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THE EFFECTS OF SIDR HONEY ON HEPATOTOXICITY IN RATS EXPOSED TO SIDESTREAM CIGARETTE SMOKE

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Abstract

This work aimed to use Sidr honey (SHY) for the reduction of the acute effects of sidestream cigarette smoke (SCS) on the liver tissues of rats. 24 rats were divided into 4 groups: (G1) control group; (G2) rats were administered orally SHY (100 mg/kg BW/30 days; (G3) rats were exposed to the SCS for a one month, and (G4) rats were taken the SHY 100 mg/kg BW/ 14 days before the rats were exposed to SCS for a one month. The mean AST, ALT, ALP, and cholesterol values were significantly higher in the G3 than in the G1. In contrast, the G4 showed a significant decline compared to the G3 rats. Moreover, the albumin, total protein, and HDL levels showed an important reduction in the G3 compared to G1. However, these parameters improved after treatment with honey in the G4 compared to the G3. Also, the liver tissue of G4 had some histological changes and a moderate improvement in the tissue's structure compared to G3. Moreover, the G3 showed the collagen fibers meaningfully augmented in the central vein wall and portal area, while the G4 presented as nearly similar to the G1. Therefore, this study confirmed the beneficial effects of SHY in lowering hepatotoxicity in rats exposed to SCS for one month..

Keywords: *Liver, Sidr Honey, Histopathology, Rats, Cigarettes.*

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Introduction

The liver is continuously near many risky substances as the body's largest interior glandular organ, this has directed mounting attention to natural therapies that are specifically helpful in shielding the liver from toxins or giving various liver-related illnesses [1, 2].

Sidestream cigarette smoke (SCS) exposure may cause cytotoxicity, histological changes to many important organs, and increasing oxidative stress (OS) indicators [3, 4]. In addition, passive smoke is a complex mixture of many toxins, some of which are generated from SCS for tobacco smoke (TS) that is burning [5]. Moreover, more than 4000 different complexes, 400 of which are carcinogenic, are found in cigarette smoke (SC) [6], additionally, contain oxidants including oxygen-free radicals and unstable aldehydes, which may be the main perpetrators producing harm to biomolecules [7]. On the other hand, the byproducts of TS lead to structural abnormalities in tissues due to deficiencies in microcirculation, low oxygen concentration, inflammatory processes, and tissue repair mechanisms [8]. Many studies reveal that TS contains a wide range of risk materials, such as nicotine, tar, carcinogens, and gaseous components including carbon monoxide, and important free radicals (FRs) that can cause or exacerbate oxidative injury [7]. Furthermore, TS reasons OS because of the radicals that are produced from the smoke [3]. Also, the wide obstetrics of reactive oxygen species (ROS) and the possible synthesis of inflammatory intermediaries make TS products hazardous [6], where a situation recognized as OS happens after the body produces too many FRs, or ROS, beyond the protective aptitude of normal antioxidants [9], so taken antioxidants that could bind to FRs could decrease the hazard of disease and are beneficial in plummeting tissue damage [10]. A study by [11] found that the rats' livers exposed to TS presented inflammation and cell disintegration in hepatic tissues.

Over many years, honey used in dissimilar pathologies was mostly due to clinical observations of its hepatoprotective properties and hastening of wound therapeutic [12]. Honey, a production of honeybees, is documented to have a wide variety of therapeutic uses in many cultures. These uses contain immunostimulant, antiviral, antibacterial makings, [1], anticancer [13], anti-inflammatory, and antioxidant qualities [14]. Moreover, several components of honey include amino acids, proteins, minerals, polyphenols, flavonoids, carotenoid derivatives, vitamins, fragrance compounds [14, 13], complex mixture of proteins, enzymes, catalase, carbohydrates, organic acids, ascorbic acid, and tocopherols [15]. Thus, the current investigation aimed to use SHY to reduce the hepatotoxicity-induced SCS in rats.

Materials and Methods:

Materials:

The Karelia red cigarettes (KRC) and natural Sidr honey (SHY) were provided from the Libyan local market.

Animals:

Weighted between 180 and 200 grams were 24 male albino rats (10 weeks old) provided for the experiment by the Faculty of Science's Zoology Department at the University of Omar Al-Mokhtar in El-Beyda, Libya. After adaptation for 3 weeks, rats were preserved in standard laboratory conditions in cages at (22 ± 2 ◦C), and animals were given a regular diet and clear ad libitum water.

Design of the study:

Twenty-four male rats were divided into 4 groups (n= 6) at random, as follows: Group 1 (G1): Normal control group, animals were saved in a typical workroom with airing without exposure to KRC. Group 2 (G2): Rats were administered oral gavage of SHY (100 mg/kg BW) for a month [16]. Group 3 (G3): Rats were exposed to SCS of KRC by a connection pipe connecting [17], and the bee smoker machine to the glass box for a month [18]. The box was made locally (Figure. 1), and Group 4 (G4): For 14 days, the rats were orally by gavage with 100 mg/kg BW of SHY. Afterward, the rats were administered SHY for a month with KRC exposure [18]. The ethical standards for animal research are carried out in every animal experiment.

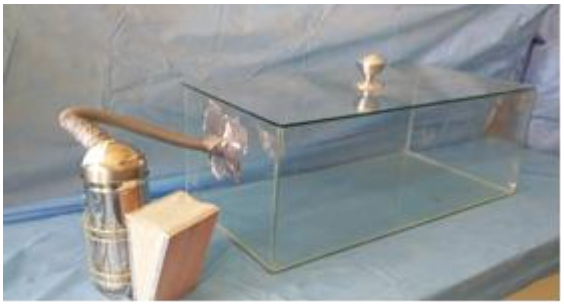

Figure. 1: The smoke exposure process box

Biochemical analysis:

Rats were sedated by diethyl ether after work (a month), and blood samples were next taken from animals [15]. After centrifuging these samples for 10 minutes at 1000 rpm, serum was composed. The enzymatic activities of the liver were measured by measuring serum levels of biochemical analyses e.g., total protein, cholesterol, albumin, high-density lipoprotein (HDL) [19], alkaline phosphatase (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) [20] of all groups were accomplished in the Al-Aseel hospital, El-Beida City, Libya.

Histological and histochemical techniques:

All groups' livers were fixed in 10% formalin before being desiccated in variable degrees of alcohol and entrenched in paraffin. Hematoxylin and eosin were applied to sections with a thickness of 5 nm by using histological protocols [21]. Masson’s trichrome staining was used to locate the levels of collagen sedimentation [22].

Analysis of data:

The data analysis was shown using Minitab version 17, with each data usually stated as the (M ±SE). A One-Way ANOVA analysis test was used to equivalence the means of all groups, with an importance verge of $P < 0.05$. Turkey's test was also practical to differentiate among the means. The T-test was also used to compare two means.

Results:

Biochemical study:

The AST, ALT, ALP, and cholesterol values of the study groups were obtainable in Table (1), where the AST displayed significant differences ($P<0.05$) in the G3 (257.8±4.48) when compared with G1 (175.7±6.4). However, there is a reduction in the AST in G4 (213.5±4.093) compared to G3. Moreover, the ALT presented, a significantly higher ($P<0.05$) in the G3 group (59±1.67) compared to G1 (34.714±1.53). However, the ALT showed a significant decrease in G4 (47.8±2.02) related to the G3. Also, the ALP presented, an important rise in the G3 (326±12.47) when compared with G1(122.7±5.1), Whereas, the ALP showed a significant reduction in G4 (281.8±7.34) as associated with the G3. In addition, the cholesterol disclosed, a considerable rise ($P<0.05$) in G3 (81.14±1.9) when related to G1(40±1.14), and G4 showed a decline in the cholesterol (56.7±2.06) as compared to the G3.

Furthermore, table (1) displays an important reduction ($P<0.05$) of the total protein level of G3 (4.62±0.174) when compared with G1 (7.67±0.125). In contrast, the total protein in G4 (5.71±.175) showed a significant increase compared to the G3. Besides, the albumin displayed, a discount in the G3 (2.22±0.11) compared with G1(3.38±0.09), and the G4 presented a significant increase in the albumin (2.71±0.051) as compared to the G3 were noticed in table (1). Also, table (1) displays a momentous decrease ($P<0.05$) in the HDL in G3 (24.8±1.23) compared with G1(39.34±1.14). However, the HDL shows a substantial increase in G4 (35.5±1.193) compared to G3.

Table 1: Means of the serum AST, ALT, ALP, total protein, albumin, cholesterol, and HDL in all groups (Mean ±SEM).

Biochemical analyzing	G1	G2	G3	G4
AST (IU/L)	175.7±6.4 ^{c}	156.2±4.07 ^{d}	257.8±4.48 ^{a}	213.5±4.093 ^{b}
ALT (IU/L)	34.714±1.53 ^{c}	34.85±1.446 ^{c}	59±1.67 ^{a}	47.8±2.02 ^{b}
ALP (IU/L)	122.7±5.1 ^{c}	122.8±3.39 ^{c}	326±12.47 ^{a}	281.8±7.34 ^{b}
Total protein (g/dl)	7.67±0.125 ^{a}	7.51±0.24 ^{a}	4.62±0.174 ^{c}	5.71±.175 ^{b}
Albumin (g/dl)	3.38±0.09 ^{a}	3.6±0.125 ^{a}	2.22±0.11 ^{b}	2.71±0.051 ^{c}
Cholesterol (mg/ml)	40±1.14 ^{c}	39±1.69 ^{c}	81.14±1.9 ^{a}	56.7±2.06 ^{b}
HDL (mg/dl)	39.34±1.14 ^{ab}	42.68±0.71 ^{a}	24.8±1.23 ^{c}	35.5±1.193 ^{b}

Histopathological study:

The liver tissues of the G1 displayed a standard lobular style and the hepatocytes from the central vein produced hepatic sinusoids and inosculating plates of liver cells divided into another by vascular gaps (Figure 2). Also, many structures were found in portal tracts, such as large-diameter portal vein branches, small-diameter hepatic artery branches, and bile duct branches (Figure 3). Moreover, G2's liver displayed a typical hepatic structure, including a regular central vein, sinusoidal capillary size without any signs of congestion or narrowing, normal hepatocytes with no alterations to their cytoplasm or nucleus, and normal portal tracts (Figures 4 & 5) as in the control group.

The liver sections of G3 presented different histopathological variations comparable to G1 including dilated, congested central vein, disintegrating hepatocytes, and degeneration of the portal region with dilated, congested portal vein (Figure 6). Also, figure (7) found a fibrotic zone, inflammatory cell penetration, and necrotic zones surrounding the dilated, and overfilled portal vein wall. This was accompanied by necrotic regions, pyknotic nuclei, hemorrhagic areas, and deteriorating hepatocytes noticed in Figure (8). Moreover, figure (9) shows harm to hepatic style with deteriorating liver cells, a hydropic bulge of cells, and inflammatory cell penetration in the portal area, necrotic zones, and karyorrhexis nuclei in some cells. After a month of exposure to KRC, the rats' livers revealed imperative tissue injury.

On the other hand, the liver in G4 manifested minimal histopathological changes comparable to the G3. Figure 10 shows enhanced hepatic architecture with hepatocyte structures with congested sinusoids, minimal congested central vein, few necrosis areas, and pyknotic nuclei. Additionally, figure (11) showed improvement of the hepatocytes, pyknotic nuclei, and few inflammatory cell infiltrations in the portal zone compared to G3. Finally, in many parts of the liver tissues, G4 attained almost normal patterns.

Histochemical study:

Normal collagen fibers were seen in the G1 hepatic tissue surrounding the major vein, and there was some slight fiber statement in the interlobular septum (figure 1). Also, figure (13) showed normal collagen fibers in the portal triad zone. Moreover, the G2 liver tissues displayed a minor fiber statement in the interlobular septum, typical collagen fibers surrounding the central vein (Figure 14), and normal collagen fibers in the portal zone (Figure 15) compared to the G1. Moreover, Masson’s trichrome staining in G3 showed, that the collagen fibers meaningfully enlarged in the liver lobules' major vein wall, and the fibrous septum of the liver lobule was shaped, and spreading in the perisinusoidal places (Figure 16). Furthermore, collagen fibers statement around the portal zone enlarged, and it is interrelated with the adjacent septa, and equally were enfolded around to each other (Figure 17).

The liver sections of G4 displayed improvement in the collagen fibers surrounding the central vein that presented nearly to the control liver shape (Figure 18). Moreover, the

improvement in the collagen fibers in the portal zone and extending in the peripheral places (Figure 19) when compared with G3.

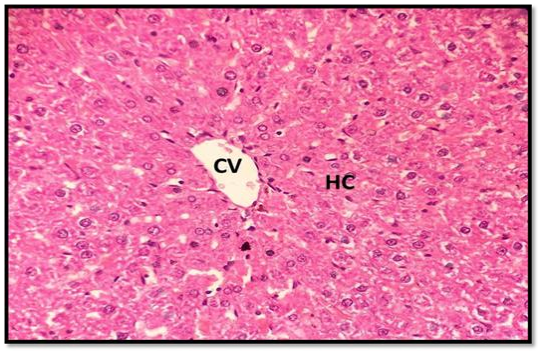

Figure 2: The G1 rats' liver section reveals the ordinary lobular architecture, normal central vein (CV), and hepatocyte structures (HC). (HE, ×40).

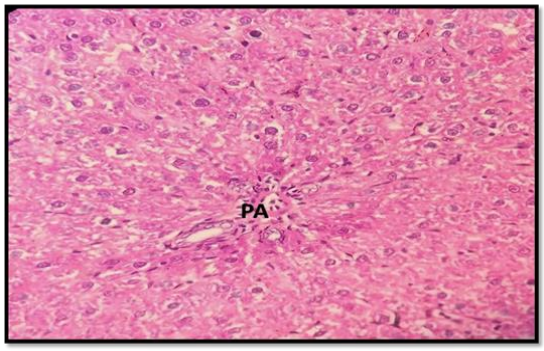

Figure 3: The G1 rats' liver section reveals the normal portal area (PA) (HE, ×40).

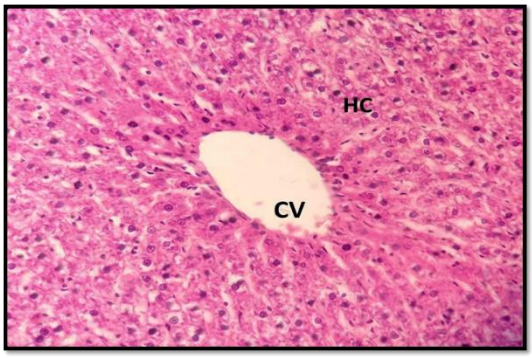

Figure 4: The G2 rats' liver section reveals the ordinary lobular architecture, normal central vein (CV), and hepatocyte structures (HC). (HE, ×40).

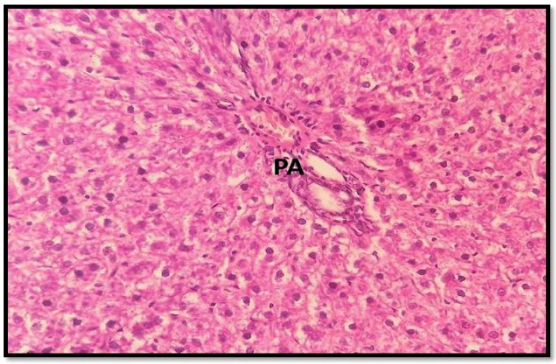

Figure 5: The G2 rats' liver section reveals the normal portal area (PA) (HE, ×40).

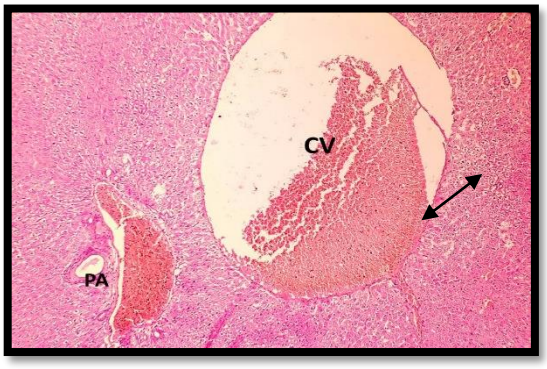

Figure 6: The G3 rats' liver section reveals the dilated, congested central vein (CV), disintegrating hepatocytes, and degeneration of the portal region with dilated (arrow), congested portal vein (PA). (HE, ×10).

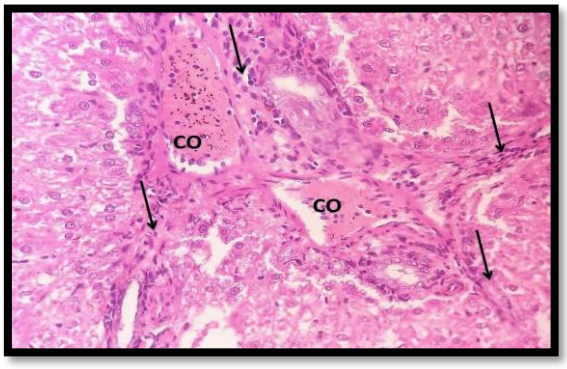

Figure 7: The G3 rats' liver section reveals the fibrotic zone, inflammatory cell penetration (arrows), and necrotic zones (NC) surrounding the dilated, and overfilled portal vein wall. (HE, ×40).

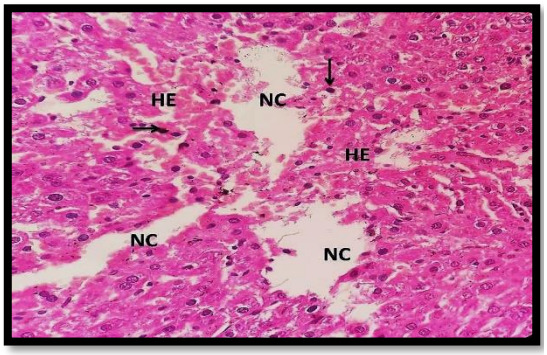

Figure 8: The G3 rats' liver section reveals the necrotic regions (NC), pyknotic nuclei (arrows), hemorrhagic areas (HE), and deteriorating hepatocytes (HC). (HE, ×40).

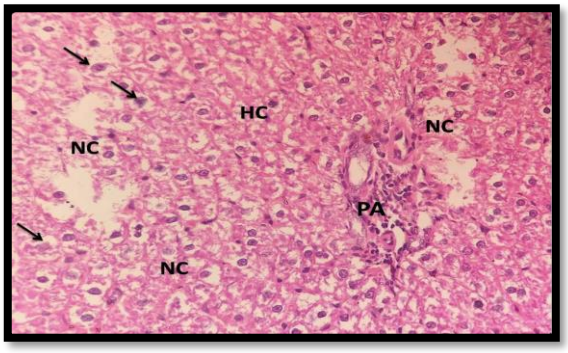

Figure 9: The G3 rats' liver section reveals the deteriorating liver cells, a hydropic bulge of cells (HC), inflammatory cell penetration in the portal area (PA), necrotic zones (NC), and karyorrhexis nuclei in some cells (arrows). (HE, ×40).

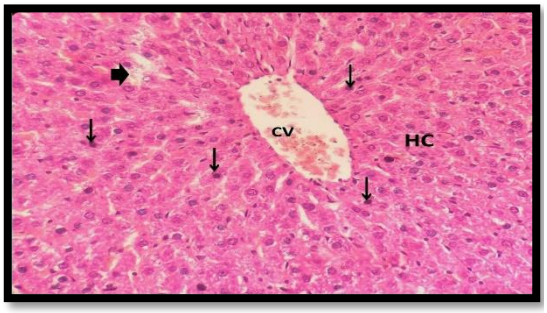

Figure 10: The G4 rats' liver section reveals the enhanced hepatic architecture with hepatocyte structures (HC) with congested sinusoids (S), minimal congested central vein (CV), few necrosis areas (thick arrow), and pyknotic nuclei (arrows). (HE, ×40).

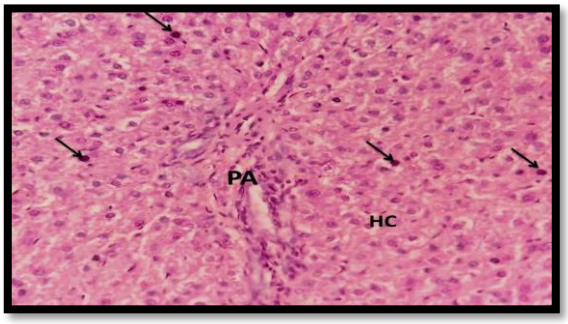

Figure 11: The G4 rats' liver section reveals the enhanced hepatocytes (HC), pyknotic nuclei (arrows), and a few inflammatory cell penetrations in the portal area (HE, ×40).

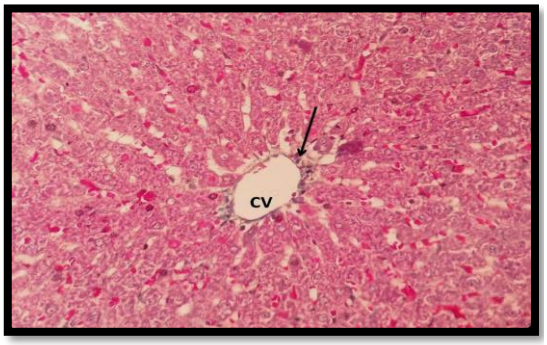

Figure 12: The G1 rats' liver section reveals the normal collagen fibers (arrow) surrounding the central vein (CV), and there was some slight fiber statement in the interlobular septum. (Masson's trichrome, ×40).

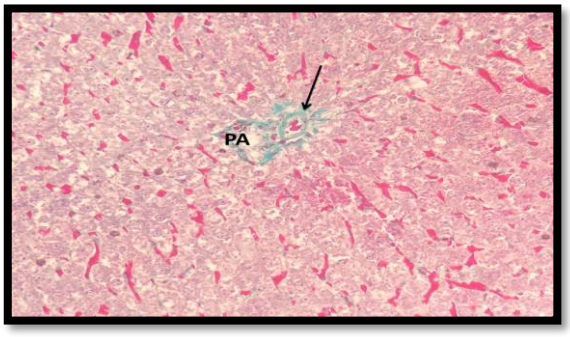

Figure 13: The G1 rats' liver section reveals the normal collagen fibers (arrow) in the portal zone (PA). (Masson's trichrome, ×40).

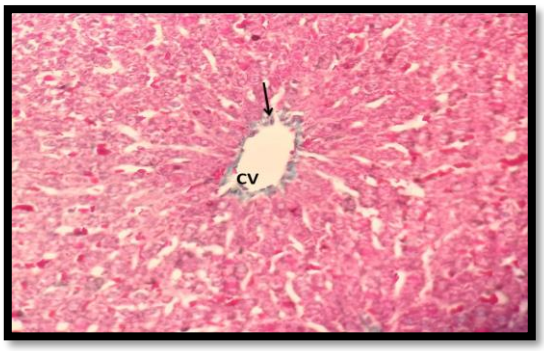

Figure 14: The G2 rats' liver section reveals the typical collagen fibers surrounding the central vein (CV), and a minor fiber statement in the interlobular septum. (Masson's trichrome ×40).

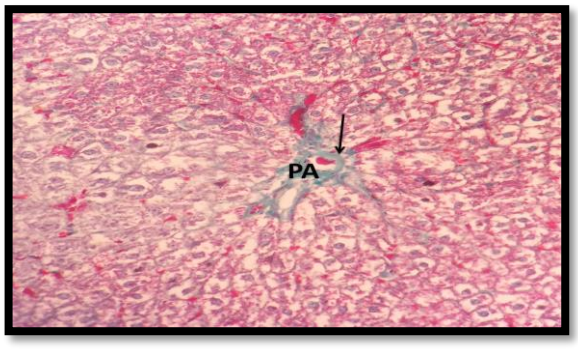

Figure 15: The G2 rats' liver section reveals the normal collagen fibers (arrow) in the portal zone (PA). (Masson's trichrome ×40).

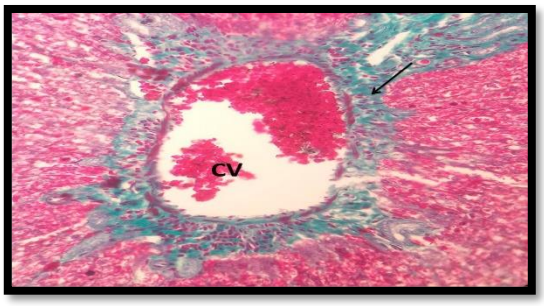

Figure 16: The G3 rats' liver section reveals the collagen fibers meaningfully enlarged in the liver lobules' central vein (CV) wall, the fibrous septum of the liver lobule was shaped (arrow), and spreading in the perisinusoidal places. (Masson's trichrome ×40).

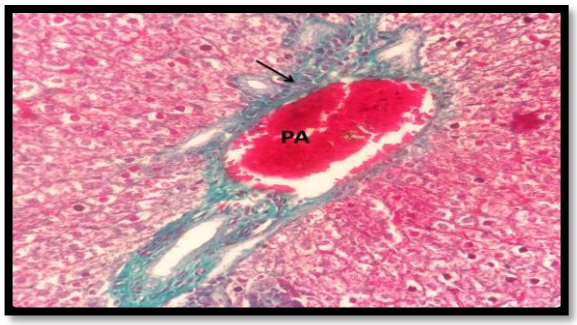

Figure 17: The G3 rats' liver section reveals the collagen fibers statement around the portal zone enlarged (PA), and it is interrelated with the adjacent septa (arrow), and equally were enfolded around to each other. (Masson's trichrome ×40).

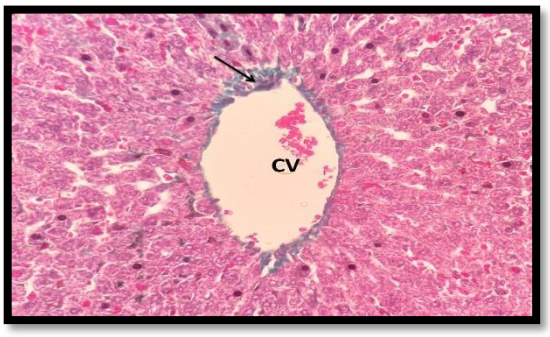

Figure 18: The G4 rats' liver section reveals improvement in the collagen fibers (arrow) surrounding the central vein (CV) that presented nearly to the control liver shape. (Masson's trichrome ×40).

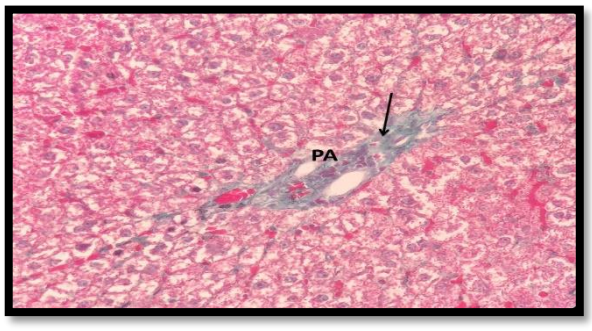

Figure 19: The G4 rats' liver section reveals improved collagen fibers (arrow) in the portal zone (PA) and extending in the peripheral places (Masson's trichrome ×40).

1. Discussion:

This study showed a substantial increase in AST, ALT, ALP, and cholesterol in G3 compared with G1. These results are in arrangement with [23] who stated that the effect of TS was significantly associated with augmented levels of ALP, AST, and GGT. Also, our results agree with [24, 25] who found that CS raised ALP, ALT, AST, and plasma total cholesterol comparable to the control animals. This has been explained by the CS which leads to OS by generating FRs via the lipid peroxidation mechanism [25]. Moreover, CS is one of the greatest shared sources of cadmium (Cd), which accrues in the liver, and kidneys [26], CS is one of the sources of Cd intoxication [27]. Additionally, the significant rise in liver enzymes in the CS group strengthens the destructive induced of SCS [28]. Liver enzymes are documented indicators of liver illnesses and are the primary enzymes to prove plasma examination in situations of liver cell mutilation [29, 20]. These causes are due to the many harmful chemicals in TS [28], that cause inflammation of hepatocytes [30], and the formation of oxidants, inducing OS [31, 32]. Similar results were available by [4], who confirmed that TS exposure in rats increased liver enzyme levels, a sign of liver damage.

Furthermore, the present study revealed an important reduction in total protein, HDL, and albumin values in the G3 than in the G1. Our results were reliable with [24] who found a reduction in total protein, and albumin in the CS animals comparable to the control animals. In addition, [25] who have investigated the impact of CS on liver functions, revealed a significantly declined level of total protein, and albumin through CS. Also, [33] confirmed the association between TS, and low levels of HDL in plasma, which may mediate part of the augmented risk of cardiovascular disease resulting from CS. Moreover, because the chemical compounds in TS directly change albumin's binding capabilities and produce albumin due to liver damage and loss via the kidney, they can indirectly or directly affect the serum total protein [28]. Likewise, [34] said lipid profile inequity may occur systemically as an effect of TS, and these impacts on serum levels of HDL, and cholesterol were seen in this study. Besides, several FRs in CS alter the metabolism of proteins and lipids and destroy a few important macromolecules. This might result in dyslipidemia and atherogenesis [28].

On the other hand, the G4 showed important enhancement in liver functions compared with the G3. Our results are supported by [35] who stated that the physiological valuations consumed revealed the curative strength of normal honey towards toxicity impact on the enzyme's liver. Also, [36] mentioned that the administrated of SHY daily is a hopeful normal antioxidant and fibrosuppressive mediator that can heal liver fibrosis. Moreover, [4] found that by combining honey or other normal antioxidants with CS, liver damage was lessened, which was seen through lesser liver enzyme levels after the trial. Also, numerous phytochemicals, including polyphenols, which function as antioxidants, and several indices like homocysteine, blood lipids, cholesterol, and C-reactive proteins, are rich in honey [37]. These findings argue that honey contains phenols, some vitamins, and flavonoids, which may have defensive effects on OS in tissues exposed to TS [17, 16]. Moreover, they institute that honey could enhance the healing effect against rat hepatotoxicity caused by CS. Natural honey

might deliver a normal remedy for general health such as liver functions and positive impacts on other chronicle diseases [35].

Additionally, rats exposed to SCS by KRC a month (G3) showed more significant histological alterations associated with normal rats in G1. These alterations involved a dilated, congested central vein, disintegrating hepatocytes, and degeneration of the portal region with a dilated, congested portal vein, fibrotic zone, inflammatory cell penetration, and necrotic zones surrounding the dilated portal vein wall, hemorrhagic areas, and harm to hepatic style with deteriorating liver cells, a hydropic bulge of cells. These impacts are displayed by [38]. The progressive dilatation of liver vessels may be understood as a variation in reply to elevated prostaglandin (PG) synthesis, whereby these PGs either directly or indirectly endorsed smooth reduction following vasodilatation by discharging other vasodilator materials into the bloodstream. Whereas, portal vein congestion and enlargement occur by portal hypertension, brought on by degenerative ballooning hepatocytes obstructing the sinusoidal level. Also, the permeated cells, in the portal zones and nearby the lobules might be a protection response against toxicity of inhaled tobacco by metabolic in the liver [39]. Besides, the quantity of lobular inflammation and hepatocellular swelling was significantly affected by CS, which raised the rats' non-alcoholic fatty liver disease score [40]. Also, [41] said that one of the damaging detrimental effects of SC is OS transports toxic chemicals to cells and produces fibrosis. Furthermore, CS damages hepatic cells by increasing the proinflammatory cytokines interleukins e.g., IL-1, IL-6, IL-8, and tumor necrosis factor (TNF)-α [42]. This procedure has been associated with direct hepatotoxic and hepatocarcinogenic effects where the CS causes FRs, which produce ROS. So, OS in the hepatocytes produces ROS via cytochrome P450 pathways [43], and due to the increasing damage to DNA, hepatocyte necrosis and apoptosis, abnormalities in the distribution of free fatty acids, cholesterol, and lipid metabolism, and epigenetic alterations [44]. This was shown by the clear variations in the AST and ALT levels of liver injury where acute liver injury is recognized to be the source of elevated enzymes which is the greatest marker of liver harm because it out into the bloodstream after hepatic cell injury [45]. Furthermore, the indirect OS in the liver cells might be carried on by nicotine, raised carboxyhemoglobin (COHb), and declined oxygen stock. Also, carbon monoxide caused by TS exposure raises COHb content and reasons hepatocellular damage [46].

On the other hand, Masson’s trichrome staining in G3 presented, that the collagen fibers meaningfully enlarged in the liver lobules' major vein wall, and the fibrous septum of the liver lobule was shaped, and spreading in the perisinusoidal places. Also, collagen fibers statement around the portal zone enlarged, which is interrelated with the adjacent septa. This is demonstrated by OS, which plays a vital part in liver illnesses and rising collagen fibers in the liver tissues [47]. Furthermore, cellular necrosis and inflammation are the two main reasons for liver damage. These situations are brought on by augmented OS of mitochondria from triglyceride breakdown and the manufacture of FRs in peroxisomes [48]. Additionally, increased making of adipokines, such as TNF-α and leptin [49], also contributes to liver damage. These chemical mediators formed during inflammation and cell necrosis stimulate

hepatic stellate cells, which express collagen, connective tissue development issues, and a combination of extracellular matrix mechanisms [50]. This results in fibrosis, increased appearance, biogenesis, statement of connective tissue, and collagens [51].

In addition, this study found that the hepatic tissue of G4 revealed enhanced hepatic architecture with hepatocyte structures with improvement of the hepatocytes, and few inflammatory cell permeations in the portal zone compared with G3. On the other hand, the liver tissues of G4 displayed improvement in the collagen fibers surrounding the central vein that presented nearly to the control liver shape with enhanced collagen fibers in the portal zone and extending in the peripheral places compared with G3. These outcomes might be accredited to the antioxidant properties of SHY [6], the attendance of phenols and vitamins [52], and its protective effects on OS in rat tissues exposed to TS [16]. Also, many compounds and distinct physicochemical features of honey, like flavonoids [53], contribute to its anti-inflammatory and FRs scavenging properties, and its healing therapeutic in injured tissues [16]. Moreover, [18] indicates that the pretreatment with SHY acted as antisecretory, cytoprotective, and antioxidant agents, demonstrating an antiapoptotic impact against tissue damage.

Conclusion:

The current results show that SHY has a protective effect on reducing hepatotoxicity. It is also an improved healing choice that can improve products regarding tissue injury induced by SCS. So, it is important to deliberate the safeguarding properties of organic antioxidants to keep the health of organs, assist them in functions optimally, and prevent apoptosis.

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