**16**, 293-311. DOI: 10.1093/humupd/dmp047.
- [34]- Sushma, M., Ramalingam, K., Naidu, N. J., Lakshmi, V. T., (2014), Lipid profile alterations and fasting blood glucose levels in primary hypothyroidism. *IJRMS.*, **2**, 1694-1698. DOI:10.5455/2320-6012.IJRMS20141185.
- [35]- Nakamura, H., Noh, J. Y., Itoh, K., Fukata, S., Miyuchi, A., Hamada, N. (2007), Comparison of methimazole and propylthiouracil in patients with hyperthyroidism caused by Grave's disease. *J Clin Endocrinol Metab.*, **92**, 2157-2162. DOI:10.1210/jc.2006-2135.
- [36]- Torun, A. N., Kulaksizoglu, S., Kulaksizoglu, M., Pamuk, B. O., Isbilen, E Tutuncu, N. B., (2009), Serum total antioxidant status and lipid peroxidation marker malondialdehyde levels in overt and subclinical hypothyroidism. *Clin Endocrinol.*, **70**, 469-474. DOI: 10.1111/j.1365-2265.2008.03348.x.
- [37]- Kandasamy, B., Jayaraai, I. A., Prabhakar, J., (2021), Effect of abnormal thyroid hormone changes in lipid peroxidation and antioxidant imbalance in hypothyroid and hyperthyroid patients. *Highlights on Med Med Res.*, **12**, 159-167. DOI: 10.9734/bpi/hmmr/v12/2178F.
- [38]- Barakat, H., El-Masry, S., (2015), Impact of folic acid on the neurotransmitters and oxidant-antioxidant balance in hypothyroid and hyperthyroid rats. *Int J Pharma and Bio Sci.*, **6**, 1155-1165.
- [39]- Saxena, V., Dharamveer, G. R., & Saraf, S. (2012). *Ficus carica* leaf extract in regulation of thyroidism using Elisa technique. *Asian J Pharm Clin Res.*, **2**, 77-84.
- [40]- Tanvir, E. M., Afroz, R., Chowdhury, A. Z., Ibrahim Khalil, I., Hossain, S., Rahman, A., Rashid, H., Ganc, S. H., (2015): Honey has a protective effect against chlorpyrifos-induced toxicity on lipid peroxidation, diagnostic markers and hepatic histoarchitecture. *EuJIM.*, **7**, 525-533. DOI: 10.1016/j.eujim.2015.04.004.
- [41]- Kurus, M., Söğütü, G., Firat, Y., Otlu, A., (2009), The histopathological effect of resveratrol in thyroid tissue of rats exposed to cigarette smoke. *Turkiye Klinikleri J Med Sci.*, **29**,1183-1190.
- [42]- Bhanja, S., Chainy, G. B. N., (2010), PTU-induced hypothyroidism modulates antioxidant defence status in the developing cerebellum. *Int J Dev Neurosci.*, **28**, 262-251. DOI: 10.1016/j.ijdevneu.2010.01.005.
- [43]- Morariu, I. D., Avasilcai, L., Cioanca, O., Morariu, B., Vieriu, M., Tanase, C., (2022), The Effects of Honey Sulfonamides on Immunological and Hematological Parameters in Wistar Rats. *Medicina*, **58**, 1558. DOI:10.3390/58111558.
- [44]- Awhin, P. E., Uzuegbu, U. E., Ojugheli, E., Otomewo, L. O., (2021), Brain levels of reduced glutathione and malondialdehyde in honey-fed Wistar rats. *Biosci Biotech Res Asia.*, **18**, 517-522. DOI:10.13005/bbra/2936.
- [45]- Erejuwa, O. O., Sulaiman, S. A., Ab Wahab, M. S., (2012), Honey: A novel antioxidant. *Molecules*, **17**, 4400-4423. DOI:10.3390/molecules17044400.