Trop J Nat Prod Res, May 2024; 8(5):7220-7224 ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Hepatoprotective Effects of Honey Against Tobacco Smoking Toxicity in Wistar Rats

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ARTICLE INFO

Article history:
Received : 12 December 2023
Revised 28 April 2024
Accepted 03 May 2024
Published online 01 June 2024

ABSTRACT

Honey has been used as a nutritional diet and traditional medicine due to its antimicrobial and antioxidant properties. This study aims to evaluate the hepatoprotective potential of honey against the hepatoxic effect of cigarette and waterpipe tobacco smoking in rats. Honey was purchased from the local market. A daily dose of 1.2 g/kg of honey was administered orally to 48 male Wistar rats, and a digital smoking device was used to expose rats to waterpipe and cigarette tobacco smoke before dissection. The enzymatic activities of the liver using a serum sample were quantified, and liver tissue was isolated and prepared for light microscopy for histopathological examination. The hepatoprotective effect of honey was studied in rats in combination with cigarette and waterpipe smoking. The current investigation demonstrates that honey exhibits a substantial protective effect on liver tissue in rats exposed to cigarette and waterpipe smoking. Based on the study findings, honey exhibits a hepatoprotective effect.

***Keywords*:** Honey, Hepatoprotective, Cigarette, Waterpipe, Smoking

Introduction

Tobacco consumption is accountable for more than 8 million fatalities annually and results in more impairment and poor health compared to any behavioral risk factor.[1] It is predicted that developing countries will see millions of deaths annually by 2030.[2] Tobacco can be smoked in cigarettes, chewed, or used in waterpipes (also known as nargile, arghileh, shisha, goza, hookah, and hubbly-bubbly). Cigarette smoking has been the most common route of nicotine intake since the beginning of the 20th century. It has been reported that each cigarette contains about 10 mg of nicotine. [3]
Secondhand exposure to tobacco smoking could induce cytotoxicity and histological alterations to several vital organs in animal models and elevate oxidative stress biomarkers.[4] Two cases of squamous cell carcinoma and keratoacanthoma of the lower lip have been reported among Egyptian nargile smokers.[5] Tobacco use is a leading cause of fatal illnesses worldwide, including lung cancer, cardiovascular disease, and chronic obstructive pulmonary disease (COPD).[6] Nicotine, a component of tobacco, has been reported to cause oxidative stress in both in vitro and in vivo environments. It also depletes antioxidant defense mechanisms.[7] Neuronal nicotinic acetylcholine receptors mediate the addictiveness of nicotine in the central nervous system.[8] Honey has been scientifically proven to possess antioxidant, anti-inflammatory, antibacterial, antiviral, antiulcer, anti-lipid, and anticancer properties.[9]

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Citation: Khwaldeh A, Shraideh Z, Badran D, Shoiab AA, Alsarhan AA, Al-Fawaeir S, Alzbeede A, Aljamal A. Hepatoprotective Effects of Honey Against Tobacco Smoking Toxicity in Wistar Rats. Trop J Nat Prod Res. 2024; 8(5):7220-7224. https://doi.org/10.26538/tjnpr/v8i5.25

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

These activities are attributed to the phenolic compounds, such as flavonoids, found in all types of honey in different proportions.[9] Honey contains various polyphenols and phenolic acids that function as antioxidants. These phytochemicals differ depending on the location and climate conditions.[10] Some polyphenols found in honey, such as caffeic acid, chrysin, and quercetin, show potential in cancer treatment.[11]
Honey lowers various parameters, such as plasma glucose, cholesterol, triglycerides, plasma insulin, blood lipids, C-reactive protein, and homocysteine.[12] Honey may reduce the risk of cardiovascular illness in healthy people and those with increased risk factors.[12] Honey has been shown to have several positive effects on the brain. It increases brain-derived neurotrophic factor, which benefits brain health. Honey also helps to reduce brain oxidative stress and has protective effects on learning and memory. It is believed to enhance the morphology of memory-related brain areas, decrease acetylcholinesterase activity in brain homogenates, and increase the acetylcholine concentration.[9, 13, 14]
This study aims to examine the histological alterations in the liver tissues of rats following an extended period of exposure to waterpipe and cigarettes. A light microscope will be employed to analyze liver tissue in the investigation. Furthermore, the present study aims to assess the possible preventive properties of natural antioxidants present in honey to mitigate liver damage from cigarette or waterpipe smoking.

Materials and Methods

Forty-eight male Wister rats weighing 150–180 g were acclimatized in the animal room at the University of Jordan for a week before they were divided into six experimental groups, eight animals per group. Group 1 was the negative control and was only exposed to fresh air. Group 2 was the positive control exposed only to honey treatment, and Groups 3 and 4 were exposed to one red LM cigarette per day for 30 consecutive days. Groups 5 and 6 of rats were subjected to flavored

waterpipe smoke from the combustion of 20 g of moassal for thirty days. One session a day for the whole body.
Five groups were in the experiment, and all rats received the doses below once a day for one month, according to their weight, which was recorded weekly. The rats who smoked red LM cigarettes were divided into distinct categories. Group (3) was exposed to cigarettes for one month. Group (4) was subjected to cigarette exposure and concurrently administered honey orally via gavage at a dosage of 1.2 g/kg body weight/day for one month. The groups exposed to flavored waterpipe smoke were divided as follows: Group (5) was exposed to flavored waterpipe smoke for one month. Group (6) was exposed to flavored waterpipe smoke and treated simultaneously with (1.2 g/kg) honey orally by gavage for one month. The institution of research ethics approved the study protocol and animal care procedure (MLS-R-10/01/2017).

Preparation of Selected Antioxidants
Honey Manuka was from the local market in Jerash city-Jordan Jarash City-Jordan, north of Jordan 48 kilometers (30 mi) north of Amman's capital. Latitude: 32° 16' 12.12" N Longitude: 35° 53' 17.41" E, in April 2017, and oral gavage was administered 1.2 g/kg of honey daily.[15]

The Digital Smoking Machine
The digital smoking gadget developed by Shraideh *et al.*[16] was employed to generate tobacco smoking. This experimental setup is appropriate for subjecting rats to controlled exposure to waterpipes or cigarette smoke. The smoking apparatus consists of several essential elements, including an inhaling chamber, a time controller, a valve, and a vacuum pump.

Protocol for Light Microscopy
The liver tissue of rats was collected, washed using phosphate-buffered saline, and fixed in a 10% formalin solution for 48 hours. A sequence of procedures was employed to treat the tissues, which included dehydration, clearing, infiltration with paraffin wax, and embedding in paraffin blocks. The microtome was utilized to cut thin sections of 3–5 µm. These sections were deparaffinized using xylene, stained with hematoxylin and eosin and finally mounted with DPX for light microscopy.[17]

Periodic Acid–Schiff Staining
The periodic acid–Schiff (PAS) method involves exposing the tissue to periodic acid, which acts as an oxidizing agent to produce dialdehydes from compounds with free hydroxyl or amino/alkylamine groups. These dialdehydes form an insoluble magenta-colored complex when exposed to Schiff's reagent.[18]

Liver Biochemical Tests
Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) kits were quantified according to kit manual (Teco Diagnostics, CA, USA). The LDH test was operated through an automated clinical chemistry analyzer (HumaStar 600, Human Diagnostics, Germany).

Oxidative Stress Biomarkers
One common test to evaluate oxidative stress is reduced glutathione (GSH) levels in the body. GSH is a tripeptide that acts as a major antioxidant in cells, neutralizing ROS and preventing oxidative damage. Reduced glutathione levels were estimated according to the kit manufacturer's instructions (Sigma, CS0260-1K glutathione assay kit).

Statistical Analysis
Data management and analyses were conducted using SPSS Statistics version 20 (IBM Corp., Armonk, NY, USA). All data sections are presented as means ± SEM. Differences were considered significant at $P<0.05$.

Results and Discussion

The Effect of Honey, Cigarette Smoking, and Waterpipe Smoking on Liver Enzymes of Rats Exposed to Sub-chronic Smoking

Tables 1, 2, and 3 present a statistical analysis of the levels of three serum liver enzymes (AST, LDH, and ALT) after exposure to cigarette and waterpipe smoking for a short period, along with honey treatment. Table 1 presents the impact of honey therapy on alanine aminotransferase (ALT) levels. The administered intervention yielded a notable impact on both cigarette and waterpipe smoking. In the control group, the level of ALT was 34.9 ± 0.263 U/L, while in the honey group, it was 35.2 ± 0.201 U/L. However, after being exposed to cigarette smoking (44.9 ± 0.748, p = 0.0) and waterpipe smoking (39.61 ± 0.282, p = 0.0), the level of ALT considerably rose. In both the cigarette smoking group (40.37 ± 0.111, p = 0.01) and the waterpipe smoking group (39 ± 0.152 U/L, p = 0.019), the administration of honey significantly reduced ALT levels.
The impact of honey treatment on the AST level is illustrated in Table 2. The administered intervention yielded a notable effect on both cigarette and waterpipe smoking. In the control group, the level of AST was 33.3 ± 0.115 U/L, whereas in the honey group, it was 34 ± 0.050 U/L. Exposure to cigarette smoking (92.12 ± 0.046, p = 0.0) and waterpipe smoking (88.12 ± 0.063, p = 0.0) significantly raised the level of AST. Significant reductions in AST levels were observed in both the cigarette smoking group (85.8 ± 0.212, p = 0.00) and the waterpipe smoking group (80 ± 0.577 U/L, p = 0.0) following treatment with honey.
Table 3 displays the effect of honey treatment on LDH levels. The treatment had a significant effect on both cigarette and waterpipe smoking.

Table 1: Effect of honey ALT level in albino rats exposed to cigarette and waterpipe smoking. *Mean significant P.

Animal groups	ALT (U\L)	P- value
Control (fresh air)	34.9 ± 0.263	0.00
Honey	35.2 ± 0.201	0.00
Cigarette	44.9 ± 0.748	0.00*b
Waterpipe	39.61 ± 0.282	0.00*c
Cigarette+ Honey	40.37 ± 0.111	0.01*b
Waterpipe + Honey	39.0 ± 0.152	0.019*c

*Mean significant P<0.05.

Table 2: Effect of honey AST level in albino rats exposed to cigarette and waterpipe smoking. *Mean significant P.

Animal groups	AST (U\L)	P- value
Control (fresh air)	33.3 ± 0.115	1.00
Honey	34.0 ± 0.050	0.00
Cigarette	92.12 ± 0.046	0.00*b
Waterpipe	88.12 ± 0.063	0.00*c
Cigarette+ Honey	85.8 ± 0.212	0.00*b
Waterpipe + Honey	80.0 ± 0.577	0.00*c

*Mean significant P<0.05.

Table 3: Effect of honey LDH level in albino rats exposed to cigarette and waterpipe smoking. *Mean significant P.

Animal groups	LDH (U\L)	P- value
Control (fresh air)	452.0 ± 1.15	1.00
Honey	450.0 ± 0.51	0.00
Cigarette	479.2 ± 1.31	0.03*b
Waterpipe	670.0 ± 0.577	0.00*c
Cigarette+ Honey	440.0 ± 0.577	0.00*b
Waterpipe + Honey	297.0 ± 0.577	0.00*c

*Mean significant P<0.05.

The level of LDH was 452 ± 1.15 U/L in the control group and 450 ± 0.51 in the honey group and was significantly increased following exposure to cigarette smoking (479.2 ± 1.31, p = 0.0) and waterpipe smoking (760 ± 0.577, p = 0.0). Treatment with honey decreased the level of LDH significantly in the cigarette smoking group (440 ± 0.577, p = 0.0) and the waterpipe smoking group (297 ± 0.577 U/L, p = 0.0).

The Effects of Sub-chronic Smoking on Oxidative Stress Biomarkers

The statistical analysis of oxidative biomarker (GSH) activity after sub-chronic exposure to waterpipe and cigarette smoking, with the treatment of honey, is presented in Table 4. The administered intervention yielded a notable impact on both cigarette and waterpipe smoking. Following exposure to cigarette smoking (0.21 ± 0.02, p = 0.02) and waterpipe smoking (0.18 ± 0.03, p = 0.01), the level of GSH in the control group (0.34 ± 0.07 nmol/ mL) considerably dropped. In both the cigarette smoking group (0.55 ± 0.14) and the waterpipe smoking group (0.52 ± 0.11 nmol/mL, p = 0.0), the administration of honey resulted in a substantial rise in the level of GSH.

Effects of Sub-chronic Smoking on Liver Sections

Control Group

The liver sections of the control group showed histologically normal liver tissue displaying a typical histological structure, normal central vein (C.V.), sinusoids, and hepatocytes (Figure 1).

Cigarette Smoking Group

The histological sections of the livers of the smoking group showed that Kupffer's cells became prominent and increased in number, sinusoids were dilated, and inflammatory cells aggregated around the portal and central veins (Figure 2).

Effect of Honey Treatment on Tissues of Cigarette Smoking Group

The liver tissue of rats treated with honey is represented in Figure 3. Sinusoids were dilated, and Kupffer cells became prominent and more abundant. Pyknotic nuclei are noted in some hepatocytes.

Waterpipe Smoking Period Group

Figure 4 shows the abnormal histological features of a rat liver exposed to cigarette smoke, including dilated sinusoids crowded with Kupffer cells. Furthermore, inflammatory cells were aggregated around the portal triad and central veins. Pyknotic nuclei were seen in some hepatocytes.

Effect of Honey Treatment on Waterpipe Smoking

Rats exposed to waterpipe smoking and treated with honey are represented in Figure 5. The histological sections of the liver show Kupffer cells, which are present in sinusoids in increased numbers. Otherwise, sinusoids were dilated, and inflammatory cells aggregated in the portal vein.

PAS Stain of Liver Tissue

Periodic acid–Schiff (PAS) staining was used to detect glycogen in liver tissue. Figure 6 demonstrates the liver tissue of a rat exposed to cigarette and waterpipe smoking. Fewer glycogen deposits are present than in the liver of a rat treated with honey.

Tobacco use is a leading cause of various fatal diseases, including lung cancer, chronic obstructive pulmonary disease, and cardiovascular disease.[6] the increasing prevalence of smoking worldwide has led to the development of solutions to mitigate its severe risks. In this research, we studied the effect of using natural antioxidants such as honey on smokers' histological and biochemical levels.

Results showed that sub-chronic exposure to cigarette and waterpipe smoke significantly increased the levels of the main serum liver enzymes (LDH, ALT, and AST) compared to the control group. Ababneh et al.[19] reported similar results in which they showed that rat exposure to smoking elevated liver enzymes, which indicated liver damage. Ultrastructural evaluation of hepatic injury in rats subjected to cigarette smoking exhibited polymorphic mitochondria, accompanied by mild deterioration in cristae and matrix.[20] The nuclei displayed chromatin condensations close to the inner edges of the nuclear envelope, displaying an irregular shape.[20] The rough endoplasmic reticulum seemed scattered, while the cisternae appeared dilated. The cytosol continent displayed distortion, and vacuolization was observed.[20] Tobacco smoking releases toxic chemical compounds leading to cellular injury and inducing the production of proinflammatory cytokines (TNF-α, IL-1, and IL-6).[21]

Table 4: Effect of honey on GSH level in smoking Albino rats

Animal groups	GSH (nmole\mL)	P- value
Control (fresh air)	0.34 ± 0.07	0.00
Cigarette	0.21 ± 0.02	0.02*a
Waterpipe	0.18 ± 0.03	0.01*a
Cigarette+ Honey	0.55 ± 0.14	0.00*b
Waterpipe + Honey	0.52 ± 0.11	0.00*c

*Mean significant P<0.05.

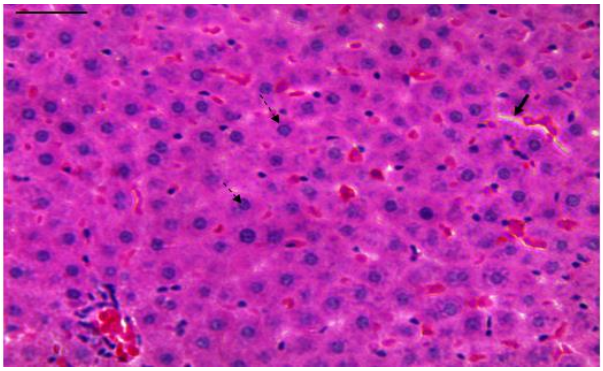

Figure 1: The liver of the control group showed normal architectures of the liver: sinusoids black arrows and hepatocyte dash arrow (Bar = 50µm). (H&E)

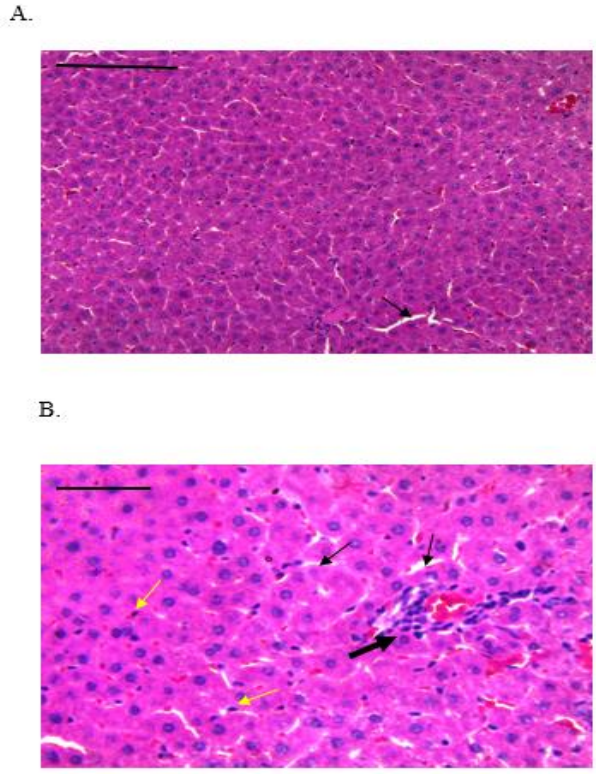

Figure 2: Liver of rat exposed to cigarette smoke showing dilated sinusoids (black arrows), Kupffer cells (yellow arrow),

and infiltration of inflammatory cells (bold arrow). A. (Bar = 200 µm) B. (Bar = 50 µm)

Using natural antioxidants such as honey with the smoking process ameliorated liver injury, as reflected by decreased liver serum enzymes at the end of the experiment. Honey is rich in many phytochemicals, including polyphenols that act as antioxidants.[10] Honey reduces many parameters, such as triglycerides (TG), cholesterol, blood lipids, C-reactive proteins, plasma glucose, and homocysteine.[12] In the current study, treatment with honey as a natural antioxidant slightly reduced the levels of liver enzymes (ALT, AST, and LDH). Smoking caused a significant reduction in GSH-specific activities. However, treatment with honey increased the activities of GSH. Exposure to smoking activates inflammatory cells, ending with large amounts of ROS. Smokers will likely have a higher oxidative stress status with an imbalance between oxidants and antioxidants.[22] Our findings showed a decreased level of GSH, which confirms the results of Alzoubi et al.[23]

Study results showed that exposure to smoking affects liver histology as demonstrated by inflammatory reactions, dilation, congestion of the sinusoids, and the increase in Kupffer cells. Furthermore, aggregation of inflammatory cells around the portal and central veins and necrosis in some hepatocytes occurred. The exposure of albino rats for a month to cigarette smoke caused many histological changes, such as infiltration of inflammatory cells in the portal area and necrosis of hepatocytes.[20] Other studies on the histological effect of cigarette exposure in the liver showed changes and degeneration in hepatocytes, cellular infiltration, and congestion of both the central and portal veins.[24] Treatment of the smoking group with honey was also protective against inflammatory reactions. Honey has been tested and approved for its antioxidant, anti-inflammatory, antibacterial, antiviral, and anti-ulcerous effects.[9] these activities are credited to the phenolic compounds, such as flavonoids, found in all types of honey in different proportions.[9]

Liver tissue stained by PAS stain showed an increase in glycogen deposited in the livers of rats exposed to smoking and treated with honey compared to those exposed to smoking only. Oral administration of honey diminished liver fibrosis and reduced glycogen stores in non-diabetic rats.[25] Honey may play a significant role in reducing oxidative stress via its antioxidant properties.[11]

Conclusion

The data of this work confirms that smoking of either cigarettes or waterpipes is associated with health effects. At all levels of study, the harmful effects of smoking on liver tissue were demonstrated. According to all the tests employed, treatment with honey was protective against the adverse effects of smoking. However, further studies are necessary to determine their mechanism of action. Nevertheless, we suggested combining the effects of honey with other antioxidants, such as vitamin C and vitamin E, to reduce the cytotoxicity of both waterpipe and cigarette smoking.

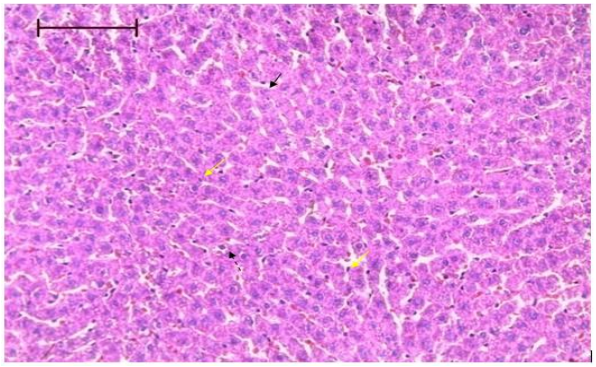

Figure 3: Liver of rat exposed to cigarette smoke and treatment by honey: Sinusoids (black arrows), |Kupffer cells (yellow arrows), and necrotic hepatocytes (dash arrow). (Bar = 100µm). (H&E)

The liver tissue of rats treated with honey is represented in Figure 3. Sinusoids were dilated, and Kupffer cells became prominent and more abundant. Pyknotic nuclei are noted in some hepatocytes

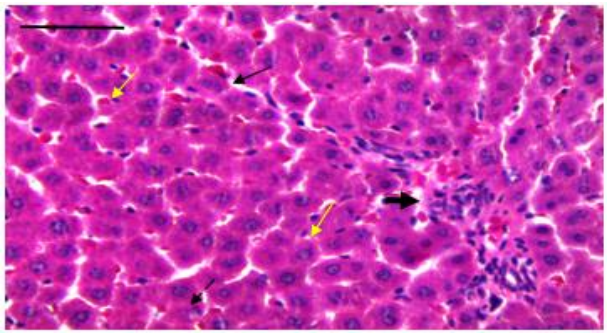

Figure 4: Liver of rat exposed to waterpipe smoke: sinusoids (black arrows), |Kupffer cells (yellow arrows) inflammatory cell (bold arrow) necrotic hepatocytes (dash arrow). (Bar = 50 µm).

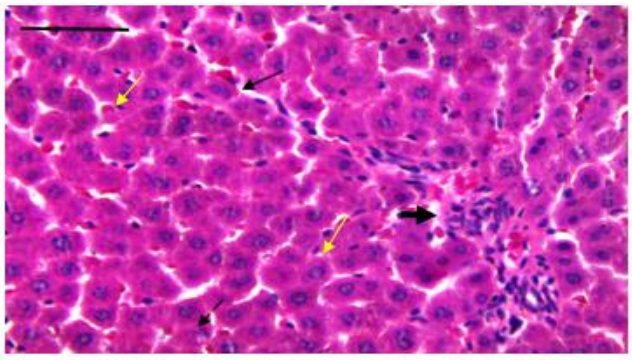

Figure 5: Liver of rat exposed to waterpipe smoke: sinusoids (black arrows), |Kupffer cells (yellow arrows) inflammatory cell (bold arrow) necrotic hepatocytes (dash arrow). (Bar = 50 µm).

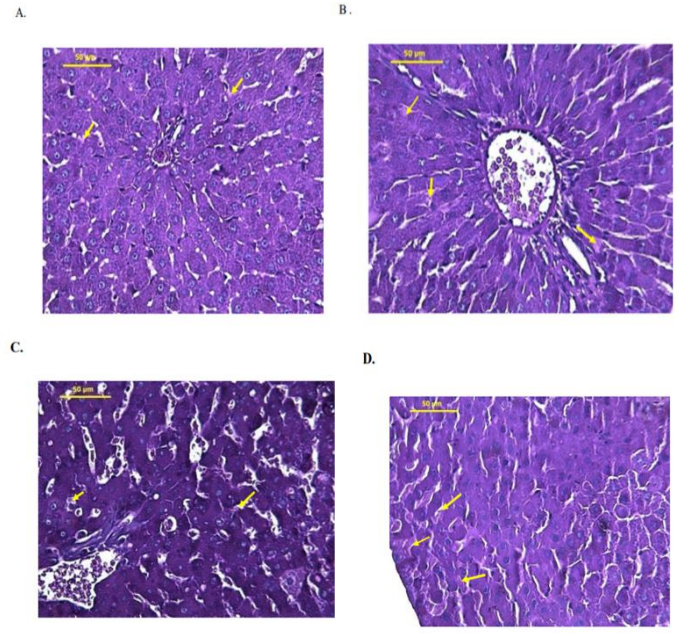

Figure 6: A. Liver of rat exposed to cigarette smoke B. Liver of rat exposed to cigarette smoke treated with honey C. Liver of rat exposed to waterpipe smoke D. Liver of rat exposed to waterpipe smoke treated with honey. Yellow arrow indicates the glycogen deposit. (Bar = 50 µm). (PAS) stain

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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