International Journal of Scientific Trends- (IJST)
ISSN: 2980-4299
Volume 2, Issue 5, May, 2023
Website: https://scientifictrends.org/index.php/ijst
Open Access, Peer Reviewed, Scientific Journal

Biochemical and Histopathological Study of the Toxic Effects of Electronic and Conventional Cigarette Smoke on the Liver of Rats

Hakim bahlok jebur1,
Raghad Jawad Salman2
Khalid Alyodawi3
1Department of Pharmacology, College of Medicine,Wasit University ,Iraq
2Department of Anatomy and Medical Biology, College of Medicine Wasit, University
3Department of Anatomy and Medical Biology, Wasit University
*Corresponding author: hjabr@uowasit.edu.iq

Abstract:
The present study was conducted to investigate the biochemical and histopathological changes in rats' liver induced by the toxic effects of exposure to electronic vapour and conventional cigarette smoke. The experimental work was performed using 25 rats; 10 rats were exposed to electronic cigarette vapour (ECV), 10 rats were subjected to conventional cigarette smoke (CCS), and 5 rats were recruited as a control group with no treatment. One hour daily of exposure to the ECV and CCS was followed for 40 days. The ECV and CCS were delivered to the animals via the use of a smoke chamber and were locally modified for this study. When the endpoint of the experiment was reached, the blood and liver specimens were collected from all animals for examining biochemical and histological changes. The findings of the work highlighted that significant ($p<0.05$) reductions occurred in the serum amounts of albumin and cholesterol of the ECV and CCS groups when both were compared separately to the data of the control group. Moreover, serum amounts of lactate dehydrogenase (LDH) were increased significantly ($p<0.05$) in the ECV and CCS groups when both were compared separately to the control group. Furthermore, significant ($p<0.05$) decreases were seen in the serum amounts of the alkaline phosphatase (ALP) and aspartate transaminase (AST) of the CCS group with no significant ($p>0.05$) alterations in the alanine aminotransferase (ALT). For the histological alterations, both ECV and CCS induced hepatic cell-related hydropic degeneration and intercellular sinusoidal dilation. In detail, the CCS caused some hepatic cell vacuoles, in which blood filled some of these vacuoles, however, no blood was observed in any of these vacuoles induced by the exposure to ECV. In addition, both ECV and CCS caused focalized infiltration of lymphocytes; though, CCS was

identified with a less degree, especially in the portal vein and surrounding regions. Moreover, both ECV and CCS caused congestion in the portal vein region. The present findings highlight the dangerous effects of exposure to electronic and conventional cigarette smoke, affecting both biochemical and histological features in the studied rats.

Keywords: Biochemical effects, conventional smoking, electronic smoking, histological changes.

INTRODUCTION

There does not seem to be any reasonable answer to justify the phenomenon of perpetual smoking. Despite knowing the risks and reporting aversion to smoking, many people continue to smoke (1). One possible explanation for this is that the nicotine in cigarettes leads to cravings, which raises concerns about the dangers of smoking as quitting requires willpower. Although many countries have made progress in reducing smoking rates, it remains a leading cause of preventable disease and death worldwide (2,3).

About six million people worldwide die prematurely each year due to tobacco use. A person is said to have died prematurely from smoking when they fall victim to a smoking-related disease before their natural time of death. Many of these fatalities take place in ex-smokers whose health was already compromised by the habit. Smokers who don't quite have a life expectancy that is on mean ten years shorter than that of nonsmokers, and they develop age-related illnesses about ten years sooner than that of nonsmokers (4). Lung cancer, COPD, and heart disease account for the vast majority of cigarette-related fatalities. One of the major causes of stroke, loss of vision, loss of hearing, backache, and peripheral vascular disease is smoking. Smokers, on the mean, experience more pain and impairment after the time of life of 40 than nonsmokers (5).

Many bodily systems, including the liver, are negatively impacted by smoking despite having no direct relationship to the habit. The liver is an important organ because it is responsible for the bioconversion of various toxic chemicals (6). Tobacco use is linked to several forms of liver cancer and is a cause of other chronic liver diseases. The effectiveness of antiviral therapy may be reduced, and there is evidence from epidemiological research that smoking raises the danger of primary biliary cirrhosis and possibly boosts the threat of liver fibrosis in sick people with persistent hepatitis B or C viruses (7).

The present experimental study was conducted to investigate the biochemical and histopathological changes induced by the toxic effects of exposure to the electronic vapour and conventional cigarette smoke in rats.

Materials and Methods

Ethical Criteria

Practices for the care and use of laboratory animals were conducted in line with the relevant legislation and international standards.

Experiment and Tests

This study was conducted at the Waist University/College of Medicine from July 10th to September 10th, 2021. Twenty-five fully adjusted Wistar rats (mean weight 170gm) were used, 10 rats were exposed to ECV, 10 rats were subjected to CCS, and 5 rats were recruited as a control group with no treatment. All of the rats were housed in hygienic 33*20.5*19cm cages, and they experienced a standard atmosphere with a routine light period, temperature, and humidity (12hrs, 28-31°C, and 50-55%, respectively).

During the period of exposure to ECV and CCS, electronic and conventional cigarette smoke was introduced into the compartments using a locally customized anaerobic box chamber (25.9*23.4*20.9cm), comparable to that developed by Montanari and Christian (8).

For 40 days, the rats were exposed to cigarette vapour that was blown in their direction by a special motor (ECV: E-vape controller at (4.5watts) and CCS: the equivalent of 10 cigarettes smoked in 60mins per day). The smoke was eliminated from the chambers via the use of a specific vacuum. There was no smoking exposure for the control rats at any point during the experiment. Microscopic slides of the liver tissues were prepared after the animals were sacrificed and started by using chloroform-induced anaesthesia at the end of the experiment period. Histopathological slides were prepared using the methods of Disbrey and Rack (9).

For the identification of the serum levels of biochemical parameters, a spectrophotometric technique was followed as mentioned by Huang *et al* (10). Spectrophotometric procedures were used for the determination of the serum level of some parameters of hepatic diseases. The liver parameter in serum may be measured quickly using spectrophotometry in a short-interval enzyme-activity analyzer. The device is simply a spectrophotometer with a variety of different wavelengths. The test cuvette's altered absorbance is measured in comparison to water. The technique made it possible to precisely determine the reaction's beginning velocity, which was inversely proportional to the enzyme concentration. The technique was used to measure the enzyme activity in a variety of materials, including rat serum (10).

Statistical Analysis

SPSS built-in *t*-test was followed for the analysis of the current study data, and the use of mean ± standard deviation (SD) was completed to highlight any changes in the data after treatments. If *p* was <5%, the data change was considered significant.

Results

The findings of the work highlighted that significant ($p<0.05$) reductions occurred in the serum amounts of albumin and cholesterol of the ECV group when compared with the data of the control rats. Moreover, increases in a significant ($p<0.05$) way were seen in the serum amounts of the LDH of the ECV (Table 1 and figure 1). However, no significant ($p>0.05$) changes were seen in the serum amounts of the ALP, AST, and ALT of the ECV group (Table 1 and figure 1) when compared with the data of the control group.

International Journal of Scientific Trends- (IJST)
ISSN: 2980-4299
Volume 2, Issue 5, May, 2023
Website: https://scientifictrends.org/index.php/ijst
Open Access, Peer Reviewed, Scientific Journal

Table 1: Biochemical parameters in electronic cigarette-exposed and control rats

Parameters	Group	Mean	Std. Deviation	*P* value
Albumin	**Control**	3.0182	0.19242	0.0001*
	ECV	2.4214	0.39545	
ALP	**Control**	609.2750	487.50385	0.516**
	ECV	716.3500	155.06917	
LDH	**Control**	620.40	77.775	0.006*
	ECV	2211.30	1620.791	
AST	**Control**	65.8945	14.47874	0.052**
	ECV	120.6954	81.81316	
ALT	**Control**	78.1510	6.04335	0.154**
	ECV	126.1220	101.79860	
Cholesterol	**Control**	141.80	13.927	0.0001*
	ECV	109.40	4.195	

*Significant $p< 0.05$, **Non-significant $p> 0.05$

The results of the work also determined that significant ($p<0.05$) reductions occurred in the serum amounts of albumin and cholesterol of the CCS group when compared to the data of the control rats. Moreover, increases in a significant ($p<0.05$) way were seen in the serum amounts of the LDH, ALP, and AST of the CCS groups (Table 2 and figure 1) when compared with the data of the control rats. No significant ($p>0.05$) alterations were seen in the serum amounts of the ALT of the CCS group (Table 2 and figure 1) when compared with the data of the control rats.

Table 1: Biochemical parameters in conventional cigarette-exposed and control rats.

Parameters	Group	Mean	Std. Deviation	*P* value
Albumin	**Control**	3.0182	0.19242	0.0001*
	CCS	2.4809	0.24257	
ALP	**Control**	609.275	487.50385	0.023*
	CCS	1096.85	386.00144	
LDH	**Control**	620.40	77.775	0.00001*
	CCS	2855.3	379.144	
AST	**Control**	65.8945	14.47874	0.002*
	CCS	149.507	70.26729	
ALT	**Control**	78.151	6.04335	0.559**
	CCS	83.7	28.85955	
Cholesterol	**Control**	141.80	13.927	0.0001*
	CCS	121.20	6.408	

*Significant $p< 0.05$, **Non-significant $p> 0.05$

International Journal of Scientific Trends- (IJST)
ISSN: 2980-4299
Volume 2, Issue 5, May, 2023
Website: https://scientifictrends.org/index.php/ijst
Open Access, Peer Reviewed, Scientific Journal

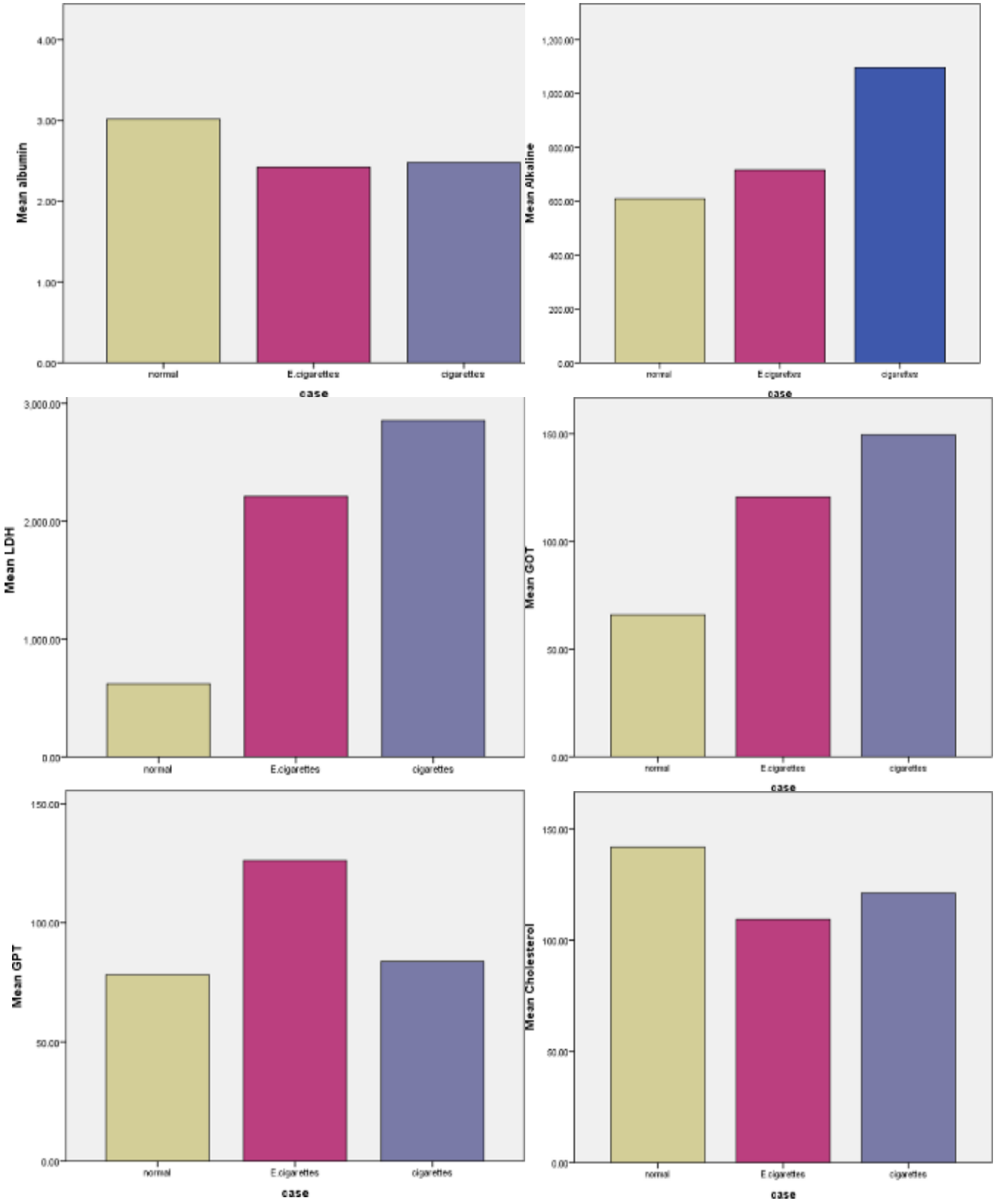

Figure 1: Effects of electronic and conventional cigarette smoke on biochemical parameters of rats.

International Journal of Scientific Trends- (IJST)
ISSN: 2980-4299
Volume 2, Issue 5, May, 2023
Website: https://scientifictrends.org/index.php/ijst
Open Access, Peer Reviewed, Scientific Journal

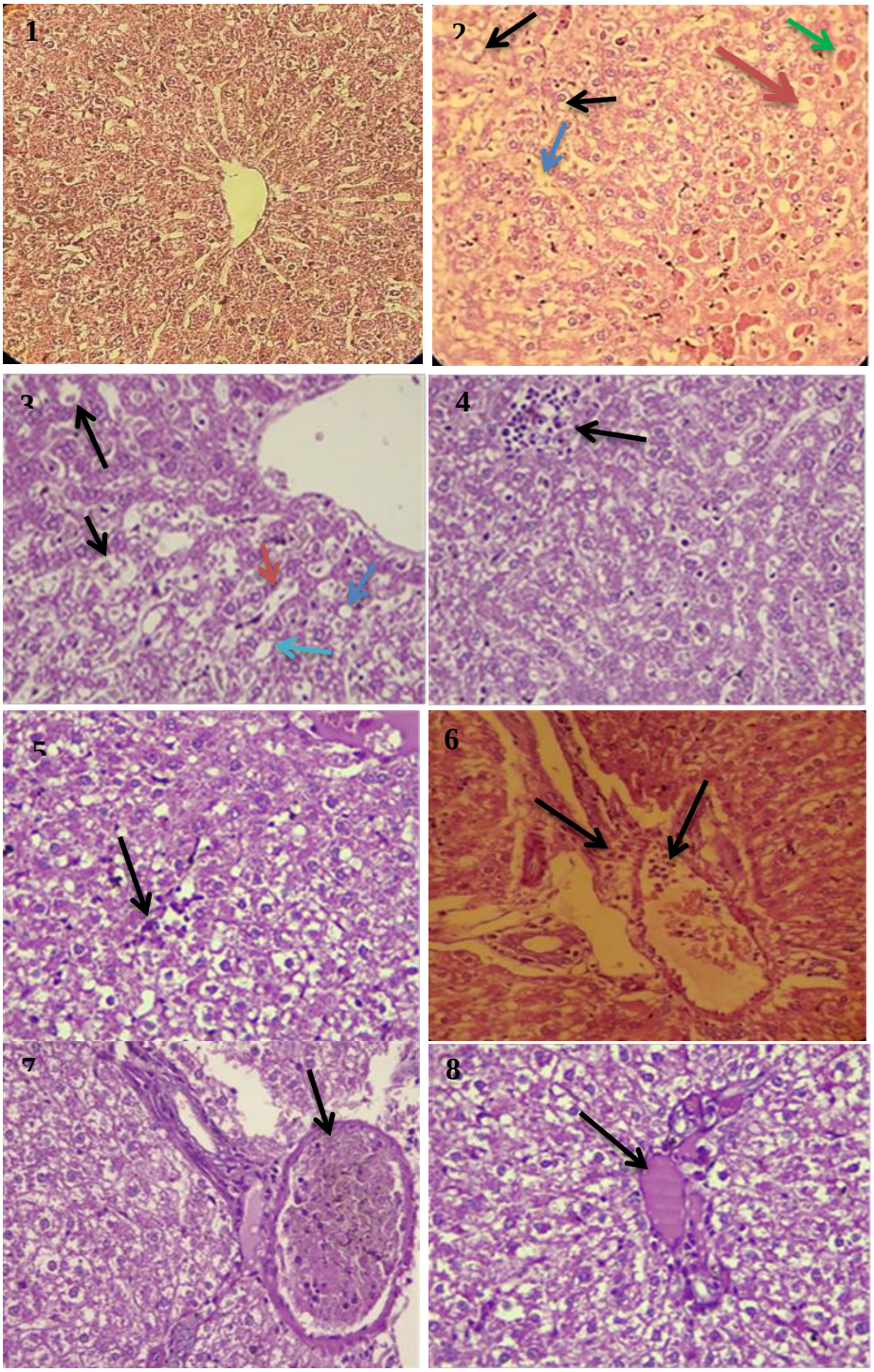

Figure 2: Hepatic histopathological features in ECV- and CCS-exposed rats (H&E 40X). 1. Control rat: Normal features. 2. CCS: Hydropic degeneration (black arrow), intercellular sinusoidal dilation (blue arrow), and vacuoles (Red arrow), in which blood completely filled some of these vacuoles (green arrow). 3. ECV: Hydropic degeneration (black arrow), intercellular sinusoidal dilation (red arrow) and some vacuoles (Blue arrow). 4. ECV: Focalized infiltration of lymphocytes (black arrow). 5. CCS: Focalized infiltration of lymphocytes (black arrow). 6. CCS: Infiltration of lymphocytes in the portal vein and surrounding regions (black arrow). 7. ECV: Portal vein congestion (black arrow). 8. CCS: Portal vein congestion (black arrow).

Discussion

Among its many roles, the liver is a critically important organ that cleanses toxins from the body including drugs and alcohol. In addition, the liver is responsible for the transformation of nicotine, which has several deleterious physiological impacts on the body. Toxic, carcinogenic, and/or liver-damaging compounds and nanostructured materials have been found in ECV aerosols, cartridges, refill liquids, and ecological emission levels (11). Tobacco alkaloids, flavourings, metals, volatile organic compounds, phenols, polycyclic aromatic hydrocarbons, and nicotine all contribute to the ECV-toxicity. There is no hepatic impact from exposure to humectant aerosols at the concentration levels located in the ECV; however, numerous dangerous substances, such as formaldehyde, acetaldehyde, and acrolein, have been discovered in the ECV liquids and the heated aerosol generated by ECV (12).

Alterations in the permeability of plasma membrane and the amounts of hepatic biomarker enzymes are considered important indicators of cellular destruction in hepatocytes (13). A rise in hepatic enzyme levels has been linked to histopathological occurrence, as indicated by multiple publications. Similar circumstances of increased liver function enzymes in the serum of rats after exposure to dichlorvos, an insecticide, were noted by Celik et al. (14), which is consistent with our findings. The toxic effects of a toxin on the hepatocytes can be made worse by the release of pro-inflammatory mediators from necrotic cells. One of the most significant and fundamental contributors to hepatocyte death is the generation of free radicals and the resulting oxidative stress. Toxic substances are known to cause oxidative stress and DNA damage. As a result of these effects, mitochondrial cytochrome C is activated, stimulating caspases and causing cell death (15). Wannamethee and Shaper (16) found alterations in the levels of liver injury indicating enzymes due to conventional cigarette smoking in examined people in the United Kingdom, which agrees with the present study outcomes. Moreover, Aljohani *et al* (17) detected that the administration of nicotine elevates liver injury indicating enzymes, such as ALT and AST. In addition, Azzalini *et al* (18) reported elevation in the ALT of rats exposed to CCS. Furthermore, Ogenyi *et al* (19) demonstrated that rats exposed to CCS showed increases in AST and ALT levels.

Salahshoor *et al* (20) found that exposure to cigarette smoke in mice led to the occurrence of infiltration of lymphocytes and enlargement in the central hepatic vein, which comes in agreement with the current findings. In addition, Bandiera *et al* (21) reported moderate to severe liver tissue damages, such as necrosis, enlargement of hepatocytes, and sinusoidal enlargement, in rats after exposure to cigarette smoke. Aljohani *et al* (17) also recorded nicotine-induced infiltration of lymphocytes in high numbers. Azzalini *et al* (18) also reported liver damage in rats exposed to CCS. In addition, Ogenyi *et al* (19) revealed severe liver damage of necrosis and deposition of fat in rats exposed to CCS for three weeks.

The present findings highlight the dangerous effects of exposure to electronic and conventional cigarette smoke, affecting both biochemical and histological features in the studied rats.

References

1. Ussher M, Brown J, Rajamanoharan A, West R. How do prompts for attempts to quit smoking relate to method of quitting and quit success? Ann Behav Med [Internet]. 2014 [cited 2022 Aug 27];47(3):358–68. Available from: https://pubmed.ncbi.nlm.nih.gov/24046150/

2. West R, McEwen A, Bolling K, Owen L. Smoking cessation and smoking patterns in the general population: a 1-year follow-up. Addiction [Internet]. 2001 Jun 1 [cited 2022 Aug 27];96(6):891–902. Available from: https://onlinelibrary.wiley.com/doi/full/10.1046/j.1360-0443.2001.96689110.x
3. Gowing LR, Ali RL, Allsop S, Marsden J, Turf EE, West R, et al. Global statistics on addictive behaviours: 2014 status report. Addiction [Internet]. 2015 Jun 1 [cited 2022 Aug 27];110(6):904–19. Available from: https://pubmed.ncbi.nlm.nih.gov/25963869/
4. West R. Tobacco smoking: Health impact, prevalence, correlates and interventions. Psychol Health [Internet]. 2017 Aug 8 [cited 2022 Aug 27];32(8):1018–36. Available from: /pmc/articles/PMC5490618/
5. Jha P, Peto R. Global effects of smoking, of quitting, and of taxing tobacco. N Engl J Med [Internet]. 2014 Jan 2 [cited 2022 Aug 27];370(1):60–8. Available from: https://pubmed.ncbi.nlm.nih.gov/24382066/
6. M. KA, O. DA, Ş. ŞS, P. M. DOUBLE THERAPY WITH PEGYLATED INTERFERON AND RIBAVIRIN FOR CHRONIC HEPATITIS C. A PHARMACOGENETIC GUIDE FOR PREDICTING ADVERSE EVENTS. Farmacia. 2017;65(6):1–8.
7. Docea A. O., Gofita E., Calina D. IZS. AUTOIMMUNE DISORDERS DUE TO DOUBLE ANTIVIRAL THERAPY WITH PEGINTERFERON AND RIBAVIRIN IN PATIENTS WITH HEPATITIS C VIRUS INFECTION ANCA OANA DOCEA 1# , ELIZA GOFITA 1# *, DANIELA CALINA 2# , ZAHARIE SORIN IOAN 3# , DANIEL IONICA VALCEA 4#. Farmacia. 2016;64:4.
8. Montanari C, Kelley LK, Kerr TM, Cole M, Gilpin NW. Nicotine e-cigarette vapor inhalation effects on nicotine & cotinine plasma levels and somatic withdrawal signs in adult male Wistar rats. Psychopharmacology (Berl) [Internet]. 2020 Mar 1 [cited 2022 Aug 18];237(3):613–25. Available from: /pmc/articles/PMC7039759/
9. Disbrey BD, Rack JH. Histological laboratory methods [Internet]. Livingstone; 1970 [cited 2022 Aug 17]. Available from: https://agris.fao.org/agris-search/search.do?recordID=US201300465541
10. Huang X-J, Choi Y-K, Im H-S, Yarimaga O, Yoon E, Kim H-S. Aspartate Aminotransferase (AST/GOT) and Alanine Aminotransferase (ALT/GPT) Detection Techniques. Sensors (Basel) [Internet]. 2006 [cited 2022 Aug 27];6(7):756–82. Available from: /pmc/articles/PMC3894536/
11. Noël A, Verret CM, Hasan F, Lomnicki S, Morse J, Robichaud A, et al. Generation of Electronic Cigarette Aerosol by a Third-Generation Machine-Vaping Device: Application to Toxicological Studies. J Vis Exp [Internet]. 2018 Aug 25 [cited 2022 Aug 27];2018(138):58095. Available from: /pmc/articles/PMC6231858/
12. Vansickel AR, Eissenberg T. Electronic cigarettes: effective nicotine delivery after acute administration. Nicotine Tob Res [Internet]. 2013 Jan [cited 2022 Aug 27];15(1):267–70. Available from: https://pubmed.ncbi.nlm.nih.gov/22311962/
13. Dhouib El-Bini, Annabi A., Montassar M.L., Gharbi N. E-FS. Anti-inflammatory effects of N-acetylcysteine against carbosulfan-induced hepatic impairment in male rats. 2015;1:29–40. Recent Adv Biolo Med. 2015;1:29–40.
14. Celik I, Yilmaz Z, Turkoglu V. Hematotoxic and hepatotoxic effects of dichlorvos at

sublethal dosages in rats. Environ Toxicol [Internet]. 2009 Apr [cited 2022 Aug 27];24(2):128–32. Available from: https://pubmed.ncbi.nlm.nih.gov/18442070/

15. Karami-Mohajeri S, Abdollahi M. Mitochondrial dysfunction and organophosphorus compounds. Toxicol Appl Pharmacol [Internet]. 2013 Jul 1 [cited 2022 Aug 27];270(1):39–44. Available from: https://pubmed.ncbi.nlm.nih.gov/23578477/
16. Wannamethee SG, Shaper AG. Cigarette smoking and serum liver enzymes: The role of alcohol and inflammation. Ann Clin Biochem [Internet]. 2010 Jul 28 [cited 2022 Aug 28];47(4):321–6. Available from: https://journals.sagepub.com/doi/10.1258/acb.2010.009303?url_ver=Z39.88-2003&rfr_id=ori%3Arid%3Acrossref.org&rfr_dat=cr_pub++0pubmed
17. Aljohani ZM, Alharbi AA, Alsaedi MF, Alahmadi AF, Alahmadi OA, Alharbi AA, et al. Evaluation of the Potential Beneficial Effects of Thymoquinone Against Nicotine Induced Toxicity. Int J Pharm Clin Res [Internet]. 2015 [cited 2022 Aug 28];7(6):395–8. Available from: www.ijpcr.com
18. Azzalini L, Ferrer E, Ramalho LN, Moreno M, Domínguez M, Colmenero J, et al. Cigarette smoking exacerbates nonalcoholic fatty liver disease in obese rats. Hepatology [Internet]. 2010 May 1 [cited 2022 Aug 28];51(5):1567–76. Available from: https://europepmc.org/article/med/20432253
19. Ogenyi SI, Choji TPP, Chimezirim A, Onyemelukwe AO, Ngokere AA, Onwuasoanya UF, et al. Histological and Biochemical Effects of Cigarette Smoke on the Liver of Wistar Rats. Annu Res Rev Biol [Internet]. 2015 May 14 [cited 2022 Aug 28];7(2):119–25. Available from: https://journalarrb.com/index.php/ARRB/article/view/25830
20. Salahshoor M, Mohamadian S, Kakabaraei S, Roshankhah S, Jalili C. Curcumin improves liver damage in male mice exposed to nicotine. J Tradit Complement Med [Internet]. 2016 Apr 1 [cited 2022 Aug 28];6(2):176–83. Available from: /pmc/articles/PMC4833467/
21. Bandiera S, Pulcinelli RR, Huf F, Almeida FB, Halmenschlager G, Bitencourt PER, et al. Hepatic and renal damage by alcohol and cigarette smoking in rats. Toxicol Res [Internet]. 2021 Apr 1 [cited 2022 Aug 28];37(2):209–19. Available from: /pmc/articles/PMC8007694/