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EFFECTS OF EXPOSURE CIGARETTE SMOKE ON THE CARDIAC TISSUES IN MALE ALBINO RATS AND THE IMPROVEMENT ROLE OF SIDR HONEY

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Abstract: The aim of this study was to investigate the effects of exposure cigarette smoke on the cardiac tissues in male rats and the improvement role of Sidr honey. Twenty eight male rats were divided into four groups; Group 1: control rats; group 2: rats were given Libyan Sidr honey (100 mg/kg b.w./d.) orally for 4 weeks.; group 3: rats were exposed to the five lit from sidestream of the Karelia red cigarettes (5 times/d.) by a machine smoking for 4 weeks.; and group 4: rats were received the Sidr honey (100 mg/kg b.w./d.) orally for 2 weeks, then the rats were exposed to the Karelia cigarettes generated by a machine smoking with given the Sidr honey for 4 weeks. The X-Ray radiography of rats showing, heart mildly enlarged size in the KC-exposed rats as compared with the NC rats. While, the POR rats showed normal heart size when compared with KC rats. Moreover, the KC group showed a significant increase ($P < 0.05$) in CK, CK-MB, and LHD as compared to the NC group, whereas the POR group showed a significant decrease ($P < 0.05$) in the CK, CK-MB, and LHD when compared with the KC group. Histological investigation of the heart tissues of the KC group showed different histopathological changes as compared to the NC group. Nevertheless, the POR group showed the marked improvement in the heart tissues as compared to the KC rats. Conclusion, results demonstrated that Libyan Sidr honey significantly reduced the toxic effects of KC-exposed on the heart structures.

Keywords: : Sidr honey, cigarette smoke, enzymes, heart tissues

Introduction

Cigarette smoke (CS) is one of the leading causes of death in many countries, which it is caused tissue damage, release of many substances in the body that have the direct potential of forming free radicals and activating inflammatory cells, such as macrophages and neutrophils, which also produces of reactive oxygen species (ROS) [1], [2], resulting in an imbalance in the cellular oxidant-antioxidant system [3], [4], and increasing the harmful substances concentration of tissues [1].

CS is a complex mixture composed of numerous harmful substances about 5000 chemical compounds. Among these substances

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are nicotine, tar, carbon monoxide [5], [6], polycyclic aromatic hydrocarbons, cyanide, carbon-monoxide, lead, cadmium nitric oxide and nitric dioxide [7], where the nicotine is rapidly absorbed through the mucous membranes, respiratory tract, and gastrointestinal tract, and it is metabolized in the liver and caused oxidative cellular damage [8]. In addition, a long-term of CS exposure may be associated with myocardial dysfunction, heart failure, and increased mortality [9].

Antioxidants keeps the cells components from damage by resolving the free radicals. So, when antioxidants are consumed through the diet block damage to cells [10].

Honey is the natural product, and it has many nutritional, therapeutic and industrial values. it is contains a lot of bioactive substances such as amino acids, alkaloids, vitamins, proteins, phenolic compounds (PC), organic acids, flavonoids, and tocophrenols, where the PC counter oxidative stress, ROS [11], [12], and roughly limiting cell damage by their chemopreventive agents [13]. Moreover, PC might also display distinctive anticarcinogenic and cardioprotective effects linked to their free radical stopping properties [14].

In the light of these findings, the aim of this study is to investigate the effects of exposure cigarette smoke on the cardiac enzymes and tissues in male albino rats and the improvement role of Sidr honey.

Materials and Methods:

1. Chemicals:

•Libyan Sidr honey (SH) used was obtained from local market and analyzed by the Centre Lab of Omar Al-Mokhtar University, El-Beyda, Libya.

•Karelia red cigarettes (KC) were obtained from the local market.

2. Experimental animals:

28 healthy male albino rats, weighing 180-200 gm (10 weeks old) were used. Rats were obtained from the Zoology Department, Faculty Science, University of Omar Al-Mokhtar, El-Beyda, Libya. Animals were acclimatized for a period of 3 weeks and were housed in cages at standard laboratory conditions of room temperature (22 ± 2° C). Rats were fed standard rat chow and water ad libitum. This experiment complied with the guide for the care and use of laboratory animals ethical guidelines.

3. Experimental design:

Male rats were randomly allocated into four groups of eight rats as follows:

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

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Group 2: The Libyan Sidr honey group (SH), rats were given Sidr honey (100 mg/kg b.w./d.) [15], orally by gavage for four weeks.

Group 3: The Karelia red cigarettes group (KC). Cigarette smoke exposure was 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 (length, 80 cm; width, 30 cm; height, 40 cm) for keeping the rats [16], [17]. 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 KC was used five lit by using a smoking machine for fifteen minutes and exposing the rats to the sidestream of the KC for five minutes, then the rats were rested to ten minutes and ventilation by removing the box cover. This operation was repeated five times a day for four weeks, where the rats were exposed to the sidestream of the KC for six days in a week [18], [19].

Group 4: The protective group (PRO), rats were given SH (100 mg/kg b.w./d.) orally by gavage for two weeks then rats treated with the sidestream of the KC generated by a machine smoking (same group 3) with taking the Sidr honey for four weeks.

Fig. 1: The smoke-exposure system.

4. X-ray dark-field radiography:

At the end of the treatments, all rats were anesthetized with diethyl ether, then rats were taken to Tepa center in El-beyda city to investigate changes to the size heart can be precisely detected with imaging technique by X-ray dark-field [20].

1. Serum biochemical analysis:

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 serum samples obtained analyzed to determine the concentration of creatine kinase (CK), lactate dehydrogenase (LDH) and creatine Kinase-myocardial (CK-MB) band of the control group and the experimental groups were performed in the Al-Beyda Laboratory for Medical Analysis, El-Beyda City by the methods of [21]. Plasma cardiac troponin T and I (cTnT and cTnI) were quantitatively

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measured by means of a highly specific enzyme immunoassay using commercially available kits.

2. Histological examination:

Part of the heart tissue from all groups were fixed process in formalin (10 %), then dehydrated in graded alcohol and embedded in paraffin, and sections (5 µm) were prepared using a microtome device (model Leitz 1512, Germany). Sections were stained with hematoxylin and eosin by using standard procedures [22].

3. Statistical analysis:

All data were analyzed using Minitab statistical analysis package program (Minitab version 17). The parametric variables were displayed as the mean ± standard error (SE). We performed a one-way analysis of variance (ANOVA). In addition, means were separated using Turkey's test at $P < 0.05$. The T test also using for compared between two means.

Results:

1.X-ray dark-field radiography:

The X-Ray radiography of rats showing, normal heart size in the NC rats and SH rats after 4 weeks (Fig. 2, A & B). Moreover, heart mildly enlarged size in the KC-exposed rats after 4 weeks (Fig. 2, C) as compared to NC rats. While, normal heart size in the POR rats (Fig. 2, D) when compared with KC rats.

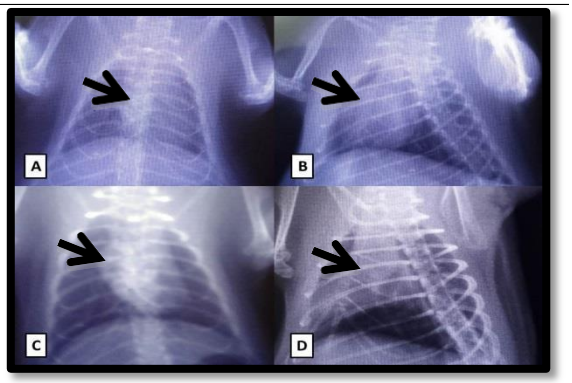

Fig. 2: X-Ray radiography of rats, showing, (A): Normal heart size (arrow), in the NC rats; (B): Normal heart size (arrow), in the SH rats; (C): Heart mildly enlarged size (arrow) in the KC-exposed rats; (D): Normal heart size (arrow) in the POR rats.

2. Determine of the enzymatic activities of heart:

2.1. Determination of the creatine kinase (CK) :

The results showed that there was a highly significant increase (P <0.05) in the expression of the mean values of CK level of the KC group (185.43 ± 3.05) as compared with the NC group (129.9 ± 1.72). Moreover, the mean values of the CK level in the PRO group (161.14 ± 2.45) showed a significant reduction (p<0.05) as compared to the KC group (Table 1).

2.2. Determine of the creatine kinase-myocardial band (CK-MB):

Data recorded for CK-MB levels were carried out in Table (1), a significant increase (P <0.05) were found in the mean values of the KC rats

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(25.43±1.161) when compared with the NC rats (15.14 ± 0.77). Whereas, the mean values of CK-MB level in the PRO rats (19.71±0.71) showed a significant decrease (P <0.05) as compared with the KC rats.

2.3. Determine of the lactate dehydrogenase (LDH):

The mean values of LDH level of control and experimental animals were presented in Table (1). The mean values of LDH showed, a highly significant increase (P <0.05) in the KC animals (188.3 ± 3.70) as compared to the NC (151.9 ± 4.99). Nevertheless, the mean values of LDH showed, a significant inhibition (P <0.05) in the POR (171.49 ± 2.60) as compared to the KC animals.

Table 1: Average of mean values of CK, CK-MB, and LDH levels in the control and experimental groups.

Parameters	NC mean ± SE	SH mean ± SE	KC mean ± SE	PRO mean ± SE
CK (U/L)	129.9 ±1.72 C	130.29± 1.63 C	185.43 ±3.05 A	161.14 ±2.45 B
CK-MB (U/L)	15.14 ±0.77 C	15± 0.76 C	25.43± 1.161 A	19.71± 0.71 B
LDH (U/L)	151.9 ±4.99 C	139.29± 3.29 C	188.3± 3.70 A	171.49 ±2.60 B

*The means with different superscript 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. SH= Libyan Sidr honey treated group. KC= Karelia red cigarettes group (PRO)= Protective group.

3. Histological investigation:

3.1. Histological preparations of the heart tissues

3.1.1. The heart sections of the NC group:

Microscopically, the heart sections of the NC group showed a normal histological architecture of the cardiomyocytes with well-organized and branched cardiac myofibers, centrally located oval nuclei, and minimal interstitial connective tissue with few interstitial fibroblasts (Fig. 3).

3.1.2. The heart sections of the SH group:

Light microscopic examination of the heart after administration of SH alone for 4 weeks revealed a normal histological structure: Normal histological architecture of the cardiomyocytes with well-organized and branched cardiac myofibers, centrally located oval nuclei, and minimal interstitial connective tissue with few interstitial fibroblasts (Fig. 4) as in the NC group.

3.1.3. The heart sections of rats exposure to KC:

Histological examination of the heart of rats after exposure to KC alone for 4 weeks showed different histopathological changes when compared with NC group such as arrangement of myocardial fibers was disordered as well as cellular oedema and breaks or necrosis were evident. In addition, perivascular mononuclear cell infiltration,

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ragmentation of sacroplasm and degeneration changes, and congestion of blood vessels (Fig. 5). Hyaline degeneration of myocardial fibers, and highly thickened wall of blood (Fig. 6). Moreover, figure (7) showed congestion and dilatated of blood vessels, and hyperemia with inflammatory cells infiltrations. Also, degeneration of myocardial fibers and congestion of blood vessels with highly thickened wall was noted in the figure (8).

In the gross level, the heart of male adult rats after exposure to the KC after 4 weeks showed severe damage in the heart tissues.

3.1.4. The heart sections of POR rats:

The heart sections of animals that treated with SH for two weeks then the animals were exposure to KC by a machine smoking with taking the SH for 4 weeks manifested minimal histopathological alterations when compared with the KC group. The marked improvement in myocardial fibers with few degeneration of myocardial fibers and hyperemic interstitial blood vessels, and small number of necrosis (Fig. 9). The myocardial fibers which almost looks like the control with few degeneration of myocardial fibers these were apparent in the figure (10).

Finally, in many areas of heart tissues in the protective rats attained almost normal patterns.

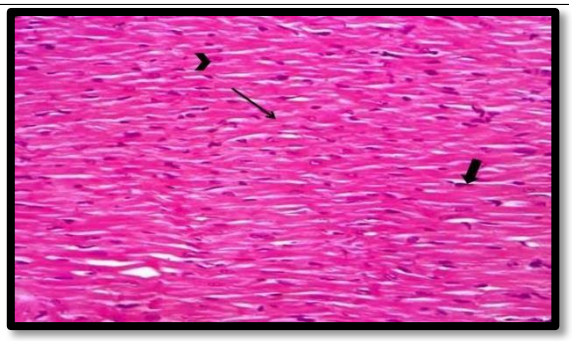

Fig. 3: Photomicrograph of heart tissues in the NC rats, showing the normal histological architecture of the cardiomyocytes with well-organized and branched cardiac myofibers (long arrow), centrally located oval nuclei (head arrow), and minimal interstitial connective tissue with few interstitial fibroblasts (thick arrow) in between. (H&E, ×400).

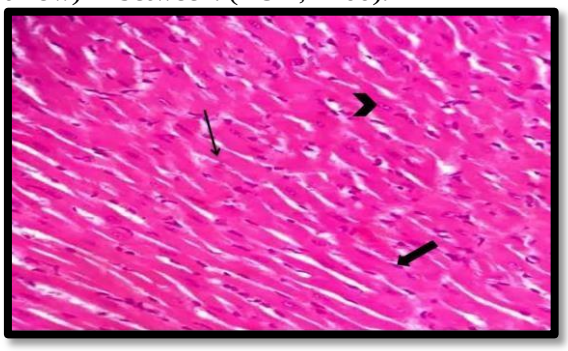

Fig. 4: Photomicrograph of heart tissues in the SH rats, showing the normal histological architecture of the cardiomyocytes with well-organized and branched cardiac myofibers (long arrow), centrally located oval nuclei (head arrow), and minimal interstitial connective tissue with few interstitial fibroblasts (thick arrow) in between. (H&E, ×400).

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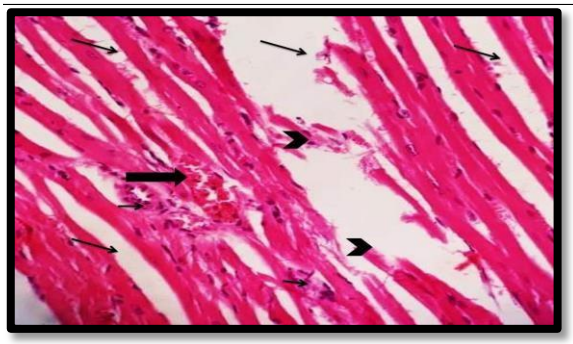

Fig. 5: Photomicrograph of heart tissues in the KC-exposed rats, showing the arrangement of myocardial fibers was disordered (long arrows) as well as cellular oedema and breaks or necrosis were evident. In addition, perivascular mononuclear cell infiltration (short arrows), ragmentation of sacroplasm and degeneration changes (head arrows), and congestion of blood vessels (long arrow). (H&E, ×400).

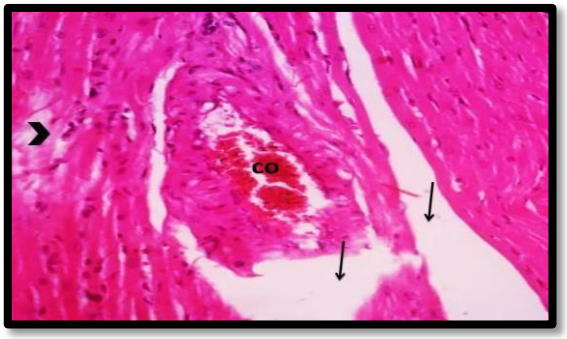

Fig. 6: Photomicrograph of heart tissues in the KC-exposed rats, showing the arrangement of myocardial fibers was disordered with oedema and necrosis (arrows), hyaline degeneration of myocardial fibers (head arrow), and congestion of blood vessels with highly thickened wall (long arrow). (H&E, ×400).

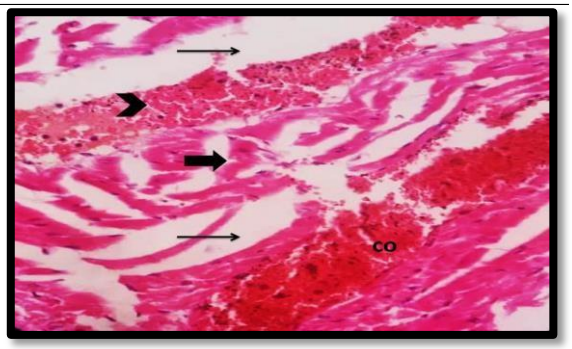

Fig. 7: Photomicrograph of heart tissues in the KC-exposed rats, showing the oedema (arrows) as well as ragmentation of sacroplasm and degeneration changes (thick arrow), hyaline degeneration of myocardial fibers (head arrow), and congestion and dilated of blood vessels (CO), and hyperemia with inflammatory cells infiltrations (head arrow). (H&E, ×400).

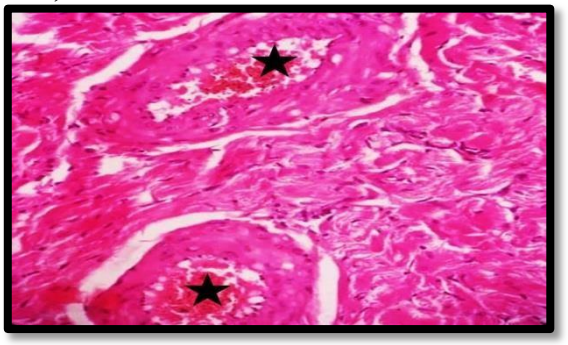

Fig. 8: Photomicrograph of heart tissues in the KC-exposed rats, showing the degeneration of myocardial fibers and congestion of blood vessels with highly thickened wall (stars). (H&E, ×400).

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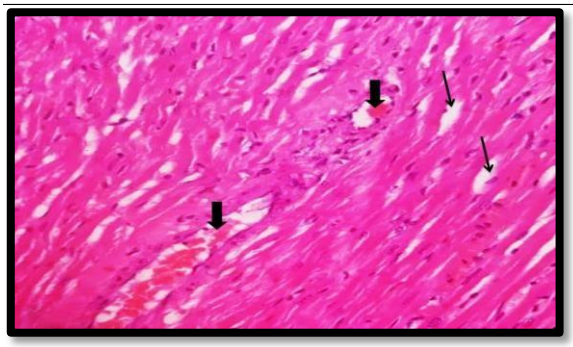

Fig. 9: Photomicrograph of heart tissues in the POR rats, showing the marked improvement in myocardial fibers with few degeneration of myocardial fibers and hyperemic interstitial blood vessels (thick arrows), and small number of necrosis (arrows). (H&E, ×400).

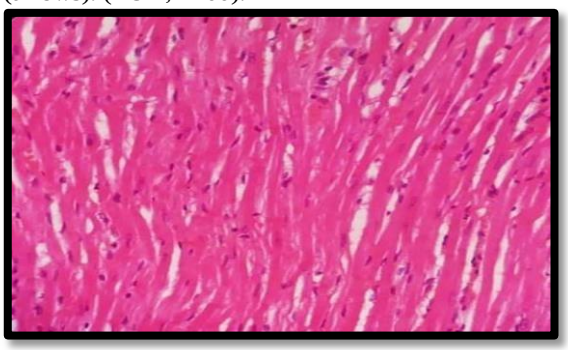

Fig. 10: Photomicrograph of heart tissues in the POR rats, showing the marked improvement in myocardial fibers which almost looks like the control with few degeneration of myocardial fibers (H&E, ×400).

Discussion:

CS exposure lead to many morphological changes in human and animal tissues [23]. Moreover, each puff of CS contains over 10^{15} free radicals, which include H_2O_2, reactive aldehydes, quinines, and benzo pyrene, because the CS had an estimated many toxic chemical compounds [24] such as nicotine, a highly addictive substance, where many of these compounds which are causally associated with deaths and diseases [25]. Furthermore, they said that about 11.1% of people deaths from cardiovascular disease occur exposed to sidestream cigarette smoke (SCS).

In this study, the X-Ray radiography of rats showed, heart mildly enlarged size in the KC-exposed rats after 4 weeks as compared with the NC rats. This is further supported by an experimental study showing that the 90 % of rats had hyperinflation in the CS exposure rats [20]. These changes may due to the toxic effect of KC on the structures of in the heart tissue. Moreover, several other factors could act as additional mechanisms to trigger cardiac hypertrophic process the generation of ROS induced by CS is cytotoxic to the myocardium, also nicotine and carbon monoxide has been shown linked to multiple effects to nervous and cardiovascular systems [26]. While, the POR rats showed normal heart size in when compared with KC rats. This might duo to by the antioxidant and radical scavenging activity of honey in endothelial cells induced by oxidative stress, where PC and flavonoids causes of the protective effect [27]. Besides that, honey has been proven reduce myocardial infarct size by reduce myocardial infarct areas [28].

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On the other hand, the results indicated that, the mean values of CK, CK-MB, and LHD showed a significant increase in the KC group when compared with the NC group. These findings come in agreement with [29], [30], and [31] who found that the treated with CS caused a significant release of heart enzymes into circulation. This first goes to confirm that CS exposure toxicity can likely generate free radicals, hence, the elevated levels of CK, CK-MB and LHD [29], [31]. Also, [31] reported that the adverse effects of CS are mediated by the tar or by the particulate phase and the gas phase, which contain many numbers of free radicals, which resulting in oxidative stress. Moreover, the production of free radicals, other reactive oxygen and nitrogen species from the tar and gas phases of CS the contributory factors to smoke-related diseases such as cardio and cerebrovascular diseases, cancers, pulmonary diseases, and several others, where the sustained release of reactive free radicals of smoke imposes an oxidant stress, promotes lipid peroxidation and consequently perturbs the antioxidant defense systems in blood and tissues [29]. Furthermore, [30] found that the increased of LDH considered as indicator of tissues damage or cell necrosis or may be attributed on the CS chemical compounds induced cell damage in the body, which might carrer cellular contents with LDH into blood by cell damage. Additionally, [32] suggested that the CS-induced cardiac damage through the substantial rise of cardiac injury biomarkers (CK, CK-MB, and LDH) activities, reflecting cardiomyocyte membrane disruption and extensive cardiomyocyte damage.

In contrast, the results in this study showed a significant decrease in PRO group in the mean values of CK, CK-MB, and LHD when compared with KC group. This is accompanied with [28], [33]. These effects may due to the honey have suppressive effects on ROS, and inhibits the production of free oxygen radicals [34]. Moreover, honey has medicinal properties that plays a role in the prevention of vascular disorders such as cardiovascular [35].

Histological investigation of the heart tissues of KC group showed different histological changes as compared to NC group such as arrangement of myocardial fibers as well as cellular oedema and necrosis. In addition, perivascular mononuclear cell infiltration, ragmentation of sacroplasm and degeneration changes, congestion of blood vessels and hyperemia with inflammatory cells infiltrations. These results were supported by [26], [36] and [37]. The CS may have acted indirectly through generation of high levels of ROS or directly as toxin to the heart thereby affecting their cellular and functional integrity and associated with higherlevels of chronic inflammation [36].

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Bocalini *et al.,* [26] stated that the effects of CS is associated with functional, structural cardiac changes and heart failure in rats. Moreover, the SCS contains a higher concentration of toxic gaseous chemicals cause vascular endothelial cell activation, dysfunction, and damage, also the CS caused an increase in oxidative stress, with effects on endothelial cells function and structure in the cardiovascular system, where, necrosis and apoptosis occur from the effect of ROS and other components of CS. Also, the necrotic death of cells leads to proteolysis of extracellular matrix through the release of lysosome proteases [25].

On the other hand, CS is associated with increases in inflammatory cells in the peripheral blood, and increased leukocyte recruitment to the vascular system [37]. However, [38] detected that the CS causes increased oxidative stress because of several mechanisms, including direct damage by radical species and the inflammatory response. Moreover, [32] established that the increase of free radicals output with excitotoxicity and lipid peroxidation accelerates inflammatory conciliators‘ synthesis and thus activates the inflammatory response in the heart tissues. So, they provoke leukocyte infiltration into the myocardium and aggravate inflammatory injury. In addition, [39] demonstrated that myocardial necrosis and oxidative stress trigger a cytokine by Tumor Necrosis Factor (TNF-α) and exacerbate myocardial injury which lead to a progressive and irreversible myocardial damage.

The POR group in the present study showed the improvement in heart tissues with the myocardial fibers which almost looks like the control with few degeneration of myocardial fibers.

The therapeutic potency of honey is due to the presence of many compounds as well as specific physicochemical properties that its associated with its wound healing effect, anti-inflammatory potency, antioxidant, and free radical scavenging ability, also it enhanced the healing process in damage tissue to rats and caused a significant decline in the levels of TNF-α. Moreover, it promote cell proliferation and neovascularization, with an overall pro-inflammatory effect [40].

Conclusion:

The present conclusions clearly demonstrate that the Libyan Sidr honey effects on protection in heart enzymes and tissues by the prevention of free radicals generation and it is an improved, and support wound healing, also it could tremendously enhance the treatment process and result in better outcomes against the tissues damage by CS.

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