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Histopathological Assessment of Cigarette Butt and Vape Oil Extracts on Rat Organs: GC-MS Identification of Active Components

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Abstract

The objective of this study was to investigate the chemical composition and histopathological effects of diverse extracts and vape oil on rat organs. Utilizing gas chromatography-mass spectrometry (GC-MS), we analyzed seven distinct extracts, comprising Vape Oil, Intact Cigarette Water Extract, Intact Cigarette Ethyl Acetate Extract, Intact Cigarette Petroleum Ether Extract, Cigarette Buds Ethanol Extract, and Cigarette Buds Petroleum Ether Extract. The findings revealed substantial variations in the chemical constituents of the tested extracts, highlighting their heterogeneous nature.

Among the identified compounds, n-Hexadecanoic acid showed higher concentrations in the Ethyl Acetate Extract of Intact Cigarette, Vape Oil, and Petroleum Ether Extract, while Oleic Acid predominated in both the Ethanol and Petroleum Ether Extracts of Intact Cigarette. Additionally, Tetrapentacontane, 1,54-dibromo-, exhibited elevated levels in the Water Extract of Intact Cigarette and Petroleum Ether Extract. The heightened levels of specific compounds in Vape Oil necessitate further investigation into their potential biological activities and toxicological profiles.

Histopathological examinations were performed on rat organs exposed to various concentrations of the extracts and vape oil. The Cigarette Buds Water Extract demonstrated a favorable effect on brain tissue, resulting in clear and well-preserved brain cells. However, the Petroleum Extract (present in both the Cigarette Buds and Intact groups) revealed concerning findings, indicating its potential association with hepatocellular carcinoma (HCC) in the liver. Furthermore, all tested extracts impacted kidney tissues, leading to notable enlargement of glomerular cells and tubules, termed super megaly. Conversely, the histopathological analysis of the vape oil group showed no indications of abnormalities or adverse effects on the organs, signifying its relative safety compared to the other tested extracts.

Overall, the study provides valuable insights into the chemical diversity and histopathological effects of various extracts and vape oil on rat organs. The findings underscore the importance of further research to assess the safety and potential risks associated with these extracts. The identification of HCC in the liver tissue underscores the significance of evaluating the safety of these extracts for consumers. Moreover, the relative safety of vape oil compared to the tested extracts suggests its potential as a less harmful alternative for organ health. This research contributes essential knowledge regarding the potential risks linked to these extracts and offers valuable guidance for regulatory bodies and researchers in making informed decisions to ensure consumer safety.

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Keywords: *GC-MS, Cigarette extraction, Vape oil, Histopathologic Vital Organs.*

Introduction

Tobacco smoking has posed a substantial public health challenge for many years, as traditional cigarettes have been well-documented to have detrimental effects on human health[1, 2]. In recent times, electronic cigarettes (e-cigarettes) have emerged as a popular alternative to smoking, raising additional concerns about potential health risks[3, 4]. Both smoking and vaping involve inhaling diverse chemical compounds, leading to the deposition of harmful substances within the respiratory system and vital organs [5, 6]. While the adverse effects of traditional cigarette smoking have been extensively studied, the health implications of vaping remain a subject of ongoing research[7, 8].

This study aims to investigate the histopathological impacts of cigarette butt and vape oil extracts on rat organs and to identify the active components responsible for these effects using Gas Chromatography-Mass Spectrometry (GC-MS) [9, 10]. Rats serve as experimental models for toxicological studies due to their physiological similarities to humans and relatively short lifespan, enabling the observation of long-term effects within a reasonable timeframe[11].

Analyzing the histopathology of rat organs exposed to cigarette butt and vape oil extracts will yield valuable insights into the potential toxicological consequences of both smoking and vaping [12]. Examining the cellular and tissue changes caused by these extracts will enhance our comprehension of the underlying mechanisms and pinpoint specific vulnerable organs [13].

Additionally, applying GC-MS analysis to the extracts will facilitate the identification and quantification of active components present in the samples [14, 15]. This advanced analytical technique allows the separation and characterization of complex mixtures, enabling the detection of a wide range of compounds, including volatile and semi-volatile substances[16, 17]. Identifying these active components will establish links between specific chemical compounds and the observed histopathological changes in rat organs, providing critical information on potential toxicants associated with smoking and vaping [18, 19].

In conclusion, this study aims to contribute to existing knowledge about the health impacts of smoking and vaping by investigating the histopathological effects of cigarette butt and vape oil extracts on rat organs. The GC-MS analysis of these extracts will further our understanding of the active components involved, informing public health policies and raising awareness about potential risks associated with these practices.

Materials and Methods

1. Collection and Preparation of Cigarette Butts and Vape Oil Extracts

Cigarette butts were procured from local smokers using standardized collection protocols. Vape oil was sourced from a reliable supplier and verified to be free of contaminants. Four distinct extracts were derived from cigarette butts, namely water extract, ethyl acetate extract, ethanol extract, and petroleum ether extract. Soxhlet extraction apparatus was employed for the extraction process, utilizing solvents tailored to each specific extract type. Vape oil was dissolved in an appropriate solvent to form the vape oil extract. Additionally, intact cigarette extracts were obtained by extracting intact cigarettes using ethyl acetate and water as the respective solvents [20].

2. GC-MS Analysis

Gas Chromatography-Mass Spectrometry (GC-MS) analysis was conducted using an Agilent 7890A GC system coupled with a 5975C MSD detector. The analysis utilized a DP5-MS column (30 m length, 0.25 mm internal diameter, 0.25 µm film thickness) coated with 5% biphenyl and 95% dimethylpolysiloxane. High-purity helium (99.999%) served as the carrier gas, operating under the following conditions: Column Oven Temperature: 60.0 °C, Injection Temperature: 280.00 °C, Injection Mode: Split (split ratio: 1:30), Flow Control Mode: Pressure, Pressure: 96.1 kPa, Total Flow: 50.0 mL/min, Column Flow: 1.55 mL/min, Linear Velocity: 45.4 cm/sec, and Purge Flow: 2.0 mL/min. [21].

3. Animal Study

3.1. Animal Ethics

Conforming to institutional guidelines, the animal study was conducted in accordance with ethical principles and received approval from the Animal Ethics Committee to ensure the well-being and ethical treatment of the animals involved.

3.2. Animal Selection

To minimize bias, healthy adult male rats, aged 8-10 weeks, and weighing between 200-250 grams were randomly chosen for the study.

3.3. Grouping and Administration

For this study, the rats were classified into ten distinct groups, with each group comprising three rats (n=3 per group). The control groups were exposed to intact cigarette extracts using different solvents, namely ethyl acetate (Control Group 1), water (Control Group 2), petroleum ether (Control Group 3), and ethanol (Control Group 4). On the other hand, the experimental groups received various extracts derived from cigarette butts: water extract (Experimental Group 5), ethyl acetate extract (Experimental Group 6), ethanol extract (Experimental Group 7), and petroleum ether extract (Experimental Group 8). Furthermore, the experimental groups also included rats that were given vape oil extract (Experimental Group 9) to evaluate the potential effects of vaping. A control negative group (Experimental Group 10) was included, where no extract administration took place. Throughout the study, each group was meticulously monitored to ensure accurate observations and precise data collection.

3.4. Dosage and Administration

The extracts were administered to the rats through the oral cavity using a lavage needle at varying concentrations (0.1, 0.2, and 0.4) over a two-week period. The administration schedule involved alternating days of extract application and rest to simulate human smoking and vaping patterns.

4. Histopathological Assessment

At the conclusion of the two-week administration period, the rats were sacrificed, and their organs (lungs, liver, kidneys, and heart) were harvested and fixed in 10% formalin. Subsequently, paraffin-embedded tissue sections were prepared and stained with hematoxylin and eosin (H&E) for histopathological evaluation. A qualified pathologist conducted microscopic examination in a blinded manner to avoid potential bias [22, 23].

5. Ethical Clearance

The study, bearing reference Sep. 2022, has obtained ethical clearance from the Scientific Committee within the Department of Environmental Science at the University of Mosul.

6. Statistical Analysis

Histopathological data were subjected to appropriate statistical methods, including one-way analysis of variance (ANOVA) followed by post-hoc analysis, to determine the significance of differences between groups. Statistical significance was considered for p-values less than 0.05.

Results

The study utilized gas chromatography-mass spectrometry (GC-MS) to perform a comparative analysis of chemical components in seven distinct extracts, which included Vape Oil, Intact Cigarette Water Extract, Intact Cigarette Ethyl Acetate Extract, Intact Cigarette Petroleum Ether Extract, Cigarette buds Ethanol Extract, and Cigarette buds Petroleum Ether Extract (table1 & figure 2). Notably, n-Hexadecanoic acid was found in higher concentrations in the Ethyl Acetate Extract of Intact Cigarette, Vape Oil, and Petroleum Ether Extract. In contrast, Oleic Acid dominated in both the Ethanol and Petroleum Ether Extracts of Intact Cigarette. Tetrapentacontane, 1,54-dibromo-, exhibited elevated levels in the Water Extract of Intact Cigarette and Petroleum Ether Extract (figure2).

Significantly, Vape Oil demonstrated elevated levels of Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester, Eicosanoic acid, isobutyl ester, and Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester.

These findings underscore the chemical diversity across the various extracts and emphasize the potential health implications of the identified compounds. Further investigation is warranted to explore the biological activities and toxicological profiles of these chemical components to assess their safety and potential impact on human health. The insights gained from this study hold importance for regulatory bodies and researchers in comprehending the intricacies of these extracts and their potential effects on consumers.

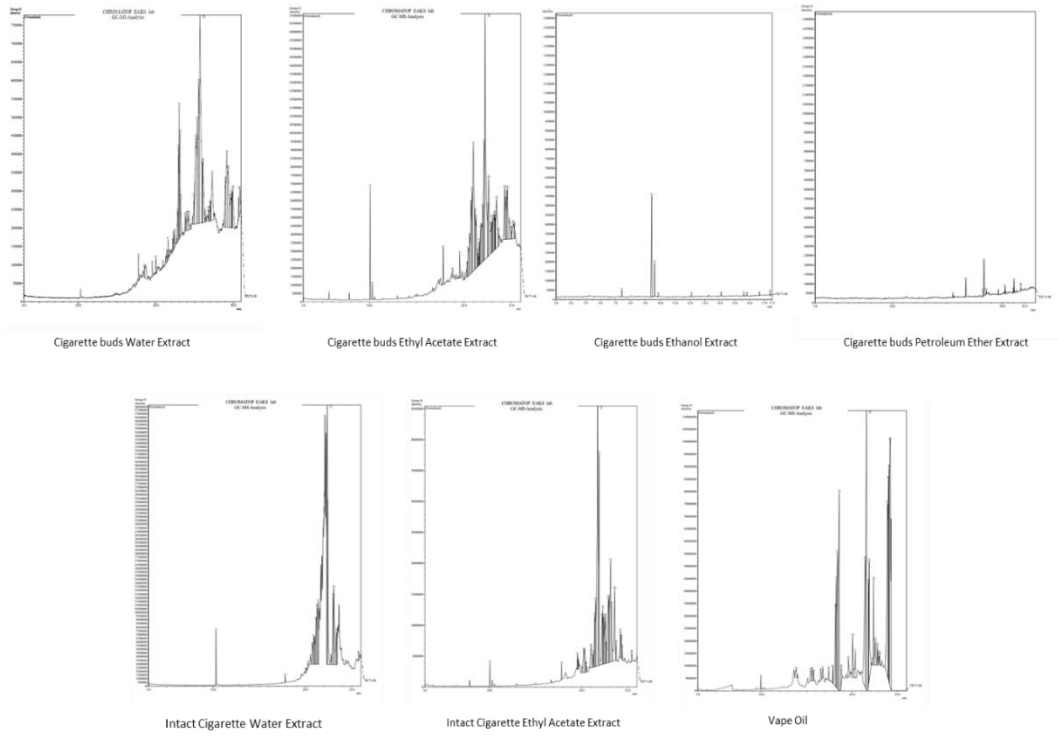


Figure1. GC-MS results curves, the peaks represented to chemical components in seven extracts: Vape Oil, Intact Cigarette Water Extract, Intact Cigarette Ethyl Acetate Extract,

Intact Cigarette Petroleum Ether Extract, Cigarette buds Ethanol Extract, and Cigarette buds Petroleum Ether Extract.

Table 1. Comparative analysis of chemical components in seven extracts using GC-MS. Results are presented as peak number, R.Time, Area, and Area%.

Peak %	**Compound Name**	**Cigarette buds Water Extract (%)**	**Cigarette buds Ethyl Acetate Extract (%)**	**Cigarette buds Ethanol Extract (%)**	**Cigarette buds Petroleum Ether Extract (%)**	**Vape Oil (%)**	**Intact Cigarette Water Extract (%)**	**Intact Cigarette Ethyl Acetate Extract (%)**
1	n-Hexadecanoic acid	0.55	3.5	1.17	14.94	0.32	2.32	0.48
2	(6Z,9Z)-6,9-Pentacosadiene	0.67	0	0	0	0.51	0	0
3	Tetrapentacontane, 1,54-dibromo-	1.04	0	0	3.07	0.58	1.04	0
4	Oleic Acid	0.42	0.98	45.47	45.47	0.6	0.42	0.5
5	Hexadecanoic acid, butyl ester	0.24	0	0	0	0	0	
6	6-[2-(3-Hydroxy-2,2,5a-trimethyl-7-methylidene-4,5,6,8,9,9a-hexahydro-3H-benzo[b]oxepin-6-	0.35	0	0	0	0	0	0
7	Z-12-Pentacosene	0.48	0	0	0	0	0	0
8	Tetrapentacontane, 1,54-dibromo-	0	0	0	3.5	0	0	0
9								
10	Hexadecane, 1,1-bis(dodecyloxy)-	0	2.5	0	1.29	0	0	0
11	Octadecane, 1-(ethenyloxy)-	0	0	4.12	0	0	0	0
12	Methyl 15-hydroxy-9,12-octadecadienoate	0	0	0.42	0	0	0	0
13	15-Tetracosenoic acid, methyl ester, (Z)-	0	0	2.89	0	0	0	0
14	Behenic alcohol	0	0	0	7.18	0	0	0
15	9-Octadecenoic acid (Z)-, tetradecyl ester	0	0	0	1.62	0	0	0
16	Decyl oleate	0	0	0	5.2	0	0	0
17	Erucic acid	0	0	0	5.06	0	0	0
18	Octadecanoic acid, 2,3-dihydroxypropyl ester	0	0	0	0	4.04	0	0
19	1-Pentadecene, 2-methyl-	0	0	0	0	2.07	0	0

20	1-Benzyl-2,6-dipentylpiperidin-4-one	0	0	0	0	1.22	0	0
21	2-Hydroxy-3-(6-methoxy-2-napthyl)-3-butenoic acid 1,1-dimethylethyl ester	0	0	0	0	0.56	0	0
22	2-Cyclohexen-3,5-diol-1-one, 2-[11-phenylundecanoyl]-	0	0	0	0	0.36	0	0
23	2-Cyclohexen-3,5-diol-1-one, 2-[11-phenylundecanoyl]-	0	0	0	0	0.27	0	0
24	Tritetracontane	0	0	0	0	10.4	0	0
25	Heneicosyl pentafluoropropionate	0	0	0	0	6.02	0	0
26	Cholestane-5-carbonitrile, 3-oxo-, (5.alpha.)-	0	0	0	0	7.53	0	0

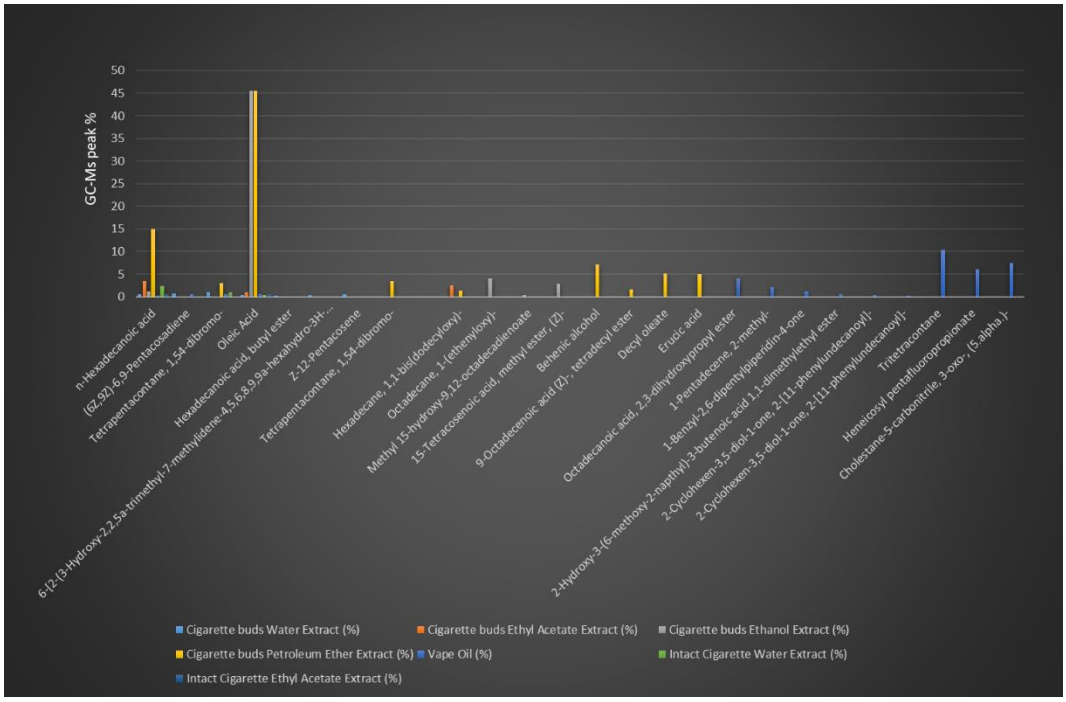


Figure 2. GC-MS histogram depicting the chemical components in seven extracts: Vape Oil, Intact Cigarette Water Extract, Intact Cigarette Ethyl Acetate Extract, Ethanol Extract, and Petroleum Ether Extract.

In this research, histopathological have examined the impacts of three different concentrations (0.1 ml, 0.2 ml, and 0.4 ml) of various extracts (Intact Cigarette Water Extract, Intact Cigarette Ethyl Acetate Extract, Intact Cigarette Petroleum Ether Extract, Cigarette buds Ethanol Extract, and Cigarette buds Petroleum Ether Extract) on rat organs (brain, liver, and kidney). Additionally, we assessed the effects of vape oil. The

study consisted of three groups (n=3) for each extract concentration, and we compared the results with normal rat organs.

Regarding the brain, the Cigarette Buds Water Extract demonstrated the most pronounced effect, with brain cells appearing notably clearer and well-preserved compared to the normal rat brain tissue. In contrast, the other extracts showed effects within the normal range.

Regarding the liver, most of the extracts showed normal histopathological features. However, the Petroleum Extract (present in both the Cigarette Buds and Intact groups) revealed some concerning findings. Both groups exhibited indications of a particular type of liver cancer, known as hepatocellular carcinoma (HCC). This indicates that the petroleum extract, whether present in cigarettes or directly in its intact form, may possess potential carcinogenic effects on the liver.

Concerning the kidney, all the extract concentrations led to notable effects on glomerular cells and tubules. These effects included significant enlargement, termed super megaly, in these structures. While the specific extract responsible for these changes was not specified, it is evident that all the tested extracts had a similar impact on kidney tissue.

On the other hand, the histopathological analysis of the vape oil group did not reveal any abnormalities or adverse effects on the organs. The brain tissue appeared normal, and there were no signs of cancer or other abnormal changes in the liver or kidney tissues when compared to the normal rat organ samples.

In conclusion, this study sheds light on the histopathological effects of various extracts on rat organs and vape oil. The Cigarette Buds Water Extract showed a positive influence on brain tissue clarity but exhibited no significant adverse effects on other organs. However, the Petroleum Extract, found in cigarette buds and intact form, was associated with hepatocellular carcinoma, a form of liver cancer. Furthermore, all the tested extracts affected kidney glomerular cells and tubules with super megaly. In contrast, vape oil did not produce any observable histopathological alterations in the organs, indicating its relative safety compared to the other tested extracts. Nonetheless, further research is required to comprehend the long-term effects of these extracts and vape oil on organ health and overall well-being.

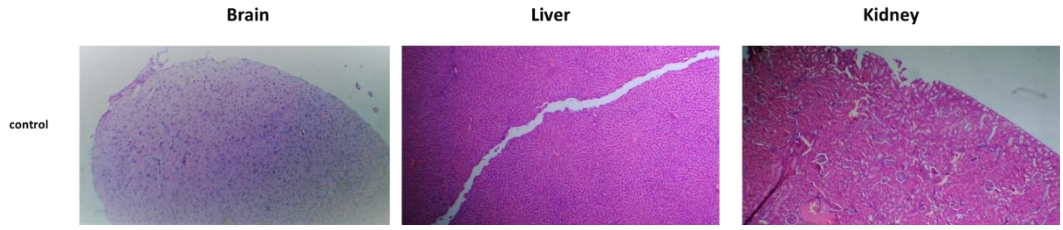
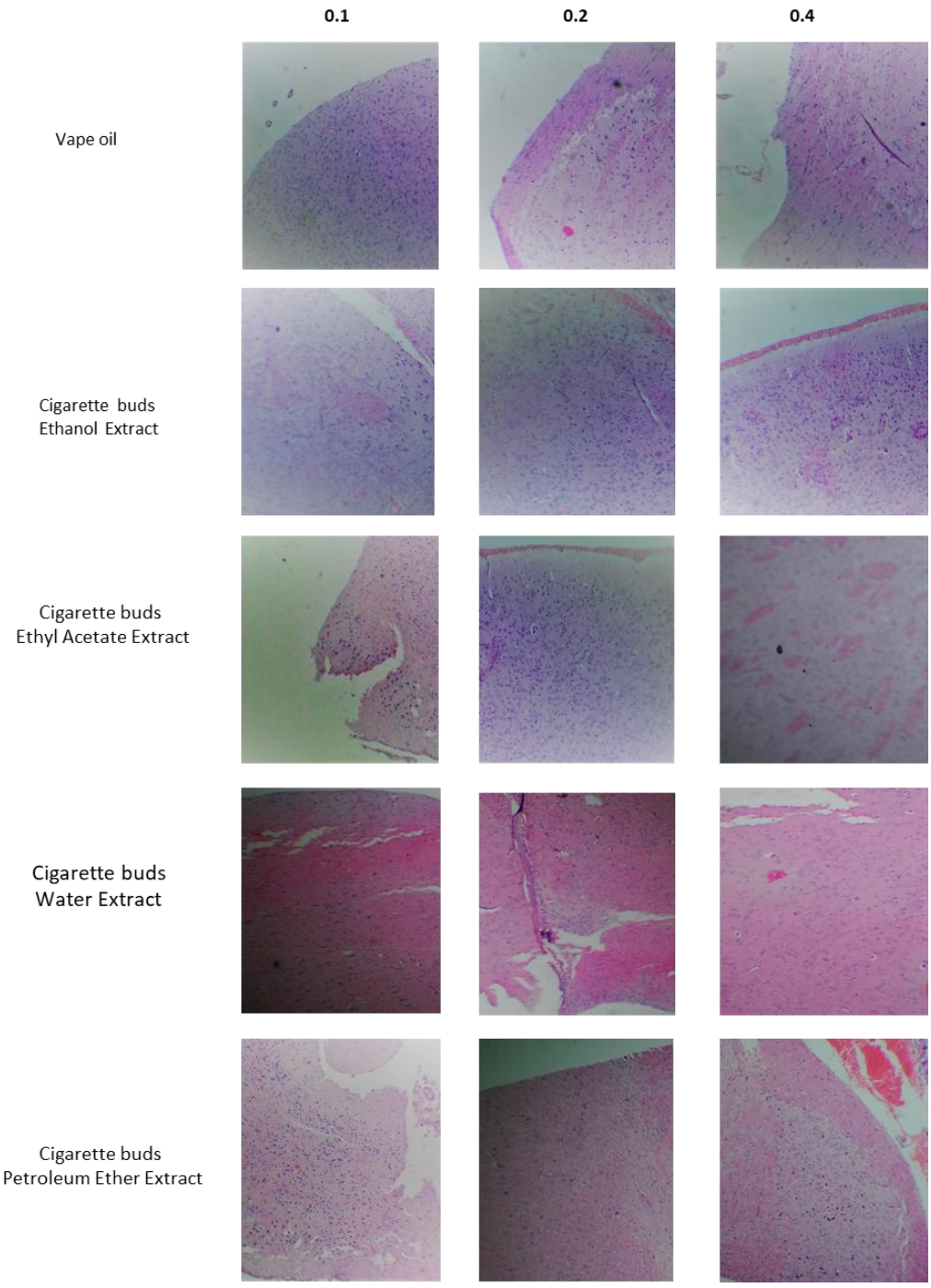


Figure 3. Histopathological sections of normal rat organs (brain, liver, and kidney) for comparison. (A) Brain tissue showing well-preserved and clear neural cells. (B) Liver tissue demonstrating normal hepatic architecture with no observable abnormalities. (C) Kidney tissue displaying intact glomerular cells and tubules without any signs of enlargement. Scale bar: 100 μm.


0.1
0.2
0.4
Vape oil
Cigarette buds
Ethanol Extract
Cigarette buds
Ethyl Acetate Extract
Cigarette buds
Water Extract
Cigarette buds
Petroleum Ether Extract

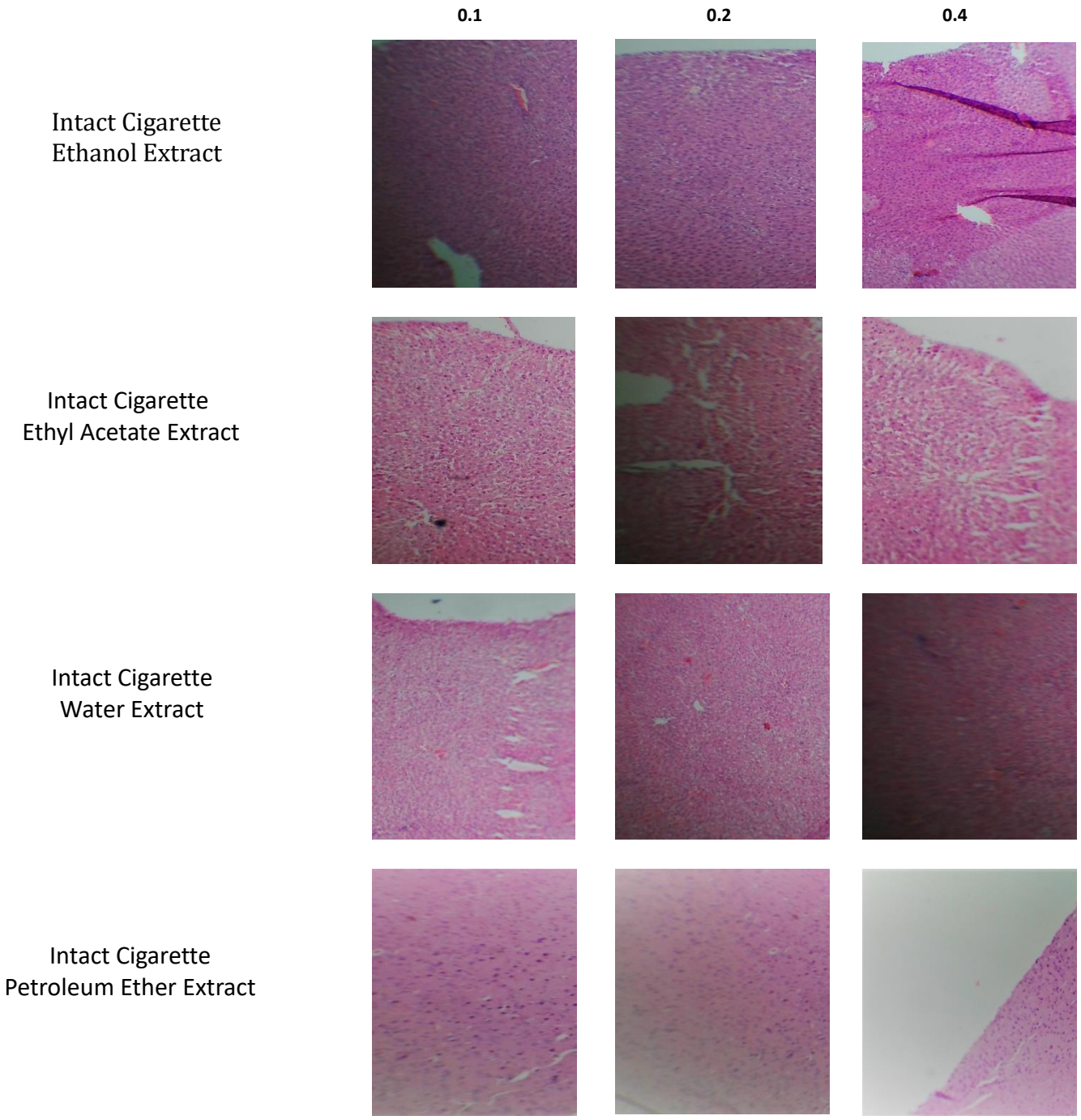
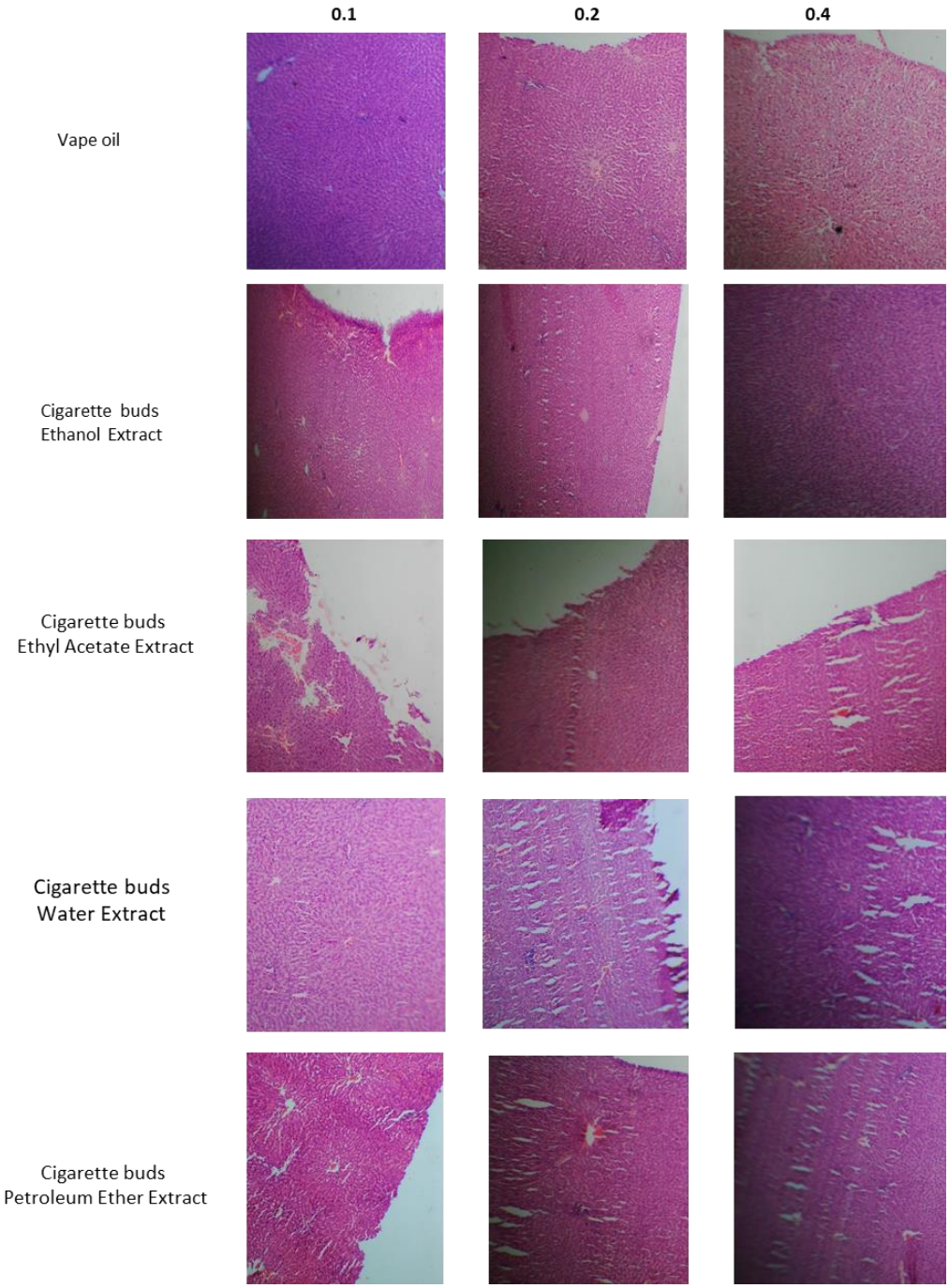


Figure 4. Histopathological sections of rat brain tissues exposed to nine different extracts, including Vape Oil


0.1
0.2
0.4
Vape oil
Cigarette buds
Ethanol Extract
Cigarette buds
Ethyl Acetate Extract
Cigarette buds
Water Extract
Cigarette buds
Petroleum Ether Extract

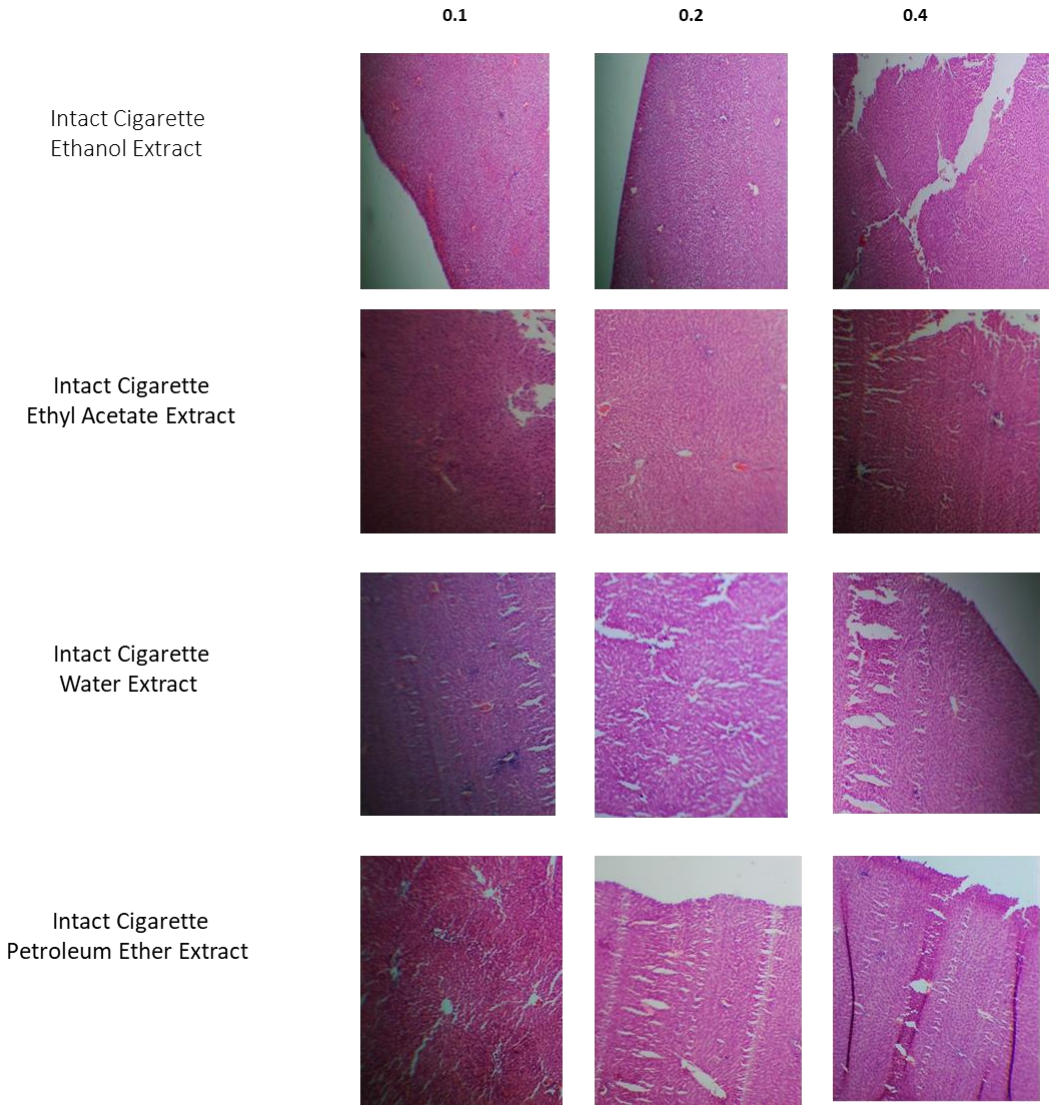
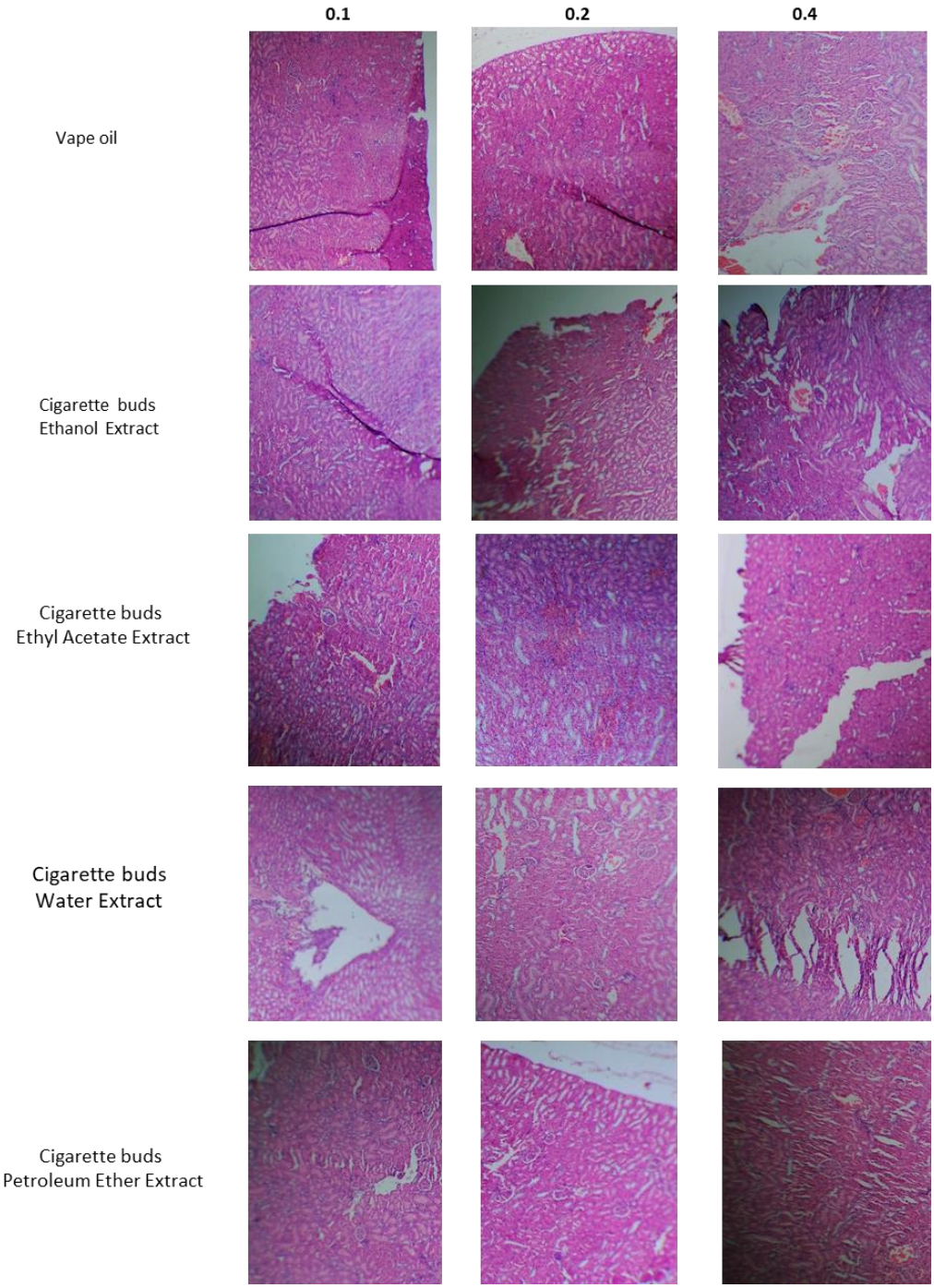


Figure 5. Histopathological sections of rat liver tissues exposed to nine different extracts, including Vape Oil


0.1
0.2
0.4
Vape oil
Cigarette buds
Ethanol Extract
Cigarette buds
Ethyl Acetate Extract
Cigarette buds
Water Extract
Cigarette buds
Petroleum Ether Extract

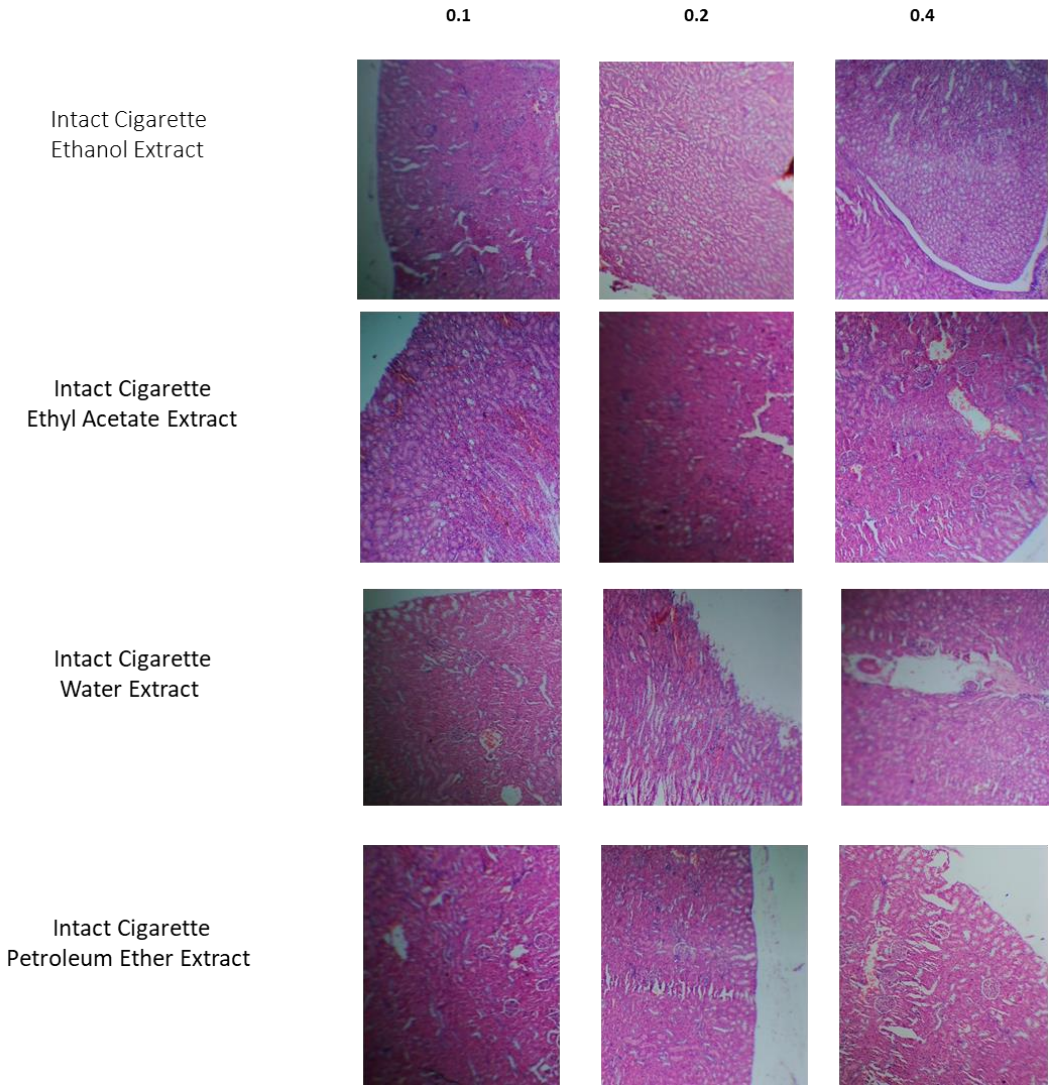


Figure 6. Histopathological sections of rat kidney tissues exposed to nine different extracts, including Vape Oil.

Discussion:

The outcomes of the chemical analysis using gas chromatography-mass spectrometry (GC-MS) unveiled notable discrepancies in the chemical compositions of the examined extracts [24, 25]. The study encompassed seven distinct extracts, namely Vape Oil, Intact Cigarette Water Extract, Intact Cigarette Ethyl Acetate Extract, Intact Cigarette Petroleum Ether Extract, Cigarette Buds Ethanol Extract, and Cigarette Buds Petroleum Ether Extract. The comparison of their chemical components showcased the diverse nature of these extracts [26].

Particularly noteworthy, the Ethyl Acetate Extract of Intact Cigarette, Vape Oil, and Petroleum Ether Extract demonstrated higher concentrations of n-Hexadecanoic acid. In contrast, Oleic Acid dominated in both the Ethanol and Petroleum Ether Extracts of Intact Cigarette. Additionally, Tetrapentacontane, 1,54-dibromo-, exhibited elevated levels in the Water Extract of Intact Cigarette and Petroleum Ether Extract. These findings underscore the chemical diversity among the tested extracts and underscore the importance of considering potential health implications associated with the identified compounds [27].

The elevated levels of specific compounds, such as Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester, and Eicosanoic acid, isobutyl ester, in Vape Oil warrant further investigation into their potential biological activities and toxicological profiles [28]. Given the increasing popularity and usage of vape oil products, understanding the safety and potential impact of these compounds on human health becomes imperative [29].

Shifting the focus to the histopathological examination of rat organs, the study explored the effects of different concentrations of various extracts and vape oil on brain, liver, and kidney tissues. Notably, the Cigarette Buds Water Extract exhibited a positive effect on brain tissue, leading to clear and well-preserved brain cells. Conversely, the other extracts demonstrated no significant adverse effects on the brain, as their impact fell within the normal range [30].

Most extracts displayed normal histopathological features in the liver tissue, except for the Petroleum Extract found in both the Cigarette Buds and Intact groups. This extract raised concerns due to its association with hepatocellular carcinoma (HCC) in the liver tissue. The identification of HCC highlights the potential carcinogenic effects of the petroleum extract when present in cigarettes or intact form [31, 32].

The kidney tissues exhibited notable effects across all extract concentrations, with glomerular cells and tubules displaying enlargement, termed super megaly. Although the specific extract responsible for these changes was not specified, it is evident that all tested extracts had a similar impact on the kidney tissue [31].

Interestingly, the histopathological analysis of the vape oil group did not reveal any abnormalities or adverse effects on the organs. The brain, liver, and kidney tissues appeared normal and comparable to the normal rat organ samples [33].

Taken together, this study offers valuable insights into the chemical composition and histopathological effects of various extracts and vape oil on rat organs. The diversity of chemical components found in the extracts underscores the need for further research to evaluate their potential health implications. The identification of hepatocellular carcinoma in the liver tissue from the Petroleum Extract accentuates the importance of assessing the safety of these extracts for consumers. Conversely, the relative safety of vape oil compared to the tested extracts indicates its potential as an alternative with fewer adverse effects on organ health. The results of this study contribute to our understanding of the potential risks associated with these extracts and can inform regulatory bodies and researchers in making informed decisions for consumer safety. However, conducting more extensive investigations is crucial to fully comprehend the long-term effects of these extracts and vape oil on organ health and overall well-being.

Conclusion

In conclusion, our research provides valuable insights into the chemical composition and histopathological effects of different extracts and vape oil on rat organs. The analysis using gas chromatography-mass spectrometry (GC-MS) revealed varying chemical profiles among the examined extracts, indicating the presence of specific compounds that may have potential health implications. Notably, Vape Oil showed elevated levels of certain compounds, which necessitates further investigation into their biological activities and toxicological profiles. The histopathological examination demonstrated a positive impact of the Cigarette Buds Water Extract on brain tissue, while the Petroleum Extract was linked to hepatocellular carcinoma (HCC) in the liver. Moreover, all extracts led to significant effects on kidney tissues. In contrast, vape oil showed no adverse effects on the organs. These findings underscore the importance of further research to comprehend the safety and potential risks associated with these extracts. Our study holds significance

for regulatory bodies and researchers in protecting consumer health and making informed decisions.

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