OXFORD Toxicology Research

Toxicology Research, 2024, **13**, 1–10
https://doi.org/10.1093/toxres/tfae002
Paper

Liver toxicity in rats after subchronic exposure to HTP aerosol and cigarette smoke

Yushan Tian[1,2,3], Hongjuan Wang[1,2,3], Shulei Han[1,2,3], Yaning Fu[1,2,3], Fengjun Lu[2,3], Wenming Wang[2,3], Xianmei Li[2,3], Shuhao Ma[2,3], Pengxia Feng[2,3], Zhihao Shi[2,3], Huan Chen[1,2,3], Hongwei Hou[1,2,3,*]

[1]Beijing Life Science Academy, Yingcai South 1st Street, Beijing 102209, China,
[2]China National Tobacco Quality Supervision and Test Center, Zhengzhou 450001, China,
[3]Key Laboratory of Tobacco Biological Effects, Zhengzhou 450001, China
*Corresponding author: China National Tobacco Quality Supervision and Test Center, No. 6, Cuizhu Street, New & High-tech Industry Development Zone, Zhengzhou 450001, China. Email: qsfctc@163.com, houhw@blsa.com.cn

Background: Heated tobacco product (HTP) considered to be a novel tobacco product which was reported safer than traditional cigarettes evidenced by lower potential harmful components released. Liver is an important detoxification organ of the body, the chemical components in aerosols are metabolized in the liver after absorbed, so it is necessary to explore the effect of HTP on the liver.
Materials and Methods: The potential effect of HTP and cigarette smoke (CS) on SD rats was explored according to OECD 413 subchronic inhalation. The rats were randomly divided into Sham (air), different dosage of HTP groups (HTP_10, 23 and 50 μg nicotine/L aerosol) and Cig_23 (23 μg nicotine/L aerosol) group. After exposure, the clinical pathology, inflammation and oxidative stress were measured.
Results: The clinical pathology results showed that both HTP_50 and Cig_23 led to abnormality of ALT for male rats. CS and HTP exposure reduced the expression of IL-1β, IL-6 and TNF-α and mitochondrial medicated oxidative stress. In addition, the ATP production was reduced in Cig_23 group. Although inflammation and oxidative stress were displayed, no apoptosis were observed by TUNEL assay and these existed obvious pathological changes only in HTP_50 group, while in CS group with equivalent nicotine, hepatocytes swelling were observed in liver.
Conclusion: CS exposure induced liver damage through mitochondrial mediated oxidative stress and inflammation, which was also observed in high concentration of HTP exposure group. For the same equivalent nicotine, HTP may show lower toxic effect on liver than CS.

Key words: heated tobacco product; cigarette smoke; subchronic inhalation; liver toxicity.

Introduction

Cigarette smoke (CS) is one of the main preventable causes of liver diseases and the proportion of people with liver disease associated with CS was approaches 40%. Harmful substances inhaled from CS, such as beta-naphthylamines and polycyclic aromatic hydrocarbons, are mainly metabolized in the liver, and several carcinogens including tobacco-specific nitrosamines (e.g. NNN and NNK) effect P450 enzyme activity in the liver.[1] It is reported CS is not cause directly hepatotoxicity, but a large number of studies suggests that it may exacerbate preexisting chronic liver disease. Studier showed that CS exacerbate alcohol-related liver disease, suggesting that chemicals in CS may accelerate alcohol-related liver damages.[2] Moreover, the exposure of CS showed profibrotic effect by promoting liver inflammation and necrosis,[3,4] which suggested that CS may exert potential harmful effect on liver health.

Heated tobacco product (HTP) is a new category of tobacco product that produces smoke by heating rather than burning.[5] The incomplete combustion of tobacco lead to the production of many harmful and potentially harmful chemicals in CS, while the heating without burning tobacco of HTP lead to significant reduction of potential harmful components in aerosol.[6] Recently, Choukrallah et al. explored the effects of CS and a candidate modified risk tobacco product (Tobacco Heating System 2.2, THS 2.2) aerosol exposure on DNA methylation in liver of apolipoprotein E-deficient mice and showed that DNA methylation in liver was largely insensitive to CS and THS 2.2.[7] Another animal study compared the effects of CS and HTP aerosol exposure and the results showed that CS induced in a higher number of necrotic cells, increased the production of reactive oxygen species and inflammatory cytokines than that of HTP aerosol exposure,[8,9] which indicated that HTP aerosol may exert lower risk than CS. Now the U.S. Food and Drug Administration had listed HTP as a modified risk tobacco product and clearly required that permission of modified risk tobacco products such as HTP must provide scientific evidence to demonstrate that the product will or is expected to benefit the health of the population as a whole. However, up to now there are few studies on its potential health risks, especially on the liver.

The liver is an important detoxification organ of the human body. During the use of tobacco product, the aerosol can enter the blood directly through inhalation and then were metabolized in the liver. It is reported that CS exacerbated oxidative damage through oxidative stress and caused production of inflammatory cytokines, and leads to the accumulation of cellular damages and apoptosis in the liver.[10] CS exposure induced the redox imbalance evidenced by up-regulation of malondialdehyde (MDA) and decrease of superoxide dismutase (SOD) and glutathione (GSH).[11] In addition, after exposure with CS, the liver was showed with the recruitment of inflammatory cells such as neutrophils and monocytes and subsequent increase of interleukin (IL-1), IL-6 and tumor necrosis factor (TNF-α), which would lead to

Received: July 24, 2024. **Revised:** December 13, 2023. **Accepted:** January 3, 2023

smoking-induced fibrosis.[12] Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are important indicators of liver function, and when the mice were exposed with CS, the content of ALT and AST in blood were increased significantly,[4,13] which indicated that oxidative stress and inflammation are important indicators of liver injury.

Given the importance of the liver as an organ of metabolism, the 90-day inhalation toxicological evaluation experiment were preformed according to OECD TG 413 and the potential toxicity of HTP on liver were explored.[14] In our study, HTP and CS were used for aerosol inhalation exposure, and the liver enzyme activity, inflammatory factors, biomarkers of oxidative stress and mitochondrial function were observed, which will reveal multiple toxicant exposure profiles on the liver. This study will provide a scientific evaluation of the HTP and the toxicological data will further support the health risks of tobacco products.

Materials and methods

Animals

Sprague Dawley (SD) rats of 7–8 weeks old were housed under controlled conditions (12 h light/dark, 23 °C ± 2 °C, 50% ± 10% humidity), with food and water provided ad libitum. The study was approved by the Laboratory Animal Management and Ethics Committee of China Tobacco Quality Supervision and Test Center (Approval number: CTQTC-SYXK-2021003). Rats were exposure with fresh air (Sham, Sham PLUS groups), HTP aerosols (HTP_10, HTP_23, HTP_50, HTP_50 PLUS groups, HTP groups of aerosol dosages with nominal nicotine concentration of 10, 23, and 50 μg nicotine/L aerosol for each group) and CS (Cig_23, Cig_23 PLUS, 23 μg nicotine/L smoke). All the groups contained twenty rats, half male and female. According to the OECD 413, the PLUS groups were employed to observe the toxic effects in the recovery period (28-day).

Aerosol/smoke generation and exposure

CS and HTP aerosols were produced with the HCI puff regime using a rotary smoking machine (Beijing HuiRongHe Technology, China) and a high-through put smoking machine (Qingdao Etsong Bioengineering, China), respectively. The puffing parameters were 55 ± 0.5 mL puff volume, 2 ± 0.5 s puff duration, and one puff every 30 ± 0.5 s. The aerosol produced was diluted with filtered and conditioned air to obtain the target nicotine exposure concentrations. The rats inhaled CS and HTP aerosols through the nose-only exposure system (Beijing HuiRongHe Technology, China) for 90 days (6 h/day, 5 days a week).

Biochemical measurements

After the exposure, the rats (except the recovery period) were anesthetized and blood were collected and centrifuged to obtain serum, which was tested for clinical chemistry biomarkers by using an automatic biochemical analyzer BS-830 (Mindray Bio-Medical Electronics, China), including AST, ALT and total protein (TP).

Detection of antioxidant and oxidative stress biomarkers

Tissue homogenate samples were prepared with normal saline using a tissue homogenizer and the supernatant was collected for BCA protein quantitative analysis (Beyotime Biotechnology, China). Then the liver homogenates were measured for interleukins (IL-1β, IL-6, and TNF-α by an enzyme-linked immunosorbent assay (Shanghai Enzyme-linked Biotechnology, China) according to the manufacturer's instructions.

Immunohistochemistry

Prepared liver paraffinized sections were incubated with primary antibodies (IL-1β, IL-6 and TNF-α, Proteintech, USA), secondary antibodies (Abcam, UK) and 3,3′-diaminobenzidine (DAB) chromogenic solution and sections were sealed with neutral resin (Solarbio, China) and observed under a microscope (COIC, China). Finally, IPP (Media Cybernetics, USA) software was used for image analysis.

Terminal Deoxynucleotidyl transferase mediated dUTP nick-end labeling (TUNEL) assay

TUNEL staining of apoptotic nuclei was performed using an in-situ cell apoptosis detection kit (Roche, Switzerland) according to the manufacturer's instructions. Briefly, paraffin sections were deparaffinized, rehydrated, and incubated with proteinase K for 25 min at 37 °C. After incubated with DAB and re-stained with hematoxylin, and the positive apoptotic cell nuclei were observed with an optical microscope.

Mitochondrial measurement

Fresh liver tissue was cut into tissue blocks less than 1 mm^3, and quickly placed in 2.5% glutaraldehyde solution, fixed for 6 h followed washing with PBS for three times. The samples were then fixed in 1% osmic acid for 1.5 h and rinsed with PBS for three times. After dehydrated, embedded, sliced, and stained, the liver tissue was observed using microscopy, and the data were analyzed by ImageJ software.

ATP detection

About 20 mg tissue were lysed with pyrolysis liquid, and then homogenized to obtain the supernatant after centrifuged at 4 °C 12,000 g for 5 min. ATP detection kit (Beyotime Biotechnology, China) was used according to the instructions and the samples were measured by a multifunctional microplate reader.

Determination of ROS

The fresh liver was obtained and embedded with OTC to prepare tissue slices. Then ROS staining (Dihydroethidium, DHE; sigma, D7008) solution was added to the slices, and incubated at 37 °C in dark for 30 min. The slices were washed with PBS and then incubated with DAPI staining solution at room temperature for 10 min. At last, anti-fluorescent quenching agent were added and the slices were observed with the fluorescence microscope. The statistical analysis of fluorescence intensity was performed with of ImageJ software.

Liver histopathology

After fixed with 4% neutral formalin (Solarbio, China), the liver was dehydrated by gradient, embedded in paraffin for sections. Sections were then stained with hematoxylin and eosin (H&E) dye or the Masson's trichrome reagent following standard procedures. Pathological changes of rat liver were observed with optical microscope.

Statistical analysis

The data are expressed as means ± SD (standard deviation). Significance of the differences was statistically analyzed using Student's t-test with the SPSS 24.0 software (IBM, USA) and $P < 0.05$ was considered to be statistically significant.

Table 1. Serum biochemical parameters of rats after 90-day inhalation.

Parameter	Sham (air)	HTP _10	HTP_23	HTP_50	Cig_23
Female					
ALT (U/L)	43.03 ± 8.05	51.79 ± 10.57	47.45 ± 8.59	46.61 ± 10.28	36.25 ± 5.02
AST (U/L)	104.92 ± 43.04	102.06 ± 33.44	126.98 ± 41.45	112.64 ± 25.56	111.29 ± 39.96
TP (g/L)	65.36 ± 5.15	61.58 ± 5.59	62.28 ± 7.00	61.48 ± 3.32	69.36 ± 1.65*
Male					
ALT (U/L)	65.48 ± 19.27	53.58 ± 17.55	77.10 ± 7.93*	86.33 ± 8.43*	82.78 ± 6.34*
AST (U/L)	141.05 ± 36.90	116.84 ± 26.68	108.38 ± 30.15	133.32 ± 33.18	80.75 ± 16.68*
TP (g/L)	54.45 ± 4.32	57.72 ± 2.59	57.30 ± 3.35	55.17 ± 2.45	52.48 ± 4.54

All data were represented as the mean ± SD (N = 10), *P < 0.05, compared with Sham group. ALT, alanine aminotransferase; AST, aspartate aminotransferase; TP, total protein.

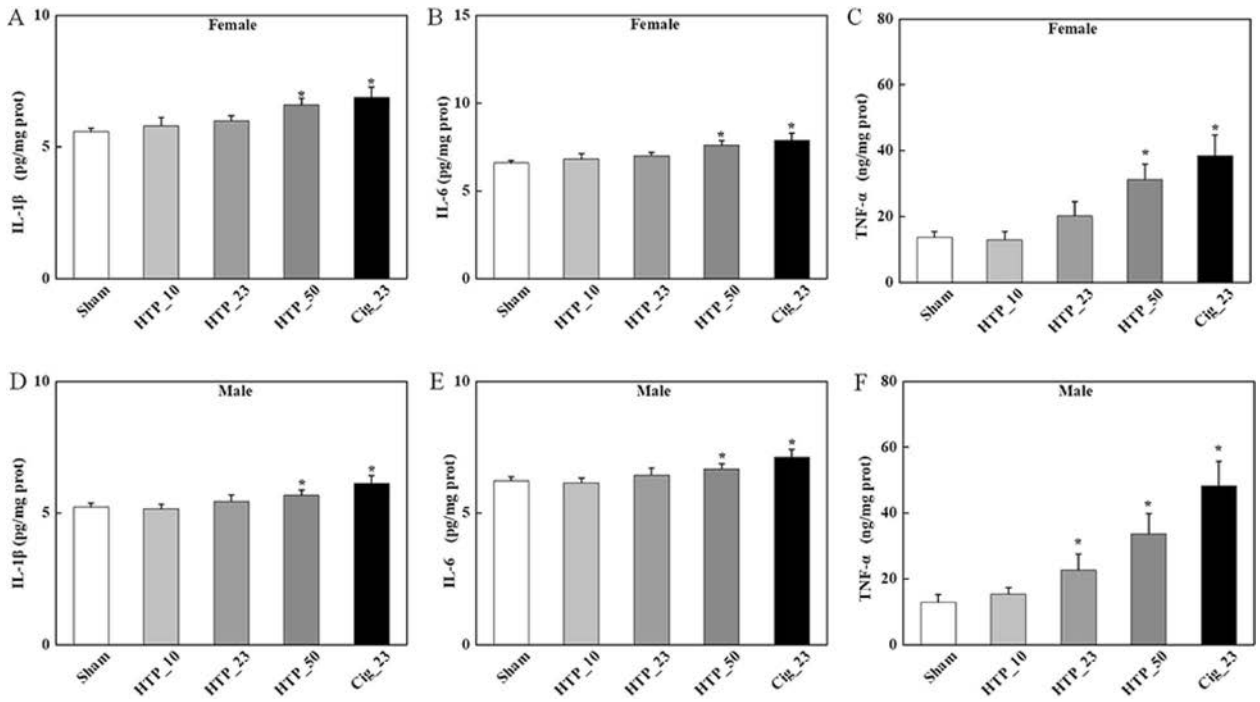

Fig. 1. The expression of inflammatory cytokines in liver. A) IL-1β in female rats. B) IL-6 in female rats. C) TNF-α in female rats. D) IL-1β in male rats. E) IL-6 in male rats. F) TNF-α in male rats. All data were represented as the mean ± SD (N = 5), *P < 0.05 compared with the sham group.

Results

Hepatic function markers

The content of biomarkers in the blood were detected such as ALT, AST, and TP following 90-day inhalation exposure. As were shown in Table 1, the content of TP in female rats and ALT in male rats increased significantly in Cig_23 group, while HTP exposure induced higher ALT in blood for female rats in the Cig_23 and Cig_50 groups compared with Sham group, which suggested HTP aerosols and CS showed potential toxic effect on liver function.

The expression of inflammatory cytokines

Inflammatory markers IL-1β, IL-6 and TNF-α were measured to assess the potential inflammation in liver. The results were shown in Fig. 1, the levels of proinflammatory cytokines including IL-1β, IL-6 and TNF-α in liver of the Cig_23 group were obviously changed than those of the Sham group ($P < 0.05$). For HTP groups, after exposure with high dose of aerosols, the inflammation was exacerbated significantly evidenced by the increase of IL-1β, IL-6 and TNF-α ($P < 0.05$). Under equal nicotine dosage levels (the HTP_23 vs Cig_23), the inflammation induced by HTP were significantly lower than the CS.[15]

Immunohistochemical markers

Immunohistochemical microscopic images of the livers from exposed male and female rats were shown in Figs 2 and 3. The expressions of IL-1β, IL-6 and TNF-α were showed very similar between the Sham and HTP_10 group. The number of IL-1β immune-positive cells in the HTP_23/50 groups and Cig_23 group were significantly greater than the Sham group. Compared with the Sham group, the expression of pro-inflammatory cytokines including IL-1β and TNF-α were significantly observed in the hepatocytes of the HTP_23 and HTP_50 groups, while the expression of IL-6 displayed by the brown marker in HTP_23 group was lower the expression of IL-1β and TNF-α. The TNF-α immunoreactivity in the HTP_10 group was not significantly different from that in the Sham group, while the numbers of positive cells in the HTP_50 and Cig_23 groups increased significantly. In total, the medium and high dose exposure to HTP aerosol caused inflammatory response was similar to CS.

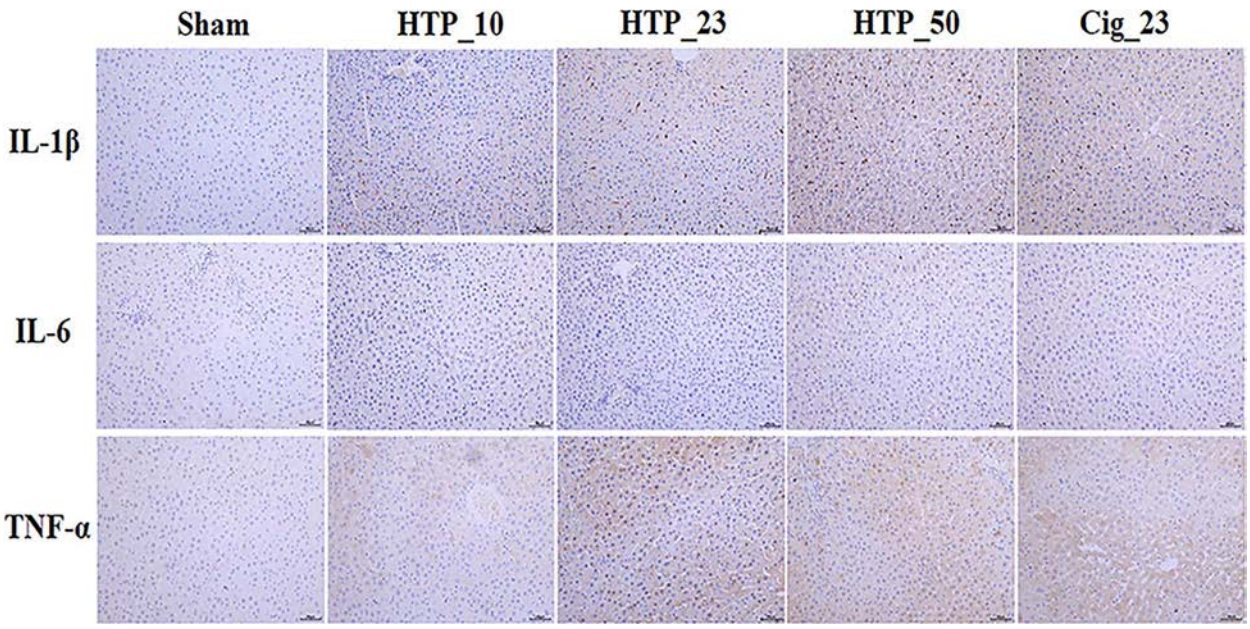

Fig. 2. Expression of IL-1β, IL-6 and TNF-α by immunohistochemistry in liver of female rats. All slides were showed at a final magnification of ×200.

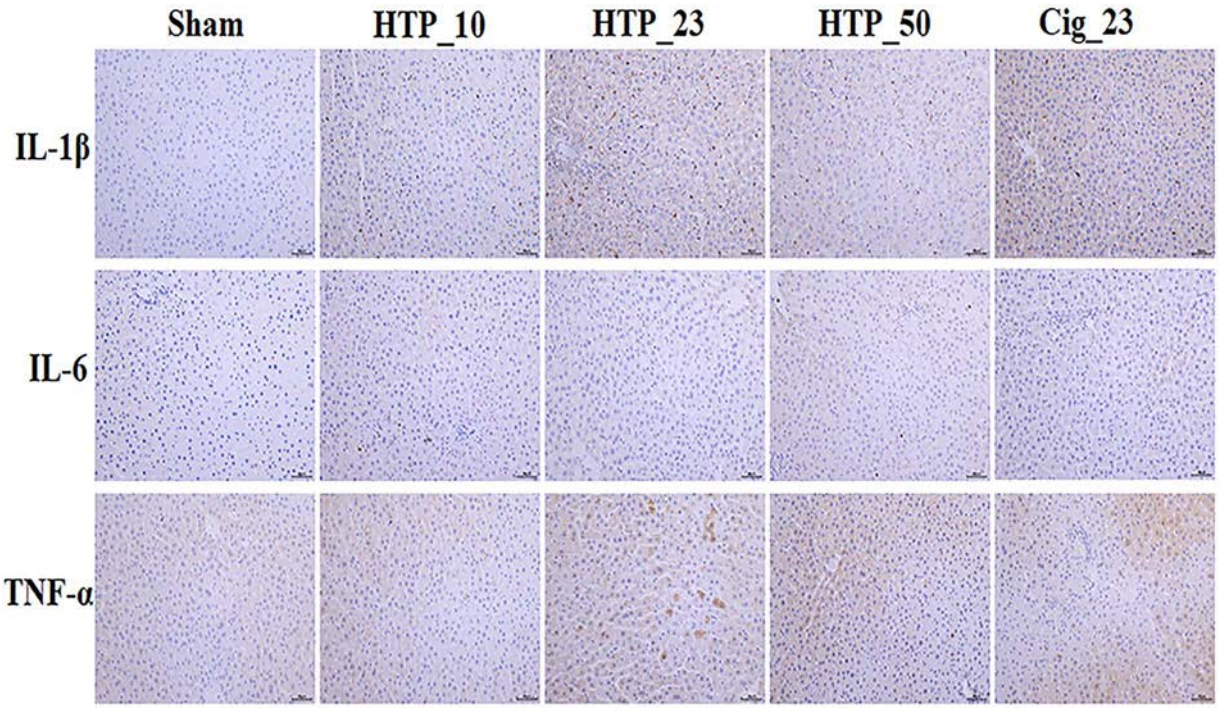

Fig. 3. Expression of IL-1β, IL-6 and TNF-α by immunohistochemistry in liver of male rats. All slides were showed at a final magnification of ×200.

Effects of HTP and CS on liver mitochondrial, ATP and ROS production

The effects of different doses of HTP and CS exposure on liver mitochondrial, ATP and ROS in SD rats are shown in Figs 4–6. The results showed that compared with the Sham group, the mitochondrial of the Cig_23 group was significantly reduced ($P < 0.05$), and when exposure with the same concentration of nicotine with HTP (HTP_23 group), no significant changes were observed. Mitochondria serve as the main organelles of ATP production, which is associated with the ATP production. In our study, Fig. 4 showed that both CS and HTP exerted certain effect on ATP production, and the effect of CS reduced obviously the content of ATP, which was much lower than that of HTP group. When mitochondrial function is impaired, a decrease in ATP is accompanied by an increase in ROS production, which was displayed in Figs 5 and 6. Both CS and HTP_50 group induced ROS production, which indicated that high dose of HTP and CS exposure could induce mitochondrial mediated ATP and ROS damage.

Antioxidant defense and oxidative stress biomarkers

CS exposure can cause lipid peroxidation damage. In our study, the imbalance of oxidative and redox were measured and the results showed that CS induced the excessive MDA, and the

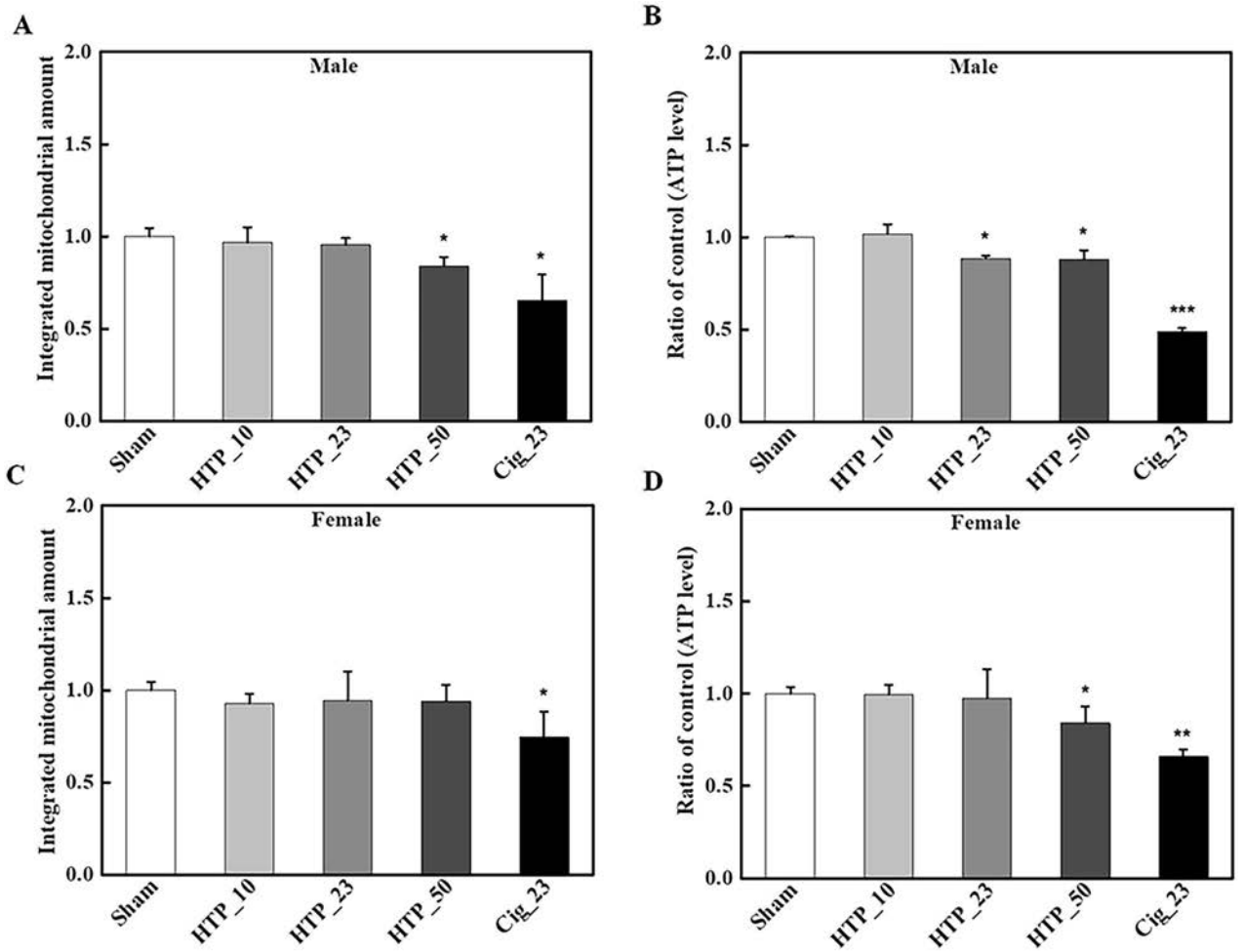

Fig. 4. The mitochondrial and ATP production in liver. A) The mitochondrial for male rats. B) The ATP content for male rats. C) The mitochondrial for female rats. D) The ATP content for female rats. All data were represented as the mean ± SD (N = 3), *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, compared with the sham group.

decrease of SOD and GSH, which were consisted with the previous study.[16] Compared with the Sham group, the lipid peroxidation of MDA in the HTP_50 group was significantly up-regulated ($P < 0.05$). SOD is a free radical scavenger, and the decrease of SOD content in the body means a decrease in the ability to scavenge harmful free radicals. As shown in Fig. 7, the SOD activities of the HTP groups (except the HTP_10 group) were significantly reduced ($P < 0.05$) and no obvious changes were observed after HTP exposure, which indicated that both HTP and CS could lead to oxidative stress in the liver, and may cause some damaging effects subsequently.

Apoptosis

Compared with the normal liver of rats in the Sham group, no apoptosis of rat hepatocytes was observed in the HTP group (Fig. 8), which indicated that HTP aerosols induced no apoptosis in the liver. Moreover, in the CS_23 group, the sporadic brown markers were observed and the results showed that CS exposure led to slight apoptosis by oxidative stress and inflammation. Although oxidative stress and inflammation were also observed in HTP groups, there existed no apoptosis.

Histopathological analysis

The pathological of the liver were shown in Fig. 9. For female rats, pathological of the liver in CS group induced swelling of hepatocyte when compared to the Sham group. Lesser vacuolar degeneration was also observed in the HTP_10 group. For male rats, liver pathological analysis of the HTP_50 revealed the same hepatocyte swelling as with the CS group (Fig. 9A), which suggested that the CS and high dose of HTP may induce the liver damage. In addition, liver fibrosis was also detected by Masson staining. The results showed in Fig. 9B, which indicated that neither CS nor HTP had any effect on liver fibrosis.

Discussion

Tobacco products in the process of use mainly rely on the generation of aerosols, which contain different types of chemical components. After aerosols is absorbed by the human, it is mainly detoxicated through liver metabolism. Therefore, understanding the mechanisms and pathophysiological processes of liver diseases is critical in metabolism of tobacco product and it is very necessary to the tobacco products that claimed reduced exposure or risk to perform their inhalation toxicity burden on the liver. In this study, the serum levels of ALT and AST (just for male rats) were higher in the subchronic smoked or aerosol-inhaled animals compared to the Sham group (Table 1). Clinical research results showed that increased activities of the liver enzymes are typically found in healthy patients following cigarette smoking,[17] which demonstrated that these markers could be used to assess the acute to

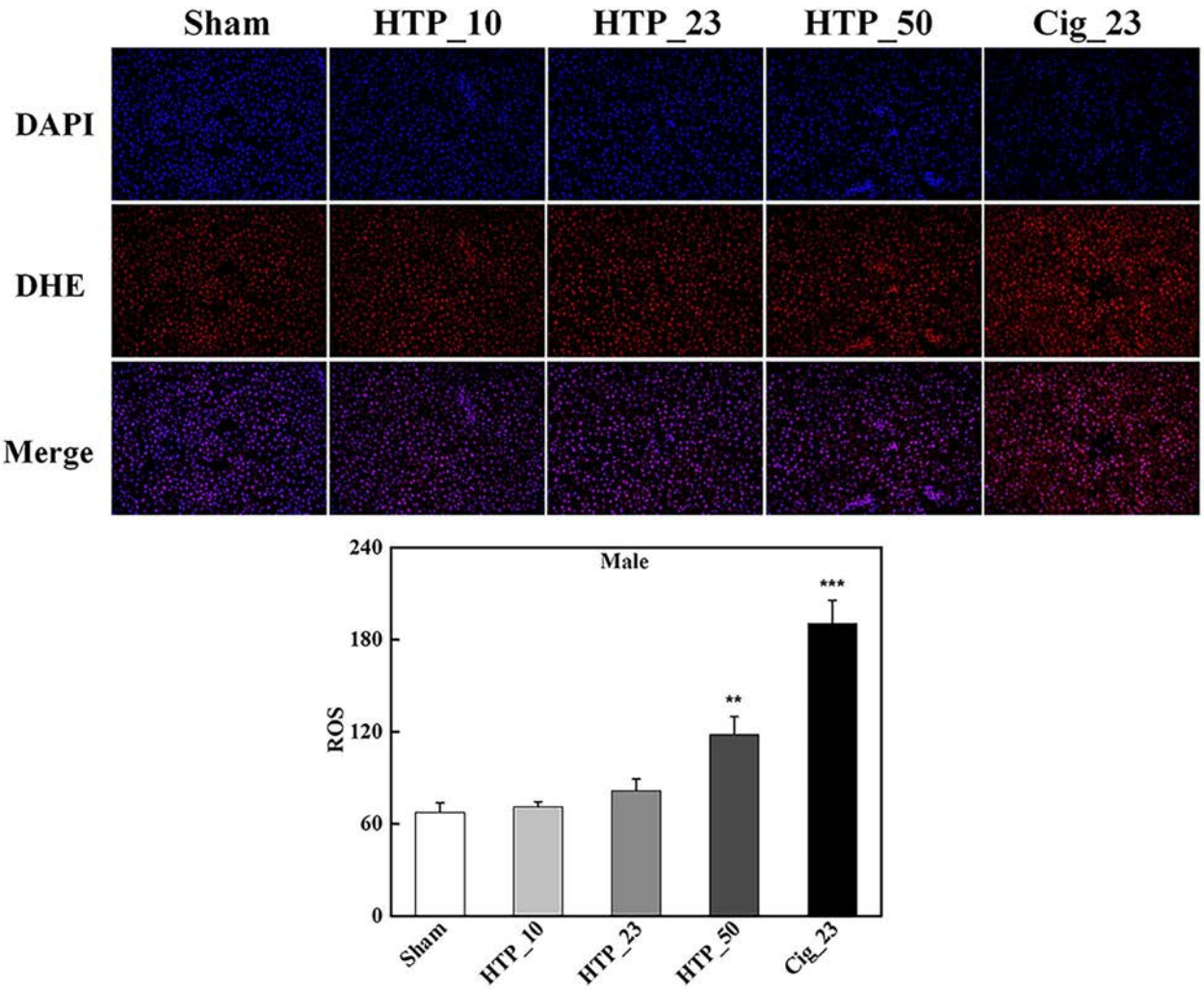

Fig. 5. The expression of ROS in liver for male rats. The nucleus was stained with DAPI and ROS were stained with dihydroethidium (DHE). All data were represented as the mean ± SD (N = 3), $^{**}P < 0.01$, $^{***}P < 0.001$, compared with the sham group.

subchronic effects of tobacco products. For the animals subjected to subchronic exposure protocol, their biochemical markers were improved after a 28-day recovery period. However, for chronical or habitual tobacco users in the previous studies, this may not be the case.

Long-term exposure to CS causes hepatic inflammation, and even irreversible apoptosis and tissue damages.[18,19] From a subchronic level, this study therefore evaluated the antioxidant defense and oxidative stress biomarkers as an early indication of potential differences and/or similarities between the HTP aerosol and CS exposures. The results showed that CS (Cig_23 group) significantly reduced SOD and GSH activities, while MDA significantly increased (Fig. 7). We observed a clear redox imbalance at the tissue level induced by the CS exposure, in agreement with previous studies.[10] The extensive influx of inflammatory cells in the Cig_23 group released ROS, which led to the activation of macrophages, the release of pro-inflammatory cytokines, and the significant formation of inflammatory cells. Further, our results indicated that pro-inflammatory cytokines (IL-1β, IL-6 and TNF-α) were significantly up-regulated in both the Cig_23 and HTP_50 groups ($P < 0.05$), perhaps in response to the combined effects of increased nicotine and other tobacco toxicants released in the HTP aerosol (Fig. 1).

The expression of inflammatory cytokines performed in this work showed that compared with the sham group, the number of immunopositivity cell for the inflammatory cytokines in the HTP_50 and the Cig_23 groups was significantly increased (Figs 2 and 3). In addition, the redox imbalance was also observed by CS exposure, leading to the accumulation of macromolecules in damaged cells (Fig. 8). In liver, a nicotine-related increase in glycogen vacuoles was detected, but there were no signs of fibrosis or lipid accumulation.[19] In our study, no fibrotic features were observed in the liver. After exposure with high concentration of tobacco extract, the blood analysis of clinical chemistry showed that increased hepatic function indexes (AST, ALT) in male and female animals were observed, as well as inflammation and damaged liver cells.[20]

By analyzing the content of reactive oxygen species (Figs 5 and 6), mitochondrial and ATP production (Fig. 4) in liver cells, we found that both CS and HTP could cause oxidative stress in liver cells, and the effect of CS was more obvious. Mitochondria, one of the main sources of ROS generation, is influenced by ROS evidenced by the decreased amount. Moreover, impaired mitochondrial function is closely related to ATP production. Therefore, a significant decrease in mitochondrial and ATP content were showed in the CS group compared with the Sham group, and the similar phenomenon occurred in the HTP high-dose group.

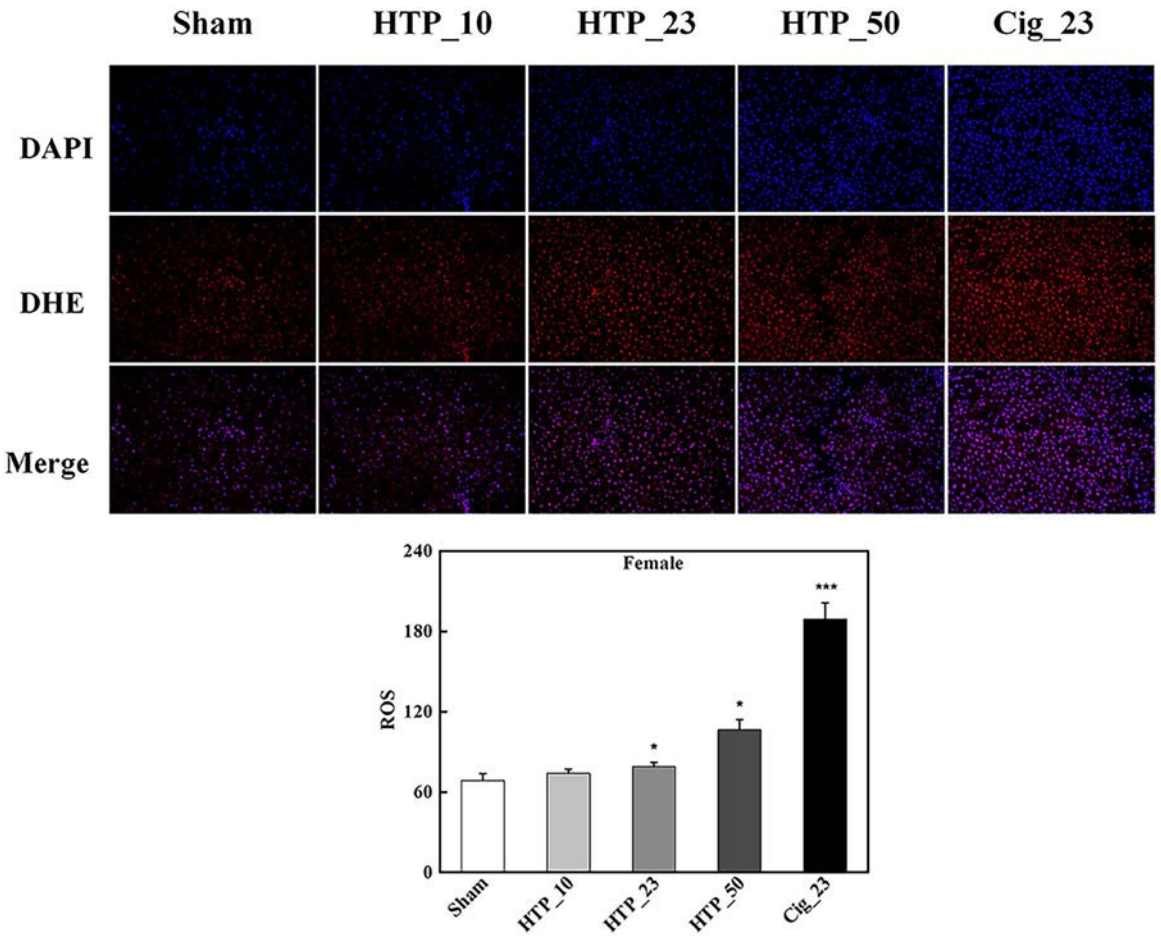

Fig. 6. The expression of ROS in liver for female rats. The nucleus was stained with DAPI and the reactive oxygen species (ROS) were stained by dihydroethidium (DHE). All data were represented as the mean ± SD (N = 3), *P < 0.05, ***P < 0.001, compared with the sham group.

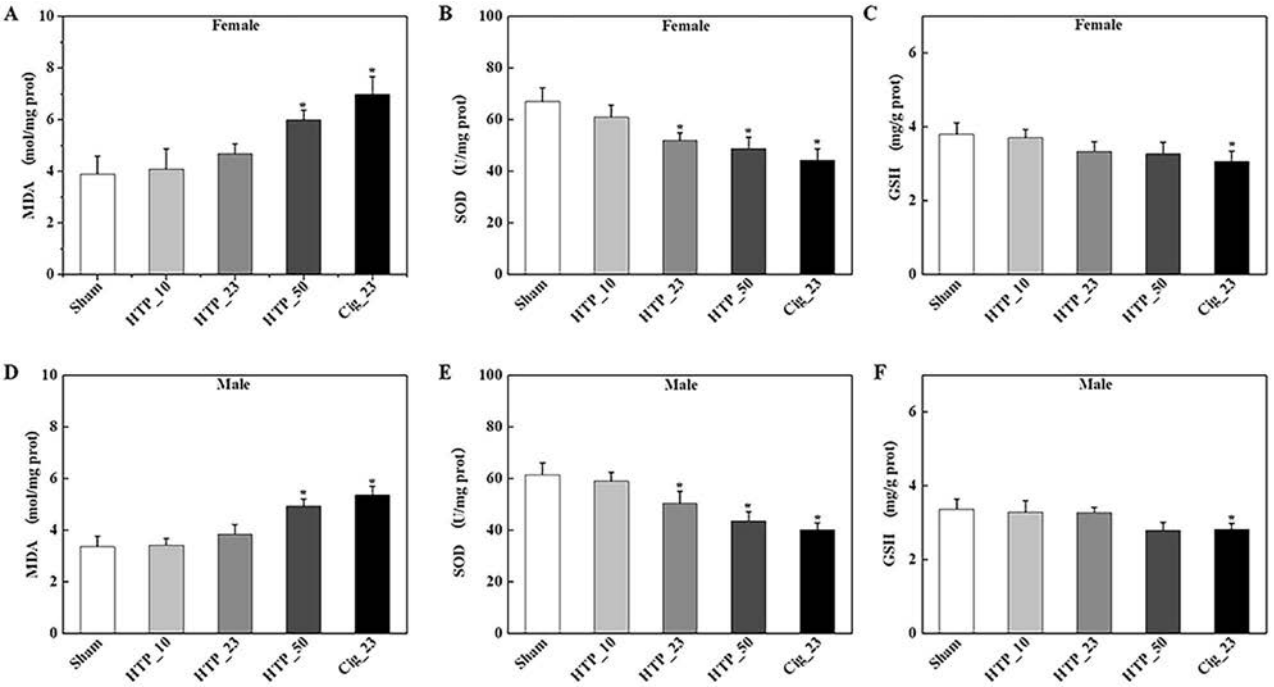

Fig. 7. Antioxidant defense and oxidative stress biomarkers in liver. A) The MDA content in female rats. B) The activity of SOD in female rats. C) The GSH in female rats. D) The MDA content in male rats. E) The activity of SOD in male rats. F) The GSH in male rat. All data were represented as the mean ± SD (N = 5), *P < 0.05, compared with the sham group.

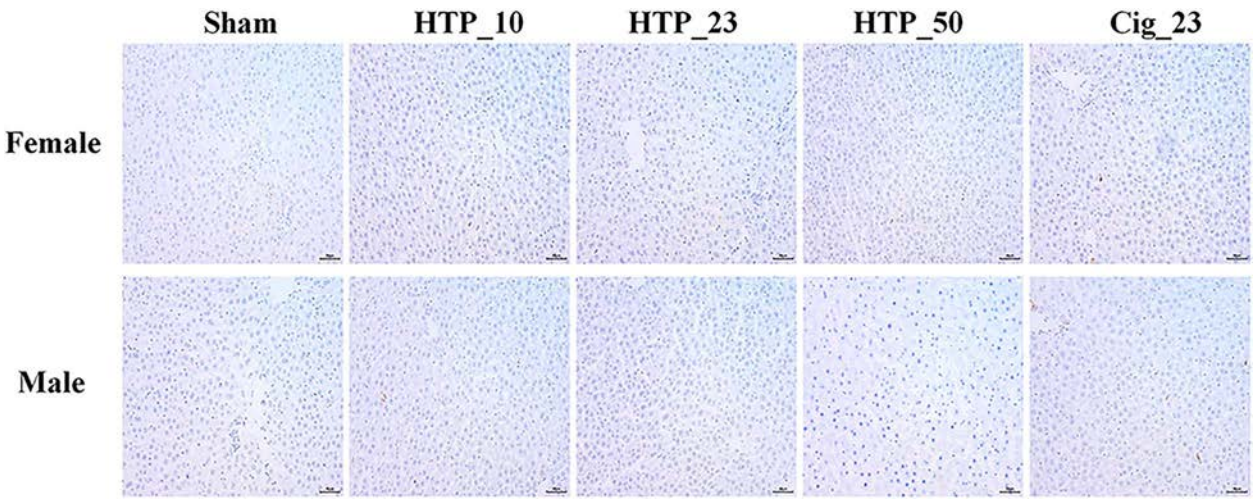

Fig. 8. The liver apoptosis of rats by TUNEL assay. All slides were shown at a final magnification of ×200.

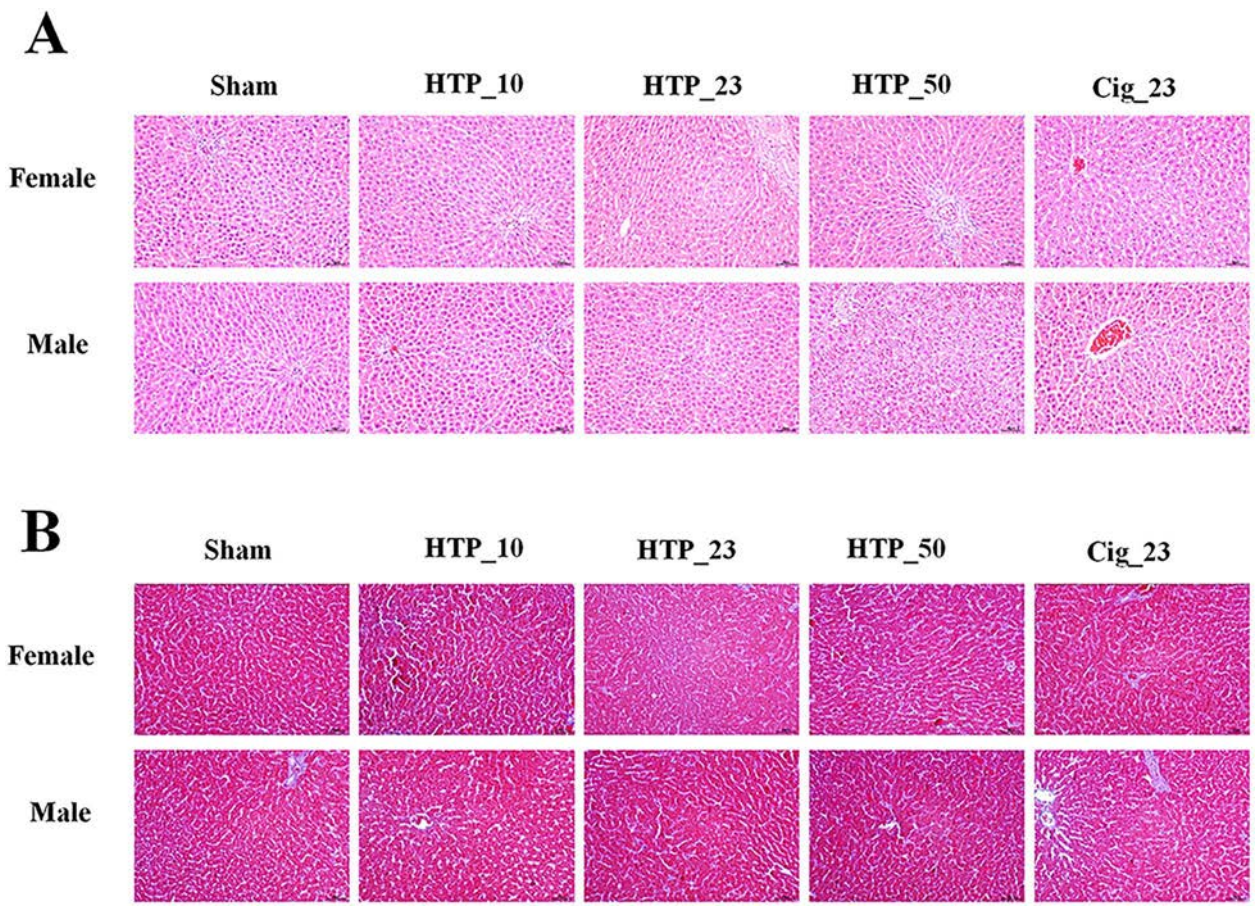

Fig. 9. Morphological changes of liver. A) H&E staining for SD rats (N = 10). B) Masson staining for SD rats (N = 10). All slides were shown at a final magnification of ×200.

During the inflammation and oxidative stress mediated by mitochondrial, apoptosis was observed in CS induced rats, while no significant apoptosis was observed in HTP group. At the same time, liver cell damage was found by pathological analysis in HTP_50 and CS groups. At equivalent nicotine concentrations, the HTP aerosols with lower dosage was unable to induce hepatocyte injury, such as hyperemia, although associated with obvious inflammation and oxidative stress. Previous studies have shown that increased collagen fiber deposition may induce CS-related liver fibrosis in animal models[21] and it has been reported that signs of sinusoidal dilation, vasodilation, areas of necrosis, and connective tissue formation have been discovered in smoking induced liver tissue damages, together with elevated ROS and pro-inflammatory effects, which subsequently lead to fibrogenesis.[22,23] This phenomenon was not observed in this study. We speculated that the exposure period may not have been long enough, and even though there were some changes in molecular after exposure, some of the changes did not result in liver tissue lesions.

Conclusion

This study investigated the potential toxicological effect of HTP on liver following the OECD 90-day subchronic inhalation exposure with fresh air, different concentrations of HTP aerosols and CS. In our study, the HTP aerosol and CS exposure both negatively induced liver inflammation and the imbalance of redox status. The liver enzyme activity, together with the histopathology associated with the liver function were also induced by CS, and HTP showed a mild effect, namely, the liver toxicity in the Cig_23 group was significantly higher than that in the HTP_23 group. On the other hand, with the decrease of aerosol exposure concentration, most of the biomarkers displayed a trend of reducing or disappeared toxicity. Meanwhile, Both CS and HTP can lead to slight changes and oxidative stress at the molecular level mediated by mitochondrial. In conclusion, the 90-day subchronic exposure in this study demonstrated that the HTP aerosol caused an observable degree of inflammation, oxidative stress, and limited levels of tissue damages compared with CS. Under equal nicotine dosage, the HTP aerosol generally displayed a reduced level of toxic effects on liver.

Author contributions

Yushan Tian (Conceptualization, Methodology, Project administration, Investigation, Writing-Review & Editing) Hongjuan Wang (Conceptualization, Methodology, Project administration, Investigation, Writing—Original Draft), Shulei Han (Methodology, Data Curation, Project administration), Yaning Fu (Investigation, Data Curation, Writing-Original Draft, Writing—Review & Editing), Fengjun Lu (Investigation, Data Curation, Writing-Original Draft, Writing—Review & Editing), Xianmei Li (Investigation, Data Curation, Writing-Original Draft), Shuhao Ma (Investigation, Data Curation, Writing-Original Draft), Wengming Wang (Investigation, Data Curation, Writing—Original Draft), Pengxia Feng (Investigation, Data Curation, Writing—Original Draft), Zhihao Shi (Investigation, Data Curation, Writing—Original Draft), Huan Chen (Methodology, Project administration, Writing-Review & Editing, Funding acquisition), and Hongwei Hou (Methodology, Project administration, Writing-Review & Editing, Funding acquisition).

Funding

This work was supported by the Provincial and Ministerial Major Project of China (grant numbers: 2019420000340623, 110202001006 (XX-02)).

Conflict of interest statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

1. Barsouk A, Thandra KC, Saginala K, Rawla P, Barsouk A. Chemical risk factors of primary liver cancer: an update. *Hepat Med*. 2021:**12**:179–188.
2. Marti-Aguado D, Clemente-Sanchez A, Bataller R. Cigarette smoking and liver diseases. *J Hepatol*. 2022:**77**(1):191–205.
3. Ampuero J, Aller R, Gallego-Durán R, Crespo J, Calleja JL, García-Monzón C, Gómez-Camarero J, Caballería J, Lo Iacono O, Ibañez L, et al. Significant fibrosis predicts new-onset diabetes mellitus and arterial hypertension in patients with NASH. *J Hepatol*. 2020:**73**(1):17–25.
4. Wang YH, Chuang YH, Wu CF, Jan MC, Wu WJ, Lin CL, Liu CJ, Yang YC, Chen PJ, Lin SM, et al. Smoking and hepatitis B virus-related hepatocellular carcinoma risk: the mediating roles of viral load and alanine aminotransferase. *Hepatology*. 2019:**69**(4):1412–1425.
5. Znyk M, Jurewicz J, Kaleta D. Exposure to heated tobacco products and adverse health effects, a systematic review. *Int J Environ Res Public Health*. 2021:**18**(12):6651.
6. Auer R, Concha-Lozano N, Jacot-Sadowski I, Cornuz J, Berthet A. Heat-not-burn tobacco cigarettes: smoke by any other name. *JAMA Intern Med*. 2017:**177**(7):1050–1052.
7. Choukrallah MA, Sierro N, Martin F, Baumer K, Thomas J, Ouadi S, Hoeng J, Peitsch MC, Ivanov NV. Tobacco heating system 2.2 has a limited impact on DNA methylation of candidate enhancers in mouse lung compared with cigarette smoke. *Food Chem Toxicol*. 2019:**123**:501–510.
8. Scharf P, da Rocha GHO, Sandri S, Heluany CS, Pedreira Filho WR, Farsky SHP. Immunotoxic mechanisms of cigarette smoke and heat-not-burn tobacco vapor on Jurkat T cell functions. *Environ Pollut*. 2021:**268**(Pt B):115863.
9. Lo Sasso G, Titz B, Nury C, Boué S, Phillips B, Belcastro V, Schneider T, Dijon S, Baumer K, Peric D, et al. Effects of cigarette smoke, cessation and switching to a candidate modified risk tobacco product on the liver inApoe−/−mice—a systems toxicology analysis. *Inhal Toxicol*. 2016:**28**(5):226–240.
10. Rocha DFA, Machado-Junior PA, Souza ABF, Castro TF, Costa GP, Talvani A, Bezerra FS, Cangussú SD. Lycopene ameliorates liver inflammation and redox status in mice exposed to long-term cigarette smoke. *Biomed Res Int*. 2021:**2021**:7101313.
11. Huang XX, Xu S, Yu LJ, Zhou YF, Zhou Y, Liu ZH. Eurotium cristatum fermented loose dark tea ameliorates cigarette smoke-induced lung injury by MAPK pathway and enhances hepatic metabolic detoxification by PXR/AhR pathway in mice. *Oxidative Med Cell Longev*. 2021:**2021**:6635080.
12. Yang SR, Chida AS, Bauter MR, Shafiq N, Seweryniak K, Maggirwar SB, Kilty I, Rahman I. Cigarette smoke induces proinflammatory cytokine release by activation of NF-kappaB and posttranslational modifications of histone deacetylase in macrophages. *Am J Physiol Lung Cell Mol Physiol*. 2006:**291**(1):L46–L57.
13. Doherty Lyons S, Blum JL, Hoffman-Budde C, Tijerina PB, Fiel MI, J. Conklin DJ, Gany F, Odin JA, Zelikoff JT. Prenatal exposure to Gutkha, a globally relevant smokeless tobacco product, induces hepatic changes in adult mice. *Int J Environ Res Public Health*. 2020:**17**(21):7895.
14. OECD. Test No. 413: Subchronic inhalation toxicity: 90-day study, OECD guidelines for the testing of chemicals, Section 4, OECD Publishing, Paris. 2018. https://doi.org/10.1787/9789264070806-en.
15. Jain D, Chaudhary P, Varshney N, Bin Razzak KS, Verma D, Khan Zahra TR, Janmeda P, Sharifi-Rad J, Daştan SD, Mahmud S, et al. Tobacco smoking and liver cancer risk: potential avenues for carcinogenesis. *J Oncol*. 2021:**2021**:5905357.
16. Kuipers I, Bracke KR, Brusselle GG, Aesif SW, Krijgsman R, Arts IC, Wouters EF, Reynaert NL. Altered cigarette smoke-induced lung inflammation due to ablation of Grx1. *PLoS One*. 2012:**7**(6):e38984.
17. Alsalhen KS, Abdalsalam RD. Effect of cigarette smoking on liver functions: a comparative study conducted among smokers and non-smokers male in El-beida city, Libya. *Int Curr Pharm J*. 2014:**3**(7):291–295.

18. Kostelli G, Kourea K, Ikonomidis I. Effects of combustible tobacco smoking and novel tobacco products on oxidative stress: different sides of the same coin? *Curr Opin Toxicol*. 2020:**20-21**: 41–47.
19. Phillips B, Esposito M, Verbeeck J, Boué S, Iskandar A, Vuillaume G, Leroy P, Krishnan S, Kogel U, Utan A, et al. Toxicity of aerosols of nicotine and pyruvic acid (separate and combined) in Sprague-Dawley rats in a 28-day OECD 412 inhalation study and assessment of systems toxicology. *Inhal Toxicol*. 2015:**27**(9):405–431.
20. Werley MS, Jerome AM, Oldham MJ. Toxicological evaluation of aerosols of a tobacco extract formulation and nicotine formulation in acute and short-term inhalation studies. *Inhal Toxicol*. 2014:**26**(4):207–221.
21. Kim JW, Zhou Z, Yun H, Park S, Choi SJ, Lee SH, Lim CW, Lee K, Kim B. Cigarette smoking differentially regulates inflammatory responses in a mouse model of nonalcoholic steatohepatitis depending on exposure time point. *Food Chem Toxicol*. 2020:**135**:110930.
22. Gumral N, Aslankoc R, Senol N, Cankara FN. Protective effect of alpha-lipoic acid against liver damage induced by cigarette smoke: an in vivo study. *Saudi J Med Med Sci*. 2021:**9**(2):145–151.
23. Roehlen N, Crouchet E, Baumert TF. Liver fibrosis: mechanistic concepts and therapeutic perspectives. *Cells*. 2020:**9**(4):875.