CHANGES IN THE LIVER OF MICE EXPOSED TO WATERPIPE AND ELETRONIC CIGARETTE SMOKE FOR 90 DAYS

Alterações em fígado de camundongos expostos à fumaça do narguilé e cigarro eletrônico por 90 dias

Access this article online	
Quick Response Code:	**Website:** https://periodicos.uff.br/ijosd/article/view/67267

Autores:
Isadora Reis de Souza
Faculdade de Ciências da Saúde, Universidade do Vale do Itajaí (Univali) – Itajaí (SC), Brazil

Jennifer Pereira Correa
Faculdade de Ciências da Saúde, Universidade do Vale do Itajaí (Univali) – Itajaí (SC), Brazil

Eduardo Padilha Correa Filho
Faculdade de Ciências da Saúde, Universidade do Vale do Itajaí (Univali) – Itajaí (SC), Brazil

Sarah Freygang Mendes Pilat
Faculdade de Ciências da Saúde, Universidade do Vale do Itajaí (Univali) – Itajaí (SC), Brazil

Instituição na qual o trabalho foi realizado: Universidade do Vale do Itajaí (Univali)

Endereço para correspondência: Sarah Freygang Mendes Pilati - Universidade do Vale do Itajaí - Rua Uruguai, 458 – Centro – CEP: 88302-901 - Itajaí (SC), Brazil
E-mail para correspondência: sarahfreygang@gmail.com

RESUMO

A utilização do narguilé e do cigarro eletrônico, comercializados como alternativas menos nocivas, tem suscitado preocupações. O fumo dos narguilés libera mais de 4800 substâncias químicas identificadas, incluindo substâncias tóxicas. Também os cigarros eletrônicos contêm elementos nocivos como o

formaldeído e metais pesados. Desta forma, ambos podem ser prejudiciais para a saúde dos utilizadores e merecem atenção e investigação, para além dos danos nas vias respiratórias. O objetivo deste estudo é avaliar possíveis alterações no tecido hepático de camundongos expostos a esses vapes e narguilés, considerando que há estudos demonstrando alterações no fígado associadas ao cigarro convencional. Trinta ratos Swiss fêmeas foram divididos em grupos de controle, cigarro eletrônico e narguilé, expostos a 4 gramas de tabaco para narguilé com sabor a maçã Mizo® e carvão vegetal Bamboo Brazil® e cigarro eletrônico-Joyetech® e VapeBoss® Salt line E-liquid 35 mg de nicotina durante 30 minutos por dia, durante 90 dias. O grupo do narguilé apresentou alterações histológicas como extravasamento hemorrágico, vacuolização de hepatócitos e alterações nas células de Kupffer. O grupo do cigarro eletrônico apresentou inflamação, extravasamento hemorrágico, aumento das células de Kupffer, dilatação vascular, vacuolização dos hepatócitos e ectasia dos vasos sanguíneos. O estudo conclui que tanto o cigarro eletrônico como o fumo do narguilé têm o potencial de induzir alterações hepáticas, enfatizando a necessidade de mais investigação sobre as consequências do seu uso para a saúde. Apesar dos danos identificados nos estudos, há uma falta de políticas ou regulamentos relativos ao uso do narguilé, destacando a importância de medidas de saúde pública para abordar estas preocupações.

Palavras-chave: Narguilé, Cigarro Eletrônico, Tabagismo, Fígado, alterações hepáticas.

ABSTRACT

The use of waterpipes and e-cigarettes, marketed as less harmful alternatives, has raised health concerns. Waterpipe smoke releases over 4,800 identified chemicals, including toxic substances. E-cigarettes, too, contain harmful elements like formaldehyde and heavy metals. In this way, both can be detrimental to the health of users and deserve attention and research in addition to damage to the airways. The aim of this study is to evaluate potential changes in the liver tissue of mice exposed to these e-cigarretes and waterpipes, considering that there are studies demonstrating changes in the liver associated with conventional cigarettes It is therefore expected that there will also be changes associated with them in liver tissue. Thirty female Swiss mice were divided into control, e-cigarette, and waterpipe groups, exposed to 4 grams of Mizo® apple-flavored waterpipe tobacco and Bamboo Brazil® charcoal and Electronic Cigarette-Joyetech® and VapeBoss® Salt line E-liquid 35 mg of nicotine for 30 minutes daily for 90 days. The Waterpipe Group exhibited histological changes such as hemorrhagic extravasation, hepatocyte

vacuolization, and Kupffer cell alterations. The E-cigarette Group displayed inflammation, hemorrhagic extravasation, Kupffer cell increase, vascular dilatation, hepatocyte vacuolization, and blood vessel ectasia. The study concludes that both e-cigarette and waterpipe smoke have the potential to induce liver changes, emphasizing the need for further investigation into the consequences of their usage on health. Despite the identified harm in studies, there is a lack of policies or regulations regarding the use of waterpipe, highlighting the importance of public health measures to address these concerns.

Keywords: Waterpipe; E-cigarettes; Tobacco; Liver; liver changes.

INTRODUCTION

A *waterpipe* (also known as a *shisha, hookah,* or *water pipe*) is a device used for smoking essences, tobacco, and other substances. It works by heating the air, which passes through the smoke and is cooled in the water present in the vessel before being inhaled (MARTINS SR, *et al,* 2014). It`s suggested that passing tobacco smoke through a small container of water before inhaling it would be less harmful. Used mainly in North African and Asian countries, in recent years its consumption has been growing worldwide, especially among young people. A study carried out in 2019 showed that waterpipe users were younger and had a higher socioeconomic status than cigarette users. In addition, recent cigarette use seems to be more associated with waterpipe use among young people than among adults (BERTONI N. *et al.* 2019).

Flavored tablet tobacco is placed in the upper part of the waterpipe and subjected to high temperatures through the combustion of charcoal. The smoke is inhaled and filtered through a container of water (TÜRKMEN S, *et al*. 2018). The composition of the tobacco used in waterpipes is variable and not standardized (INCA. 2017). Studies examining waterpipe smokers have reported high concentrations of carbon monoxide, nicotine, tar and heavy metals in the lungs (KNISHKOWY B. *et al.* 2005). During waterpipe use, not only does the charcoal burn, but also the incomplete combustion of the tobacco occurs at a temperature close to 500°C, when higher concentrations of toxic products are released. Around 4,800 chemical products have already been identified in waterpipe smoke, 69 of which have been quantified as carcinogenic. Shisha has three alarming factors: the inhalation of tobacco smoke, the inhalation of charcoal smoke and the likelihood of infection through sharing and using hoses (ZAID K, *et al.* 2005). These factors indicate similar health risks associated with sharing pipes and adding alcohol or psychoactive drugs to tobacco, comparable to the dangers of smoking and infectious diseases (KNISHKOWY B, *et al. 2005*).

Research shows that waterpipes contain a high amount of nicotine and their use involves alarming health risks. Constant nicotine consumption can also be a precursor to cigarette smoking and induce nicotine addiction (MAZIAK W, *et al.* 2015). Lung and other cancers, respiratory diseases and cardiovascular diseases are all harms caused by smoking, as well as waterpipe, because the smoke contains the same substances as tobacco (nicotine, carbon monoxide, hydrocarbons, toxins, among others) (MAZIAK W, *et al*. 2011).

Another way for young people and adults to replace conventional cigarettes is to use an electronic cigarette known as a Vape, which consists of a battery, atomizer and nicotine-containing cartridge. The Vape is a small electronic device, like a pen drive or a pen, which delivers doses of nicotine and other substances in an aerosol. However, according to the World Health Organization (WHO), there is no scientific proof of the benefits of these practices (WHO,2013).

The composition of the cartridge varies and generally has nicotine and a substance to produce the aerosol, such as propylene glycol or glycerol diluted in water. Some models have flavor-modifying components, such as fruit extract, vanilla, mint or chocolate. E-cigarette nicotine cartridges have been found to contain various toxic substances, including formaldehyde, acetaldehyde, acrolein, volatile organic compounds, heavy metals, and tobacco-derived nitrosamines (BULLEN C, *et al.* 2013).

The use of electronic cigarettes is like conventional cigarettes. When you puff, a button or suction sensor triggers a heating cycle until the liquid reaches boiling point and is transformed into vapor. The temperature can reach 40-65ºC. Part of the vapor is released with the nicotine into the vape and the other into the environment. When you exhale, it cools down, and the device will only heat up again when it is swallowed. One cartridge can generate between 10 and 250 jets, corresponding, depending on the brand, to between 5 and 30 traditional cigarettes (GONIEWICZ ML,*et al.* 2014).

It is well known that the liver has the function of filtering the blood and that it eliminates substances harmful to the body that have been ingested through the intestines via the feces. In the case of smoking, most of the carcinogenic substances and toxins present are absorbed by the lungs, enter the bloodstream and go directly to the liver, which has its function compromised as it tries to filter out these toxins. The WHO states that tobacco causes more than 8 million deaths a year (WHO, 2023) and smoking contributes to the development of various types of cancer, such as acute myeloid leukemia, bladder cancer, pancreatic cancer, liver cancer, tracheal cancer, bronchial cancer, lung cancer, cervical cancer,

esophageal cancer, kidney and ureter cancer, laryngeal cancer, pharyngeal cancer and other organs(RIGOTTI NA,2018).

It was decided to study mice because they have a fast metabolism and tissues like those of humans, with a vast array of genetic variants that are well characterized anatomically and physiologically, as well as susceptibility to the development of specific tumors, which makes them useful for oncological studies (PATIL S, *et al.* 2022). Recent studies in mices have shown cardiovascular and airway alterations resulting from exposure to waterpipes and electronic cigarettes. These showed acute and chronic inflammatory alterations, changes in endothelial cells, cardiovascular cells and lung cells (MÜNZEL T, *et al. 2020*)(NEMMAR A, *et al.* 2023)(NEMMAR A, *et al*.2024)(DEMARCHI C. *et al,* 2022).Studies demonstrated a possible association between mitochondrial dysfunction in hepatocytes and the use of electronic cigarettes (ESPINOZA-DEROUT J, *et al*.2019)(LECHASSEUR A ,*et al*. 2022) as also it can affect homeostasis and can cause cardiovascular, gastrointestinal and renal systems damage(XU A, *et al*, 2023).

The aim of this study is to evaluate possible changes in the liver tissues of mice after exposure to waterpipe and e-cigarette smoke, to investigate possible future harm to young people and adults who use them.

METHODS

This article is the result of the merger of two studies. The study on waterpipe was approved by the Ethics Committee for the Use of Animals of the University of Vale do Itajaí - CEUA/UNIVALI, under opinion no. 063/17. The second study, which analyzed electronic cigarettes, was previously approved by the Ethics Committee for the Use of Animals at the University of Vale do Itajaí - CEUA/UNIVALI, under opinion no. 010/20p. The main objective is to evaluate the potencial changes in liver tissue rats exposed to these devices.

In this experimental study, 30 12-week-old female Swiss mice were used. The gender of the animals was decided due to the availability of the animals and the experimental literature by Wang, *et al*, 2021. Martins, *et al*, 2012 who identified that females may be more prone to certain inductions. The animals were housed in conventional cages and kept on a 12-hour light-dark cycle, with daily access to food and water. The number of animals for each group was decided based on the experimental literature by Martins, *et al*, 2012, in which the authors used groups of 10 animals to study the effects of exposure to tobacco and alcohol on the mucosa of the tongue and pharynx of rats. The animals were exposed to the

experiment from May 20 to July 18, 2018, in the histology research laboratory of the Universidade do Vale do Itajaí educational institution. They were acclimatized for a week after being removed from UNIVALI's central animal house before the start of the experiment. Acclimatization took place by dividing 5 rats per cage, at a temperature of 22°C and adapted to the noise of the machine used in the experiment. The animals were randomly divided into three groups of ten animals each: a control group, an experimental group for the waterpipe study and an experimental group for the e-cigarette study. Data was collected after 90 days (LAFRANCONI M, 2021).

Based on Shraideh and Najjar, 2011, Khabour, *et al*, 2011, Flausino, *et al*, 2020 and Mayyas, *et al*, 2020, who performed whole-body waterpipe exposure. The experimental groups' animals were exposed to the whole-body exposure system by being confined in a sealed box measuring 175x170x270 mm (Figure 2a, 2b e 2c). The box was connected to the waterpipe device and common electronic cigarette by a device that sucks up the tobacco and vapor and throws it into the box. The animals in the Shisha Group were exposed to the smoke from 4 g of conventional shisha tobacco from the Mizo ® brand (Apple Waterpipe Tobacco) with a percentage of 0.5% unwashed tobacco and gunpowder charcoal from the Bamboo Brasil® brand with a diameter of 33 mm. The animals in the Electronic Cigarette Group were exposed to smoke from the eGo AIO EO 650mAh Electronic Cigarette - Joyetech® and VapeBoss® Salt line E-liquid 35 mg of nicotine in 30 ml. The duration of the session was 30 min/day (NEMMAR A, *et al, 2015*) during the daytime shift, for 90 days.

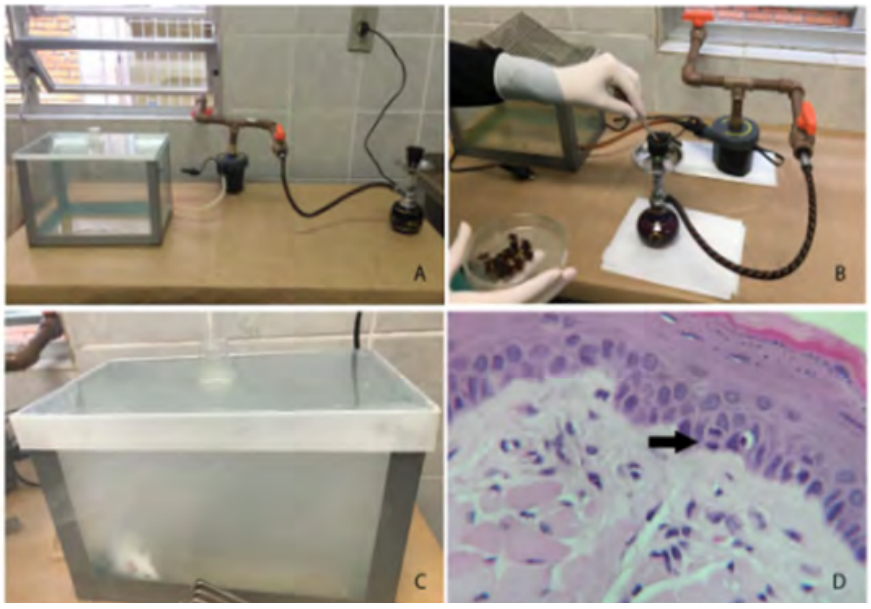

Figure 2 A: Device; B: Device in operation; C: Sealed chamber with smoke; D: Histological section of tongue showing the presence of mitosis (arrow) in the epithelium

The concentration of waterpipe tobacco smoke and VAPE vapor applied to the animals in the exposed experimental groups was 35 mL for two seconds while the other 58 seconds were fresh air (XU A,*et al.* 2023) being distributed in total in 210 mL for each mouse. The pump's flow rate was adjusted to maintain the volume of 530 mL/2 sec - the puff specified by the Beirut Method - a regime chosen because it approximates, on average, the topography of the human puff during the use of the waterpipe device (NEMMAR A, *et al*. 2023). The time the animals were exposed to the smoke was based on a study evaluating the cardiorespiratory effects of waterpipe smoke on humans (NEMMAR A, *et al*. 2023).

The animals in the control group were exposed only to air, under the same conditions as the experimental group. After the last exposure session of the experimental groups, the animals were euthanized to obtain samples. Euthanasia was carried out using an anesthetic dose consisting of 50µl of Xylazine (0.23g/ml) and 210µl of Ketamine (0.1g/ml) for every 10 grams of the animal's weight, i.e. if the animal weighed 20 grams, 100µl of Xylazine with 420µl of Ketamine was used (according to Institutional Animal Care and Use Committee). On macroscopy, at this stage, it was observed that the liver of the animals exposed to waterpipe and in e-cigarette showed changes in size and weight.

The tissues studied were fixed in 4% paraformaldehyde in phosphate buffer pH 4 to 7 and duly processed and embedded in paraffin to make the slides. The sections were stained with hematoxylin and eosin (HE) for subsequent microscopic analysis as standardized in the Histopathology Department.

First, a qualitative description of the microscope slides was carried out of the possible lesions and the presence or absence of inflammatory infiltrates, as well as describing the characteristics observed on the slides. In parallel, photographs of the slides were taken using a Nikon Eclipse Ei microscope with a built-in camera to count the Kupffer cells at 400x magnification, to evaluate the liver damage using ImageJ software version 1.41o (National Institute of Health, Bethesda, USA). The values were recorded in a spreadsheet previously prepared in Microsoft Excel® (Microsoft Corporation, Redmond, Washington, USA). The cell count for each group was blinded and was carried out by the lead researcher.

These values were subjected to statistical analysis to compare the groups. The mean, median and standard deviation of the distances obtained in each group were calculated using Primecam software, to compare whether there was a statistical difference between the groups and to assess the applicability of the experiment in a pre-established number of animals.

The Shapiro-Willks normality test was used to assess the distribution of the data which, as it did not show a Gaugassian distribution, led to the use of non-parametric tests. The Kruskal-Wallis test was used to compare the number of Kupffer cells between all the groups. Counting was carried out in 10 random fields, at 400x magnification, in blinded samples in which the evaluator did not know the origin of the sample (e-cigarette, waterpipe or control). The data was presented using the means, medians and standard deviations of the groups. Values of $p<0.05$ were considered significant. All the data collected was organized in an electronic spreadsheet and then analyzed using the software SPSS® version 11 (SPSS Inc., Headquarters, USA).

RESULTS

The histological sections of the group subjected to waterpipe showed the presence of hemorrhagic extravasation and red blood cells outside the vessels. In seven cases, hepatocyte vacuolization was observed with focal areas of Kupffer cells, indicating histopathological alteration of the tissue. (Figure 1c e 1d)

The histological sections of the e-cigarette group showed few areas of inflammation, with hemorrhagic extravasation of red blood cells, discreet vacuolization of hepatocytes as well as a focal area of intralobular inflammation, presence of a large number of Kupffer cells indicating histopathological changes in the tissue and vascular dilation (Figure 1e e 1f). There was also a discreet chronic inflammatory infiltration around the vein and the presence of ectasia in the blood vessels.

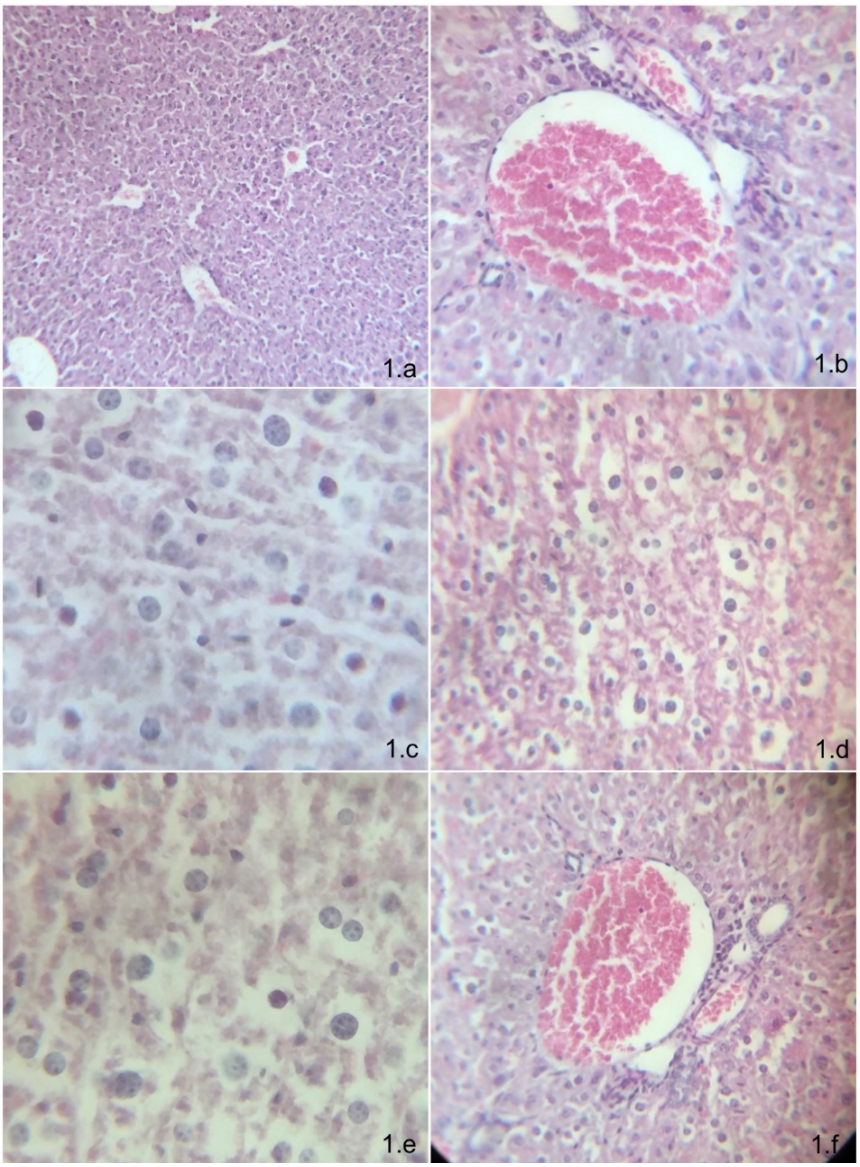

Figure 1 Histopathological observations of the animals' livers, H.E. staining: Control Group (1.a; 1.b). Normal tissue is seen (1a) (100x magnification), with the portal triad (1b-) (400x magnification); Shisha Group (1c; 1d. 100x magnification). Presence of vacuolization in hepatocytes and inflammatory cells (1.d). Kupffer cells (1c); Electronic Cigarette Group (1e; 1f). Presence of Kupffer cells and degraded hepatocytes (1e) (400x magnification). Blood stasis in vessel (1.f) (100x magnification).

The average number of Kupfer cells per field observed in the electronic cigarette group was 34.30, the median was 34 and the standard deviation was 8.97. While the average number of Kupfer cells per field observed in the waterpipe group was 14.7, the median was 13.5 and the standard deviation was 3.91. In the control group, the average number of Kupfer cells per field observed in the waterpipe

was 4.9, the median was 5 and the standard deviation was 2.42. The Kruskal-Wallis test to evaluate the statistical difference between the medians resulted in p=0.005, with a statistical difference between the groups.

DISCUSSION

The popularization of waterpipes and electronic cigarettes is due to the false impression that they are less harmful than conventional cigarettes, which has been proven that this statement is erroneous by studies that have reported the presence of toxic components in the smoke of both. Shisha smoke contains more than 4,800 chemicals, 69 of which are known carcinogens (FLAUSINO CS, *et al.*2020)such as tobacco-specific nitrosamines, polycyclic aromatic hydrocarbons, benzene, nitric oxide and heavy metals, and some contain nicotine. Various toxic substances, such as formaldehyde, acetaldehyde, acrolein, volatile organic compounds, heavy metals, and tobacco-derived nitrosamines, have been found in e-cigarette nicotine cartridges(BULLEN C, *et al*, 2013). In addition, the presence of charcoal and some toxins are produced in greater quantities by waterpipes compared to cigarettes, and the amount of smoke inhaled in a single waterpipe session can reach 150 times the amount inhaled in a single conventional cigarette.

A histologically normal pattern was observed in the liver slides of the animals in the control group, with the triad of arteries and veins showing no ectasia and hepatocytes showing no histopathological alterations (Figure 1a; 1b). Only one animal showed a discreet chronic perivascular inflammatory infiltrate and in another there was hemorrhagic extravasation. Extravasation of blood can occur in various situations, such as trauma, atherosclerosis or inflammatory or neoplastic erosions of a vessel (NEMMAR A, *et al,* 2015). In addition, Nemnar, *et al,* 2024, observed that nicotine causes damage to mice endothelial cells which may explain the hemorrhagic extravasation and de blood stasis observed in the experimental (NEMMAR A. *et al,* 2023) groups as illustrated in 1f. As also Münzel, *et al,* 2020, and Rezk-Hannah, *et al,* 2023. Brings up this discussion as these habits can cause damage to the endothelium leading to a higher risk of atherosclerosis.

The liver is the largest glandular organ in the body and receives most of its blood supply through the hepatic portal vein. This vein carries venous blood from the gastrointestinal tract, pancreas, and spleen. The liver is the first organ to be exposed to metabolic substrates and nutrients, so it is also the first organ to be exposed to harmful and toxic substances absorbed in the intestine (ROSS MH, *et al, 2012*). Thus, all the toxins present in waterpipe, and e-cigarettes are present

in the bloodstream and encounter the liver and hepatocytes via the portal triad, formed by the hepatic artery, portal vein and bile duct (DEL POZO SC, ARELLANO JLP, 2006).

The present study showed the presence of cell damage caused by the smoke from these devices, characterized by the presence of hepatocyte vacuolization and a focal area of intralobular inflammation (Figure 1c). These alterations, such as the vacuolization of hepatocytes with a ballooning appearance, are similar to the damage found in alcoholic hepatitis and steatosis, due to the abnormal accumulation of triglycerides within the parenchymal cells of the liver, causing a vacuolated appearance. The causes of steatosis or fatty degeneration include toxins, protein malnutrition and others. In developed countries, the most common causes of fatty liver degeneration are alcohol abuse and non-alcoholic fatty liver disease (KUMAR V, *et al, 2015*). One of the consequences of vacuolization is hydropic degeneration (cell edema or vacuolar degeneration), due to the accumulation of water in the intracellular medium because of changes in the sodium-potassium pump. This degeneration is the first manifestation of almost all forms of cell damage (REISNER HM, 2016).

The presence of glycogen granules and lipid droplets gives the slides stained with HE a vacuolized appearance (KUMAR V, *et al,*2015), which can also be seen in this study (Figure 1c). We also observed a loss of hepatic lobe architecture due to this vacuolization. Another finding was the presence of ectasia in blood vessels, which are localized dilations in pre-existing blood vessels, usually observed in the skin or mucous membranes, and which are associated with inflammation (REISNER HM, 2016). The presence of Kupffer cells (Figure 1d) also confirms the inflammatory process and tissue damage.

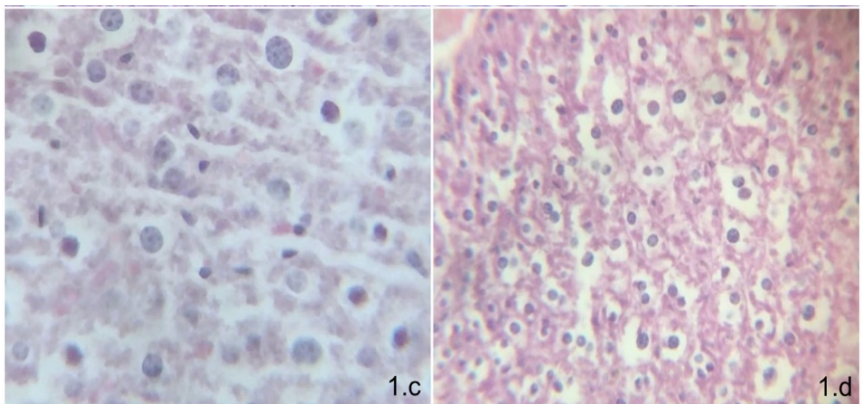

Figure 2 Shisha Group (1c; 1d. 100x magnification). Presence of vacuolization in hepatocytes and inflammatory cells (1.d). Kupffer cells (1c);

Kupffer cells are macrophages that have the function of filtering particulate matter, microbes and senescent cells. Studies show that these cells play a significant role in the pathogenesis of chronic and acute alcoholic liver disease. When activated, these cells produce high levels of reactive oxygen species, pro-inflammatory cytokines, and chemokines, which can cause liver damage (ZENG T, *et al*, 2016). Thus, the presence of Kupffer cells in the liver of the group exposed to waterpipe and e-cigarette smoke indicates the immunoregulatory response, leading to the activation of these defense cells to produce chemotactic factors also seen in the study of Nemmar, *et al*, 2024. A statistical difference was observed between groups, showing that these cells associated with the inflammatory response in the liver, and which are increased in hepatotoxicity are indeed associated with exposure to electronic cigarettes and waterpipes. Although Nemmar, *et al*, 2024. did not carry out histological studies, in his article he evaluated inflammatory markers that corroborate our findings.

Monma, *et al,* 2015, found, in a study of Winstar rats subjected to chronic alcoholism, that cellular tumefaction was the predominant liver damage resulting from the samples. Cellular tumefaction or hydropic degeneration is a reversible lesion in which cells become incapable of maintaining ionic and liquid homeostasis, resulting in the intracellular accumulation of fat, water and proteins that are normally excreted. Another study carried out with *Winstar* and vodka rats reported the same liver damage as our study, changes such as the accumulation of glycogen in the cytoplasm of hepatocytes and hydropic degeneration (SILVA AP, *et al*, 2010). The presence of vacuolization in the samples from the exposed groups in this study matches with these findings. Our study also found this same histological alteration observed by Nemmar, *et al,* 2024. in the livers of mice that were exposed to waterpipe for 30 days, that both waterpipe and e-cigarettes can alter liver morphology. This leads us to conclude that just 90 days of exposure to e-cigarettes and waterpipes in mice has already shown hepatoxicity in our samples due to toxins absorbed through the airways and circulating in the blood.

Machado Junior, 2019.evaluated mice exposed to cigarette smoke and showed that one of the most frequently observed lesions was hydropic degeneration which was observed in our study. Other alterations were also found, such as lobular and portal inflammatory process, granulomatous reaction, hyperemia, congestion of sinusoid capillaries and foci of hemorrhage. Cremonese RV, *et al*, 2001. showed that inhaling toxic substances such as carbon tetrachloride can cause cirrhosis. The authors also indicated oxidative stress as one of the main mechanisms involved in liver damage, which could lead to a discussion about the long-term consequences of inhaling toxic substances from electronic cigarettes and waterpipes.

CONCLUSION

The results of this study of Swiss mice exposed to waterpipe and e-cigarette smoke over a short period of 90 days show that their use is no safer for health than inhaling conventional cigarettes, contrary to popular belief. The study's findings align with previous research indicating that smoke inhalation induces changes in the inflammatory response and demonstrate, based on our statistical results, an increase in Kupffer cells in Swiss mice. The results suggest that longer periods of exposure could lead to more severe liver changes. Also, since young people are using these unconventional types of cigarettes, we suggest monitoring, surveying and investigating possible alterations in the liver and not just in the airways.

REFERENCES

1. Martins SR, Paceli RB, Bussacos MA, Fernandes FLA, Prado GF, Lombardi EMS, et al. Experimentation with and knowledge regarding water-pipe tobacco smoking among medical students at a major university in Brazil. J Bras Pneumol. 2014;40(2):102-10. https://doi.org/10.1590/S1806-37132014000200002

2. Bertoni N, Szklo A, De Boni R, Coutinho C, Vasconcellos M, Silva PN, et al. Electronic cigarettes and narghile users in Brazil: Do they differ from cigarettes smokers? Addict Behav. 2019;98:106007. https://doi.org/10.1016/j.addbeh.2019.05.031

3. Türkmen S, Eryigit U, Sahin A, Yeniocak S, Turedi S. Carbon monoxide poisoning associated with water pipe smoking. Clin Toxicol (Phila). 2018;56(4):264-72. https://doi.org/10.1080/15563650.2017.1375115

4. Brasil. Ministério da Saúde. Instituto Nacional do Câncer (INCA). Uso de narguilé: efeitos sobre a saúde, necessidades de pesquisa e ações recomendadas para legisladores. 2 ed. Rio de Janeiro: INCA, 2017.

5. Knishkowy B, Amitai Y. Water-pipe (narghile) smoking: an emerging health risk behavior. Pediatrics. 2005;116(1):113-19. https://doi.org/10.1542/peds.2004-2173

6. Zaid K, Azar-Maalouf E, Barakat C, Chantiri M. p53 Overexpression in Oral Mucosa in Relation to Shisha Smoking in Syria and Lebanon. Asian Pac J Cancer Prev. 2018;19(7):1879-82. https://doi.org/10.22034/APJCP.2018.19.7.1879

7. Maziak W, Jawad M, Jawad S, Ward KD, Eissenberg T, Asfar T. Interventions for waterpipe smoking cessation. Cochrane Database Syst Rev. 2015;2015(7):CD005549. https://doi.org/10.1002/14651858.CD005549.pub3

8. Maziak W. The global epidemic of waterpipe smoking. Addict Behav. 2011;36(1-2):1-5. https://doi.org/10.1016/j.addbeh.2010.08.030

9. Bullen C, Howe C, Laugesen M, McRobbie H, Parag V, Williman J, et al. Electronic cigarettes for smoking cessation: a randomised controlled trial. Lancet. 2013;382(9905):1629-37. https://doi.org/10.1016/S0140-6736(13)61842-5

10. Goniewicz ML, Knysak J, Gawron M, Kosmider L, Sobczak A, Kurek J, et al. Levels of selected carcinogens and toxicants in vapour from electronic cigarettes. Tob Control. 2014;23(2):133-9. https://doi.org/10.1136/tobaccocontrol-2012-050859

11. Rigotti NA. Balancing the Benefits and Harms of E-Cigarettes: A National Academies of Science, Engineering, and Medicine Report. Ann Intern Med. 2018;168(9):666-7. https://doi.org/10.7326/M18-0251

12. World Health Organization (WHO). Tobacco. Available from: https://www.who.int/news-room/fact-sheets/detail/tobacco

13. Patil, S., Mahuli, A. V., & Warnakulasuriya, S. Effects of smoking shisha, cancer risk, and strategies for prevention of shisha habit. J Oral Biol Craniofacial Res. 2022;12(4): 439-43. https://doi.org/10.1016/j.jobcr.2022.05.008

14. Münzel T, Hahad O, Kuntic M, Keaney JF, Deanfield JE, Daiber A. Effects of tobacco cigarettes, e-cigarettes, and waterpipe smoking on endothelial function and clinical outcomes. Eur Heart J. 2020 Nov 1;41(41):4057-4070. doi: 10.1093/eurheartj/ehaa460.

15. Nemmar A, Beegam S, Zaaba NE, Elzaki O, Pathan A, Ali BH. Waterpipe smoke inhalation induces lung injury and aortic endothelial dysfunction in mice. Physiol Res. 2023;72(3):337-47. doi: 10.33549/physiolres.935042.

16. Nemmar A, Beegam S, Yuvaraju P, Zaaba NE, Elzaki O, Yasin J, Adeghate E. Pathophysiologic effects of waterpipe (shisha) smoke inhalation on liver morphology and function in mice. Life Sci. 2024;336:122058.
doi: 10.1016/j.lfs.2023.122058.

17. Demarchi C, Souza M, Souza LS, Galli FL, Pilati SM. Lung histopathological changes in Swiss mice exposed to narghile smoke. J Bras Patol Med Lab. 2022;58:e4442022. https://doi.org/10.1900/JBPML.2022.58.444

18. Espinoza-Derout J, Shao XM, Bankole E, Hasan KM, Mtume N, Liu Y, et al. Hepatic DNA Damage Induced by Electronic Cigarette Exposure Is Associated With the Modulation of NAD+/PARP1/SIRT1 Axis. Front Endocrinol (Lausanne). 2019;10:320.
doi: 10.3389/fendo.2019.00320.

19. Lechasseur A, Mouchiroud M, Tremblay F, Bouffard G, Milad N, Pineault M, et al. Glycerol contained in vaping liquids affects the liver and aspects of energy homeostasis in a sex-dependent manner. Physiol Rep. 2022;10(2):e15146.
doi: 10.14814/phy2.15146.

20. Xu A, Duan K, Yang W, Feng G, Wu Z, Jiang X, et al. The toxic effects of electronic cigarette aerosol and cigarette smoke on cardiovascular, gastrointestinal and renal systems in mice. Sci Rep. 2023;13(1):12366.
doi: 10.1038/s41598-023-39201-7.

21. Wang Q., Khan N.A., Muthumalage T., Lawyer G.R., McDonough S.R., Chuang T.D., et al. Reparo desregulado e respostas inflamatórias por nicotina inalada derivada do cigarro eletrônico e propilenoglicol umectante de maneira dependente do sexo em pulmão de camundongo. FASEB Bioadv. 2019; 1:609–623. DOI: 10.1096/fba.2019-00048.

22. Martins RHG, Madeira SLM, Fabro AT, Rocha NS, Semenzati GO, Alves KF. Effects to exposure of tobacco smoke and alcohol on the tongue and pharynx of rats. Inhal Toxicol. 2012;24(3):153-60. https://doi.org/10.3109/08958378.2011.649190

23. Lafranconi M, Budinsky R, Corey L, Klapacz J, Crissman J, LeBaron M, et al. A 90-day drinking water study in mice to characterize early events in the cancer mode of action of 1,4-dioxane. Regul Toxicol Pharmacol. 2021 Feb;119:104819. doi: 10.1016/j.yrtph.2020.104819.

24. Shraideh ZA, Najjar HN. Histological Changes in Tissues of Trachea and Lung Alveoli of Albino Rats Exposed to the Smoke of Two Types of Narghile Tobacco Products. Jordan J Biol Sci. 2011;4(4):219-24.

25. Khabour OF, Alzoubi KH, Bani-Ahmad M, Dodin A, Eissenberg T, Shihadeh A. Acute exposure to waterpipe tobacco smoke induces changes in the oxidative and inflammatory markers in mouse lung. Inhal Toxicol. 2012;24(10):667-75. https://doi.org/10.3109/08958378.2012.710918

26. Flausino CS, Hoffmeister GF, Pilati PVF, Modolo F, Pilati SFM. A New Adapted Machine to Simulate Narghile Smoke. J Cytol Tissue Biol. 2020;7:029

27. Mayyas, F., Aldawod, H., Alzoubi, K. H., Khabour, O., Shihadeh, A., & Eissenberg, T. Comparison of the cardiac effects of electronic cigarette aerosol exposure with waterpipe and combustible cigarette smoke exposure in rats. Life Sciences. 2020;251, 117644. doi:10.1016/j.lfs.2020.117644

28. Nemmar A, Al Hemeiri A, Al Hammadi N, Yuvaraju P, Beegam S, Yasin J, et al. Early pulmonary events of nose-only water pipe (shisha) smoking exposure in mice. Physiol Rep. 2015;3(3):e12258. https://doi.org/10.14814/phy2.12258

29. Nemmar A, Beegam S, Zaaba NE, Elzaki O, Pathan A, Ali BH. Waterpipe smoke inhalation induces lung injury and aortic endothelial dysfunction in mice. Physiol Res. 2023 Jul 14;72(3):337-347. doi: 10.33549/physiolres.935042.

30. 30. Münzel T, Hahad O, Kuntic M, Keaney JF, Deanfield JE, Daiber A. Effects of tobacco cigarettes, e-cigarettes, and waterpipe smoking on

endothelial function and clinical outcomes. Eur Heart J. 2020 Nov 1;41(41):4057-4070. doi: 10.1093/eurheartj/ehaa460.

31. Rezk-Hanna M, Rossman MJ, Ludwig K, Sakti P, Cheng CW, Brecht ML, Benowitz NL, Seals DR. Electronic waterpipe (waterpipe) vaping reduces vascular endothelial function: the role of nicotine. Am J Physiol Heart Circ Physiol. 2024;326(3):H490-H496. doi: 10.1152/ajpheart.00710.2023.

32. Ross MH, Pawlina W, Barnash TA. Atlas de histologia descritiva. Porto Alegre: Artmed, 2012.

33. Del Pozo SC, Arellano JLP. Manual de patologia general. Masson, 2006.

34. Kumar V, Abbas A, Aster JC. Robbins & Cotran: Patologia - bases patológicas das doenças. 9 ed. Elsevier, 2015.

35. Reisner HM. Patologia: uma abordagem por estudos de casos. Porto Alegre: AMGH, 2016.

36. Zeng T, Zhang CL, Xiao M, Yang R, Xie KQ. Critical Roles of Kupffer Cells in the Pathogenesis of Alcoholic Liver Disease: From Basic Science to Clinical Trials. Front Immunol. 2016;7:538. https://doi.org/10.3389/fimmu.2016.00538

37. Monma CA, Silva NN, Neotti T, Coutinho VAG, Brito RB, Domingues RJS, et al. Estudo piloto: análise do fígado de ratos submetidos ao alcoolismo crônico experimental e tratados com Morinda citrifolia (noni). Rev Pan-Amaz Saude. 2015;6(4):11-18. http://dx.doi.org/10.5123/S2176-62232015000400002

38. Silva AP, Silva EL, Almeida MF, Silva IS, Souza BKM, Coelho RM, et al. Avaliação de lesão hepática causada por ingestão de álcool em ratos Wistar. 2010. Disponível em: https://repositorio.pgsscogna.com.br/

39. Machado Junior PA. Efeitos da administração da quercetina sobre o processo redox e inflamação hepática em camundongos C57BL/6 expostos a fumaça do cigarro [Conclusão de Curso]. [Ouro Preto]: Universidade Federal de Ouro Preto, 2019.

40. Cremonese RV, Pereira-Filho AA, Magalhães RB, Mattos AA, Marroni CA, Zettler CG, et al. Cirrose experimental induzida pela inalação de tetracloreto de carbono: adaptação da técnica e avaliação da peroxidação lipídica. Arq Gastroenterol. 2001;38(1):40-7. https://doi.org/10.1590/S0004-28032001000100008