ijlpr 2022; doi 10.22376/ijpbs/lpr.2022.12.1.L92-99

International Journal of Life science and Pharma Research

ISSN 2250-0480

Research Article

Red Grape As Antioxidant

Histological Study Of Red Grape Juice On Rat's Liver Exposed To Cigarette Smoke

Aisha d. Alalwani*, Sana a. Khalifa, Haifa O. Aljuhani.

Department of Biology, Science College, University of Jeddah, Saudi Arabia.
adalolwani@uj.edu.sa

Abstract: Smoking considered being one of the oldest habits. It demonstrated that smoking has many disease-causing effects. This study examined the effects of red grape juice on liver tissue, liver function, and blood parameters in rats exposed to cigarette smoke**.** Fifty rats randomly divided into five groups: Group I; Control animals, Group II; rats exposed to cigarette smoke, Group III; rats exposed to cigarette smoke and treated with red grape juice, Group IV; rats fed with red grape juice only. Group V: rats exposed to smoking for a month and then left without exposure for another month (effect of smoking withdrawal). At the end of the experiment, blood samples analyzed for liver enzymes and complete blood cell count. Liver and body weights recorded. The liver tissue stained with H&E and subjected to histopathological analysis. Statistical analysis done among all groups and significance of results compared. The level of liver enzymes and white blood cells in animals of Group II increased significantly compared to Group I. Several histopathological changes in liver tissue were observed in Group II such as necrosis, inflammatory cellular infiltration leading to granulomas, also noted dilatation, congestion in the portal tract and vascular blood vessels, while Group III showed near-normal liver tissue and biochemical measurements. Group V did not show any improvement in biochemical and histological parameters. The present study shows that red grape juice is an effective antioxidant that decreases liver tissue damage in rats exposed to cigarette smoke.

Keywords: Cigarette smoke, grape juice, hepatotoxicity, liver function, blood parameters.

***Corresponding Author**

Aisha D. Alalwani , Department of Biology, Science College, University of Jeddah, Saudi Arabia.

Received On 23 September, 2021
Revised On 4 December, 2021
Accepted On 20 December, 2021
Published On 7 January, 2022

Funding I extend my sincere thanks and appreciation to king abdulaziz city for science and technology for research No 1-18-01-009-0193.

Citation Aisha d. Alalwani , Sana a. Khalifa, Haifa O. Aljuhani. , Histological Study Of Red Grape Juice On Rat's Liver Exposed To Cigarette Smoke.(2022).Int. J. Life Sci. Pharma Res.12(1), L92-99 http://dx.doi.org/10.22376/ijpbs/lpr.2022.12.1.L92-99

1. INTRODUCTION

Smoking is well-known for harming the human body's organs, and a number of studies have linked smoking to an increased risk of cardiovascular illnesses, lung cancer, and pulmonary diseases. Moreover, cigarette smoking accelerates the development of renal and pulmonary fibrosis and correlates with accelerated progression of cirrhosis and a variety of chronic liver diseases such as primary biliary cirrhosis and chronic hepatitis [1, 2]. Nicotine is one of many substances in cigarette smoke that thought to cause substantial harm. Nicotine attaches to receptors in the tissue after being consumed leading to multiple adverse effects fluctuating between increased rates of the heart and extending to heart attacks. Several studies have suggested that exposure to cigarette smoke is detrimental to the health of smokers and non-smokers [2] in organs that do not directly affect by cigarette smoke such as the liver, especially among people with history of chronic liver disease [3]. Other studies demonstrated that smoking result in toxic effects, either direct or indirect, along with immunological impacts, and carcinogenic effects to the liver. Furthermore, it observed that the body's anti-oxidative activity decreases after exposure to cigarette smoke, resulting in liver damage [4]. Empirical evidence supports the primary role of oxidative stress in organ damage after nicotine exposure [1]. In addition, cigarette smoke effect is not limited to smokers only, it has been associated with large number of diseases to passive smokers which also known as the secondhand smokers. According to research done on male albino rats to note the changes on their liver after inhaling the cigarette smoke microscopic examination revealed a hepatocyte degeneration, periportal fibrosis, congestion of both the central and portal veins, and cellular infiltration [5]. Recently, medicinal plants such as grapes, ginger, and pomegranate have been used as medicines. The grape (Vitis vinifera) is one of the most widely planted fruits, and it shown to contain a variety of nutrients and biologically active substances such as fiber, vitamin C, phenolic compounds including flavonoids, polyphenols, anthocyanins, and minerals such as iron, potassium, zinc, manganese, and calcium [6, 7] that reduce oxidative damage. Although grape skin and seeds contain the majority of these beneficial components, grape skin and seeds are wasted annually from the daily diet, while most of the bioactive components with the significant antioxidant and free radical scavenging activity can be found in these wastes[8]. Studies have shown that consuming grape juice improves the lining of blood vessels, reduces platelet aggregation, increases antioxidants activity including DNA oxidative damage, oxidation of low-density lipoprotein, and oxidative damage to the brain [9]. Recent research identified that dietary polyphenols present in grapes and more specifically in red grapes, prevented hepatic fatty degeneration in rodents fed a high-calorie diet. Additionally, much of the anti-oxidant and anti-inflammatory properties originate from flavonoids present in grape juice as anthocyanins, quercetin, epicatechin, catechin [10]. Therefore, the present study examined the ability of dietary grape juice to reduce the histopathological and physiological changes in the liver of rats exposed to cigarette smoke.

2. Materials And Methods

2.1 Red Grape

Red grapes were selected from Jeddah city local markets; red grapes were used because of their high content of polyphenol antioxidants [11] indicated by the grapes' color, flavor, and bitterness.

2.2 The Grape-Juice Dosage

The American Dietetic Association recommends that one should consume 200 - 500 mL of grape juice per day to achieve positive physiological and histological results.[12] In the present study, rats were fed red grape juice at 10 µL/g of body weight by using a stomach tube.

2.3 Animals

Fifty 8 - 10 weeks old Wistar albino rats weighing between 250 and 350 g used. Rats placed in plastic cages and kept at 21 - 22° C with a light/dark cycle of 12 h. The cage floor covered by sawdust that changed periodically to keep the cage clean and to ensure maximum moisture absorption. The rats were free to move and had free access to food and water. The study conducted at the Animal House, King Fahd Medical Research Center, Jeddah, Saudi Arabia.

2.4 Experiment Procedure

Animals divided into five groups containing 10 rats each. Group I: control group fed regular meals and drinking water throughout the experiment. Group II: exposed to cigarette smoke for half an hour twice daily (three cigarettes at a time; a total of six cigarettes per day) for a month. Group III: treated with grape juice and half an hour later exposed to cigarette smoke for a month as Group II. Group IV: Fed red grape juice at a dose of 7 mL/kg by gavage for a month. Group V: exposed to cigarette smoke for a month and then kept without exposure for another month (representing smoking withdrawal).

2.5 Exposure to Cigarette Smoke

The rats placed in a box designed to ensure that they exposed to cigarette smoke containing 6 mg of tar, 0.5 mg of nicotine, and 7 mg of carbon monoxide. The box was made of transparent acrylic material for easy viewing of animals during exposure to record morphological and behavioral changes. The box measured 60 × 25 × 30 cm allowing movement of animals; it contained several holes at specific distances for ventilation. The box filled with cigarette smoke using a syringe with 20 mm needle to draw smoke from the cigarette, and blowing it into the box according to a published method [13]. All experiments were conducted according to the experimental protocol approved by the ethical committee of the Animal House at King Fahad Center, Jeddah, Saudi Arabia (ethics code: R-01-H 002).

2.6 Total Body Weights:

The animals observed during the experiment for morphological and behavioral changes. The rats' weight measured at the start and end of the experiment.

2.7 Liver Weights:

The animals were sacrificed 24 h after one month of exposure to cigarette smoke. The abdomen opened, and livers carefully removed and weighed.

2.8 Blood Analysis:

Blood samples were collected from all rats at the end of the experiments. About 1 mL of blood was used for complete blood count analysis (CBC) to determine the total number of white blood cells (WBCs), red blood cells (RBCs), platelets (PL), and hemoglobin (HBG) level.

2.9 Biochemical Measurements:

Approximately 5 mL of blood was centrifuged at 2500 rpm for 15 min and the serum was stored at -80 °C until used to measure the levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) using commercially available kits.

2.10 Histological Study of The Liver

The liver was fixed in 10% neutral formalin solution for 24 h at room temperature. Tissue was gradually dehydrated in ethanol (70 - 100%), cleared in xylene, and then embedded in paraffin, sliced into 2 mm sections, and stained with Hematoxylin and Eosin (H&E).[14] The sections were examined under an Olympus BX51 light microscope and photographed.

3 STATISTICAL ANALYSIS

Statistical analysis was performed using SPSS Inc., version 25, Chicago, IL, USA) software. All data were expressed as mean ± standard error of means (SEM). Student's t-test and one way ANOVA used to determine statistical significance between the results obtained for all experimental groups; p-value ≤ 0.05 considered significant.

4 RESULT

4.1 Morphological Changes and Behavioral Observation

Compared to the control group, all rats exposed to smoke (Groups II to V) moved to one of the corners of the exposure box at the start of exposure and stayed close to each other without moving during the 30-min exposure. After being taken out of the exposure box and put back into cages, there were some notable changes in the exposed animals' behavior such as drinking greedily/anxiously and not eating despite the continuing availability of food. It also noted that rats appeared to experience severe itching. After the first week of exposure, redness of the eyes and yellowing of the fur observed. After the third and fourth week of exposure to cigarette smoke, ulcers appeared on the skin especially around the nose, eyes, and ears, and hair loss in some areas of the body was seen.

4.2 Effects of Grape Juice on Total Body Weight

We observed (Table 1) that animals exposed to smoke (Group II) showed a decrease in body weight compared to the control group. Animals in Group III showed at the end of the experiment significantly increased body weight compared to Group II. Similarly, in the withdrawal Group V, showed a significantly decreased weight compared to the control group.

Table 1: Body and Liver Weights for Animals in the Experimental Groups

Experimental Groups	Group I	Group II	Group III	Group IV	Group V
Body Weighs	**272.12+8.30**	**230.52+7.31**	**282.68+8.07**	**297.4+12.34**	**226.8+16**
P value	**-**	**0.02***	**0.16***	**0.11**	**0.09***
Liver Weighs	**3.53+0.43**	**3.73+0.12**	**3.45+0.40**	**3.47+0.27**	**3.63+0.25**
P value	**-**	**0.37***	**0.44**	**0.21**	**0.31**

Significance (*) was at p ≤ 0.05

4.3 Effects of Grape Juice on The Relative Weight of The Liver

The liver of animals exposed to cigarette smoke (Group II) showed an increased weight compared to the control group (Table 1). Significant differences were observed between the liver weights of Group III and Group II.

4.4 Effect of Grape Juice Administration on Complete Blood Count:

Results presented in Table 2 show a significant increase in the number of white blood cells and platelets in Group II compared to control. Group III showed significantly more normal blood parameters compared to Group II. Group V also showed a significant increase in the level of WBC, RBC, PLT, and HCT.

Table 2: Blood Measurements of Animals in the Experimental Groups

Experimental Groups	Group I	Group II	Group III	Group IV	Group V
WBCs	**6024.4±1517**	**14752±1571**	**9368±1459**	**5590+538**	**13432+984**
p-value	**-**	**0.0004***	**0.13***	**0.05**	**0.33**
RBCs	**66406±9193**	**77410±7681**	**69660±1828**	**6640±7475**	**9236±4663**
p-value	**-**	**0.46***	**0.77***	**0.01**	**0.12***
HB	**14±1.15**	**16.20±0.9**	**14.74±1.07**	**13.42±0.7**	**17.02±077***
p-value	**-**	**0.14**	**0.58**	**0.21**	**0.16***
PLT	**493.6±107**	**820.2±117**	**507.8±74**	**455±24.5**	**800±117***
p-value	**-**	**0.01***	**0.58***	**0.04**	**0.23***

Significance (*) was at p ≤ 0.05

4.5 Effect of Grape Juice Administration on Liver Functions

Animals exposed to cigarette smoke showed a significant worsening of liver-function parameters compared to control animals Table 3; significantly increased ALP, AST, and ALT enzyme levels seen. Group III that exposed to cigarette smoke and treated with grape juice showed significantly lower levels of AST, ALT, and ALP enzymes compared to Group II. Group V also showed a significant increase in the level of the ALP but not the other two enzymes compared to control.

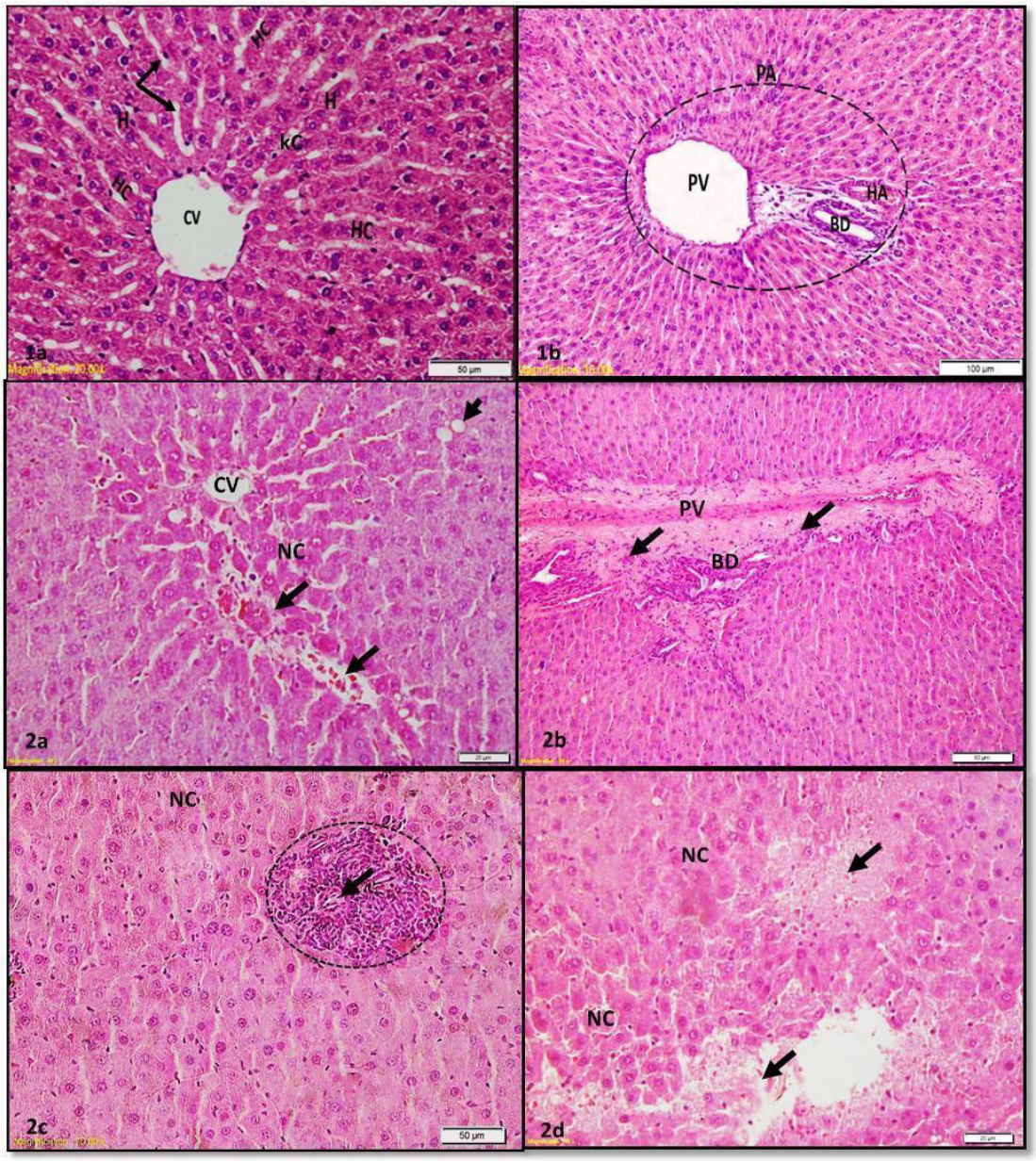
Table 3: Liver Function Enzymes in the Experimental Groups

Experimental Groups	Group I	Group I I	Group I I I	Group IV	Group V
ALT	**30.8±7.08**	**70.6±21.09**	**43.8±13.97**	**21±1.58***	**41.8±7.46**
P value	**-**	**0.04***	**0.43***	**0.01**	**0.012**
AST	**65.8±27.64**	**287.6±106.68**	**60.6±21.68**	**26.8±4.14***	**68±10.41**
P value	**-**	**0.03***	**0.35***	**0.03**	**0.11**
ALP	**104±14.3**	**548.40±60.46**	**248.8±76.87**	**93.4±6.98**	**207.6±42.23**
P value	**-**	**0.001***	**0.06***	**0.06**	**0.021***

Significance (*) was at p ≤ 0.05

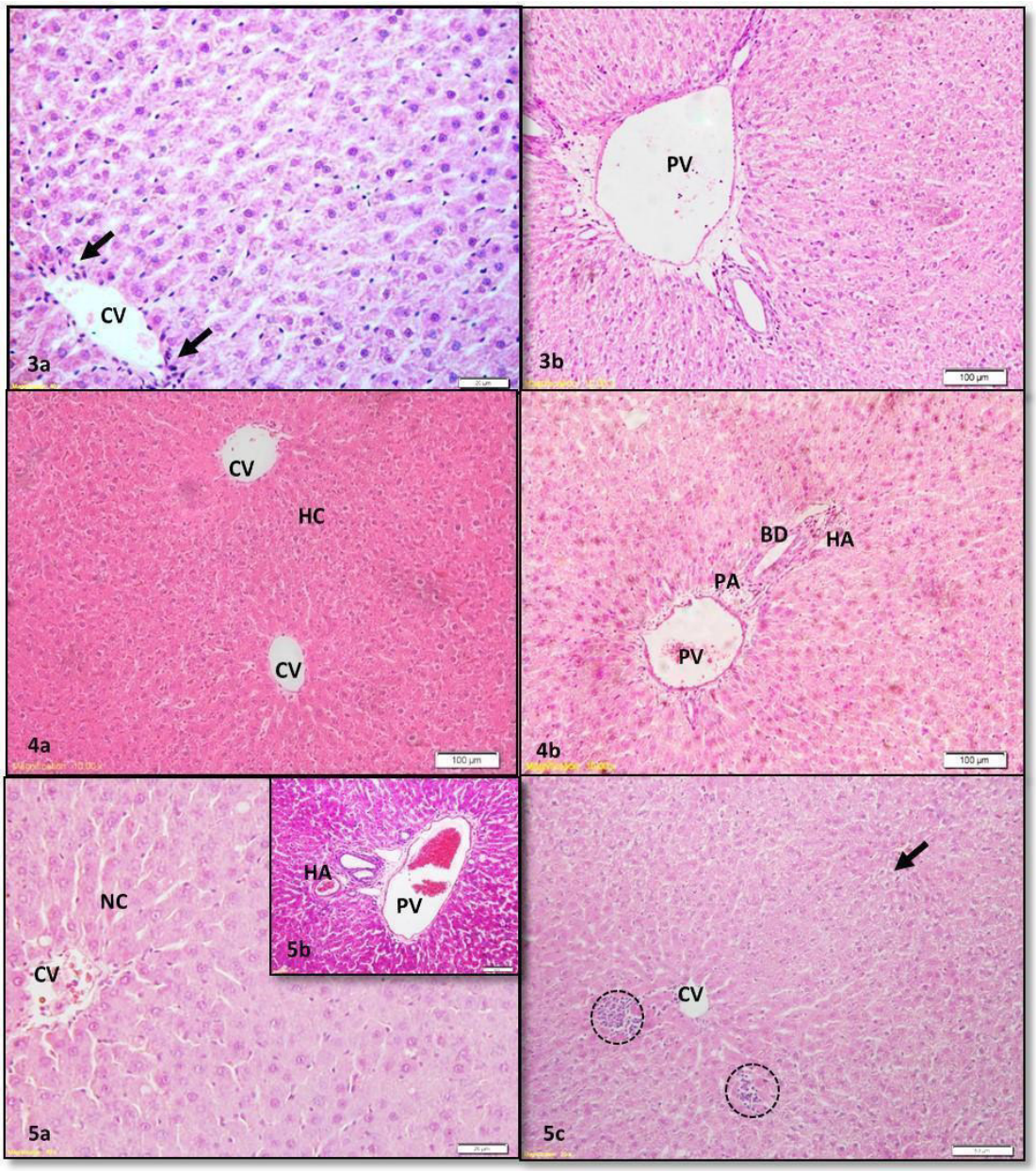
4.6 Histological Results of The Liver Examination

Histological examination of the control (Group I) liver showed lobules consisting of cords of hepatocytes forming functional and structural units and extending from the central veins Fig. 1a separated by sinusoids lined by endothelial and Kupffer cells. The portal areas at the corners of the lobule had three canals; the largest being the portal vein, hepatic artery, and bile duct Fig. 1b; fibrocytes and macrophages were also present in the portal areas. Livers from animals in Group II exposed to cigarette smoke showed several histopathological changes suggesting alterations in hepatic architecture, dilation, and congestion of sinusoids, cytoplasmic degeneration, and cellular necrosis around the central vein Fig. 2a. Also noted Figure 2b was the dilatation of portal areas due to the degeneration of the connective tissue and the circulatory disturbance, dilatation of the portal vein and hepatic artery, congestion of deformed and stagnant red blood cells, and bile duct proliferation. Infiltration of inflammatory cells into the portal area was evident, also note delimiting the granulomas and the epithelioid cells that form part of the granuloma Fig. 2b,c. Fibroblasts in the portal area showed evident hyperplasia, the fibrous septum was widened, and a typical liver fibrosis was beginning to form, which indicated it was at the stage of granulomas. Changes in the nuclei of the hepatocytes indicated by pyknosis and karyorrhexis in necrotic cells and lysis of the liver tissue in some areas were also noticeable Fig. 2d. Livers from Group III animals exhibited better hepatic architecture compared to Group II. The veins showed less severe blood congestion and less inflammatory cell infiltration around the central veins Fig. 3a. Less damage to hepatic cords, cytoplasmic granulocytes, and hepatocytes and their nuclei were evident. There was no dilatation of the portal vein, with Kupffer cells seen inside it and around the portal area Fig. 3b. No proliferation of the bile ducts was apparent. Group IV appeared normal, similar to the control group Figs. 4a, b. The hepatic architecture was normal, with the central vein and hepatic cords containing hepatocytes, one or two nuclei, and no cellular changes visible in their membranes or nuclei in the portal area. Group V portal vein and arteries were congested with inflammatory cells that spread widely in the hepatic tissue and around the central veins Figs. 5a, b. Cellular necrosis, nucleation atrophy, very dilated blood sinusoids, and an increased number of Kupffer cells were observed in the sinusoids also nots the epithelioid cells that form part of the granuloma Figs. 5c.



Figures 1a, b: Transverse liver sections of control group. Showing the normal histological structure of central vein (CV), hepatic cell (HC), sinusoidal blood (arrows), kupffer cell (KC) and portal area (PA), which contains hepatic artery (HA), portal vein (PV), and bile duct (BD).

Figures 2a-d: Transverse liver section of second group rats exposed to cigarette smoke. a: showing the central vein (CV), degeneration and cellular necrosis appears around the vein and increased staining of cells with eosin (NC) Dilatation and congestion of sinusoids around the central vein (arrow), some fatty drops were also observed (head arrow). b: showing dilation and congestion of the portal vein (PV) fibrosis could be observed in the portal area (arrows) also note proliferation of bile ducts (BD). c: showing many areas of necrosis of hepatic cells (NC), delimit the granulomas. Note the epithelioid cells (arrow) that form part of the granuloma d: necrosis of hepatic cells (NC) with pyknosis, karyorrhexis or Karyolysis nuclei and tissue lysis (arrows).



Figures 3a, b: Transverse of liver section of third group treated with grape juice and exposed cigarette smoke. a: showing Improvement in the central vein (CV), hepatocytes while still showing inflammatory infiltration around the central vein and in sinusoidal (arrows). b: showing dilation of portal vein (PV) in portal area but less congestion and stasis of red blood cells.

Figures 4a, b: Transverse of liver section of fourth group treated with grape juice only. Showing central vein (CV), hepatic cell (HC), sinusoidal blood, and portal area (PA), which contains hepatic artery (HA), portal vein (PV), and bile duct (BD) similar to the control group.

Figures 5a, b: Transverse of liver section of fifth group exposed to cigarette smoke for a month and then without a withdrawal exposure. a: Showing necrotic cells (NC), deformed central vein (CV) and stasis of red blood cells. b: dilation of portal vein (PV) and hepatic artery (HA) congestion and stasis of blood, also note the peripheral fibrosis in the portal area could be observedc: showing vacuolar cytoplasmic degeneration (arrows), and the formation of fibrous septum could be observed locally and still delimit the granulomas, blood sinusoids collapsed, and nuclei Pyknosis.

5. DISCUSSION

The present study confirmed that inhaling cigarette smoke causes a decrease in body weight as reported previously.[2] Smokers lose weight and then gain it after quitting smoking. This effect has been attributed to nicotine in cigarettes that is one of the most pathological and toxic agents that causes loss of appetite, dysphagia, poor digestion and absorption of food, digestive disorders, and weight loss [15]. The increase in liver weight (Group II) may be attributed to hematopoiesis of the liver parenchyma, hepatic veins, and dilatation and bleeding in portal areas caused by cigarette smoke [2]. In Group III, the liver-weight increase attenuated due to the positive effect of grape juice on the liver, possibly indicating that grape juice assists in the removal of metabolic waste harmful to the body; it has been suggested that grape juice aids the fat removal from the liver including cholesterol and help to eliminate free radicals [16]. The present study also showed elevated levels of AST, ALT, and ALP enzymes in Group II. High ALP enzyme indicates blockage of bile ducts;

the bile accumulation in the bile ducts leads to increased levels of this enzyme that is then released into the bloodstream; cigarette smoke inhalation is one of the causes of high ALP enzyme level [17, 18]. Numerous studies have indicated that nicotine causes a rise in serum cortisol levels that subsequently decrease over time; this has been seen throughout the early stages of smoking withdrawal in animals after their exposure to cigarette smoke was stopped. It reported [19] that elevated AST and ALT liver enzymes indicate liver damage caused by exposure to cigarette smoke leading to the formation of free radicals that affect the destruction of cell membranes. Alterations in hepatic architecture, fat accumulation, and release of enzymes into the bloodstream indicate cellular necrosis of hepatic tissue. It confirmed [20] that nicotine in cigarette smoke causes damage to hepatocellular membranes, and thus increases the release of AST and ALT enzymes that can cause changes in hepatocytes membrane permeability; this leads to an increased level of free radicals in tissues affected by chemicals present in cigarette smoke. The free radicals switched on the unsaturated lipid membrane peroxidation, induced serious destruction of the hepatic cell membrane and structure of organelles, and caused hepatic cell injury, degeneration, necrosis, and liver fibrosis formation induced by a long-term repeated stimulus. Animals treated with grape juice showed a reduced level of AST and ALT enzymes that support the liver structure [8]. Grape juice is rich in antioxidative polyphenols that are the first line of defense against damage by free radicals [12, 21]; the antioxidative activity of grape juice can be attributed to the presence of polyphenols.[22] The results of blood analysis in the present study reinforce this observation. Cigarette smoke also increases the risk of thrombosis (blood clots) caused by platelet aggregation and may be enhanced when the number of white blood cells are high. The improvement in blood parameters in Group III compared to Group II may be attributed to the effect of resveratrol, the main polyphenol found in grapes that act as an anti-inflammatory and vasodilator [23]. It also improves lipoprotein metabolism, inhibits platelet aggregation, and decreases inflammation. Cigarette smoke contains carbon monoxide that may lower oxygen levels in the blood and contribute to arterial blood clotting [22]. Grape juice contains antioxidants and thus increases the body's defense against free radicals [21]. Reduced severity of histopathological changes in animals of Group III and the recovery of the near-normal structure of liver tissue demonstrates the effectiveness of grapes in reducing hepatic damage and may be attributed to the high content of antioxidants that act as free-radical scavengers and reduce damage to cellular organelles [24]. The antioxidative effect of grapes attributed to the presence of active substances including resveratrol, and proanthocyanidins found at high levels in the grape skin and seeds, making grapes effective in limiting the toxic damage caused by exposure to cigarette smoke. These findings also suggest that the mechanism of action of grape juice in reducing hepatic damage may play an important role in altering the biological properties of membranes leading to cell damage, activation of fibroblasts, induction of cytokines, and increased collagen production. According to Rho KA and Kim MK[25], grape juice can reduce lipid peroxidation and DNA damage and significantly delay the onset of hemolytic symptoms. The lack of inflammatory cells in rats that consumed grape juice when subjected to cigarette smoke inhalation may be attributed to the body's improved immune reaction against toxins facilitated by an increased content of antioxidants [24]. Exposure to cigarette smoke generates free radicals in the body [1], that may be at least in part nullified by red grape juice acting as a powerful antioxidant. The increased number of Kupffer cells in Group III was probably due to their function as cells that release biological agents in response to infection. Hepatocellular damage also increases the population of Kupffer cells to protect liver tissue. Kupffer cells activate phagocytic cells by releasing biochemical agents that direct phagocytic cells to remove free radicals.[26] White blood cells are dominant during the subsequent stage of liver damage as active body responders/defenders. The proliferation of epithelial lining of the bile ducts is due to bile accumulation and is also a part of the body's immune response against inflammation caused by pollutants; cells respond by producing antioxidants. The cells lining the bile ducts respond and participate in the anti-inflammatory reaction of the liver through their ability to function as primary cells of the liver [(27)].

6. CONCLUSION

Our study identified the effectiveness of red grape juice and its antioxidants properties, which improved the damage to liver tissue in rats exposed to smoking. Within this study, we noticed the consequences of cigarette smoking in rats as it caused liver dysfunction and histopathological changes. However, Grape juice showed more improvement regarding the rearranged hepatic cords and their liver function.

7. AUTHORS CONTRIBUTION STATEMENT

Dr. Alalwani, AD Write and review the Manuscript, Dr. Khalifa, SA Designed Experiments, photo of Light Microscope, and Aljuhani, HO contributed the methodology and analyzed these data of tests of all experiment. Authors discussed the results and approved the final version.

8. CONFLICT OF INTEREST

Conflict of interest declared none.

9. ACKNOWLEDGEMENT

I extend my sincere thanks and appreciation to king abdulaziz city for science and technology for research No 1-18-01-009-0193.

10. REFERENCES

1. Dhouib H, Jallouli M, Draief M, El-Fazaa S, Bouraoui S. The effect of nicotine and its interaction with ethanol on biochemical parameters, oxidative damage and histological changes in the rat's liver. IOSRJESTFT. 2014;8(1):72-82. doi: 10.9790/2402-08157282.

2. Ogenyi S, Choji T, Chimezirim A, Onyemelukwe A, Ngokere A, Onwuasoanya U, Akulue J. Histological and biochemical effects of cigarette smoke on the liver of Wistar rats. Annu Res Rev Biol. 2015;7(2):119-25. doi: 10.9734/ARRB/2015/17292.

3. El-Zayadi AR. Heavy smoking and liver. World J Gastroenterol. 2006;12(38):6098-101. doi: 10.3748/wjg.v12.i38.6098, PMID 17036378.
4. Al-Easawi N, Al-Azzawi M. A histological study in the liver of albino mice posts is exposing to shisha smoke. World J Exp Biosci. 2015;3:30-5.
5. Ghaly MA, Khedr ESG, Abdel Aleem A. A comparative study of nicotine effect on the liver of albino rat. Egypt J Hosp Med. 2003;10(1):130-44. doi: 10.21608/ejhm.2003.18738.
6. Justesen U, Knuthsen P. Composition of flavonoids in fresh herbs and calculation of flavonoid intake by use of herbs in traditional Danish dishes. Food Chem. 2001;73(2):245-50. doi: 10.1016/S0308-8146(01)00114-5.
7. Shin MO, Moon JO. Effect of dietary supplementation of grape skin and seeds on liver fibrosis induced by dimethylnitrosamine in rats. Nutr Res Pract. 2010;4(5):369-74. doi: 10.4162/nrp.2010.4.5.369, PMID 21103082.
8. Al-Amoudi WM. Effect of grapefruit juice on aluminum-induced hepatotoxicity in albino rats: histological, ultrastructural and histochemical assessment. Adv Biosci Biotechnol. 2017;08(12):463-77. doi: 10.4236/abb.2017.812034.
9. Eshraghi-Jazi F, Alaei H, Azizi-Malekabadi H, Gharavi-Naini M, Pilehvarian A, Ciahmard Z. The effect of red grape juice and exercise, and their combination on Parkinson's disease in rats. Avicenna J Phytomed. 2012;2(2):90-6. PMID 25050236.
10. Buchner I, Medeiros N, Lacerda Ddos S, Normann CA, Gemelli T, Rigon P, Wannmacher CM, Henriques JA, Dani C, Funchal C. Hepatoprotective and antioxidant potential of organic and conventional grape juices in rats fed a high-fat diet. Antioxidants (Basel). 2014;3(2):323-38. doi: 10.3390/antiox3020323, PMID 26784874.
11. Burin VM, Falcão LD, Gonzaga LV, Fett R, Rosier JP, Bordignon-Luiz MT. Colour, phenolic content and antioxidant activity of grape juice. Ciênc. Tecnol Aliment. 2010;30(4):1027-32. doi: 10.1590/S0101-20612010000400030.
12. Rodrigues AD, Scheffel TB, Scola G, Dos Santos MT, Fank B, Dani C, Vanderlinde R, Henriques JA, Coitinho AS, Salvador M. Purple grape juices prevent pentylenetetrazol-induced oxidative damage in the liver and serum of Wistar rats. Nutr Res. 2013;33(2):120-5. doi: 10.1016/j.nutres.2012.12.002, PMID 23399662.
13. Azzalini L, Ferrer E, Ramalho LN, Moreno M, Domínguez M, Colmenero J, Peinado VI, Barberà JA, Arroyo V, Ginès P, Caballería J, Bataller R. Cigarette smoking exacerbates nonalcoholic fatty liver disease in obese rats. Hepatology. 2010;51(5):1567-76. doi: 10.1002/hep.23516, PMID 20432253.
14. Bancroft JD, Gamble M. Theory and practice of histological techniques. Elsevier health sciences; 2008.
15. Mineur YS, Abizaid A, Rao Y, Salas R, DiLeone RJ, Gündisch D, Diano S, De Biasi M, Horvath TL, Gao XB, Picciotto MR. Nicotine decreases food intake through activation of POMC neurons. Science. 2011;332(6035):1330-2. doi: 10.1126/science.1201889, PMID 21659607.
16. Aguiar O, Gollücke APB, de Moraes BB, Pasquini G, Catharino RR, Riccio MF, Ihara SS, Ribeiro DA. Grape juice concentrate prevents oxidative DNA damage in peripheral blood cells of rats subjected to a high-cholesterol diet. Br J Nutr. 2011;105(5):694-702. doi: 10.1017/S0007114510004368, PMID 21324234.
17. Hyder MA, Hasan M, Mohieldein AH. Comparative levels of ALT, AST, ALP and GGT in liver associated diseases. Eur J Exp Biol. 2013;3(2):280-4.
18. Hamad A-WR, Khaled N, Al-Daline SM, Al-Ani F. Effect of cigarette smoking on serumand SALIVALIVER enzymes function; 2015.
19. Omotoso GO, Enaibe BU, Akinola OB, Kadir RE, Akinlolu AA, Oyewopo AO. Lipid profile and liver histochemistry in animal models exposed to cigarette smoke. J Basic Appl Sci. 2012;8(1):20-5. doi: 10.6000/1927-5129.2012.08.01.04.
20. Salahshoor M, Mohamadian S, Kakabaraei S, Roshankhah S, Jalili C. Curcumin improves liver damage in male mice exposed to nicotine. J Trad Complement Med. 2016;6(2):176-83. doi: 10.1016/j.jtcme.2014.11.034, PMID 27114942.
21. Orak HH. Total antioxidant activities, phenolics, anthocyanins, polyphenoloxidase activities of selected red grape cultivars and their correlations. Sci Hortic. 2007;111(3):235-41. doi: 10.1016/j.scienta.2006.10.019.
22. Barua RS, Sy F, Srikanth S, Huang G, Javed U, Buhari C, Margosan D, Ambrose JA. Effects of cigarette smoke exposure on clot dynamics and fibrin structure: an ex vivo investigation. Arterioscler Thromb Vasc Biol. 2010;30(1):75-9. doi: 10.1161/ATVBAHA.109.195024, PMID 19815816.
23. de Moura CFG, Ribeiro FAP, de Jesus GPP, da Silva VHP, Oshima CTF, Gollücke APB, Aguiar O, Ribeiro DA. Antimutagenic and antigenotoxic potential of grape juice concentrate in blood and liver of rats exposed to cadmium. Environ Sci Pollut Res Int. 2014;21(22):13118-26. doi: 10.1007/s11356-014-3257-1, PMID 24996944.
24. Mahmoud GS, Amer AS. Protective effects of vitamin C against nicotine-induced oxidative damage of rat liver and kidney. IOSRJESTFT. 2014;8(12):50-63. doi: 10.9790/2402-081245063.
25. Rho KA, Kim MK. Effects of different grape formulations on antioxidative capacity, lipid peroxidation and oxidative
26. McCuskey R, Eddie Wisse E. Hepatic sinusoidal cells: endothelial cells, Kupffer cells, fat-storing cells and liver-associated lymphocytes. Cell Mol Toxicol. 2010.
27. Strazzabosco M, Fabris L. Functional anatomy of normal bile ducts. The anatomical record. Anat Rec (Hoboken). 2008;291(6):653-60. doi: 10.1002/ar.20664, PMID 18484611.