The Oxidative Stress Induced by the Vapours of Electronic- Hookah on Mice Liver Tissues

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ABSTRACT

Background: In the past few years, the increased use of Electronic- hookah is popular among youth in Iraq, raises the need for assessment of these products on liver tissue oxidative stress. The current study aims to examine the oxidative stress induced by the vapours of Electronic- hookah on mice liver tissues.

Material and Methods: Twenty mice were randomly separated into two groups. The first experimental group included albino mice were subjected to vapours of Electronic- hookah (1 hour per day for one month), and the other as a control group. At the end of the experiment, several organ weights were measured, and liver tissues were collected for all animals. Liver tissue contents of malondialdehyde, total protein carbonyl, nitric oxide were estimated.

Results: The experimental data showed that Electronic- hookah (1 hour per day for one-month) had significant differences between the two groups on the relative weight of the liver and relative weight of spleen. All of the estimated oxidative damage products (malondialdehyde, total protein carbonyl, nitric oxide) were significantly higher (P<0.0001) in the E- hookah group when compared to the animals in control group.

Conclusion: Vapours of Electronic- hookah have a harmful toxic effect that can promote worse oxidative stress, especially on liver tissue, which could elevate the risk for the development of liver diseases in a long time.

INTRODUCTION

Electronic- hookah (E-hookah) is a tool that could produce a vape inhaled with by the users, belong to electronic nicotine delivery system, that is a battery-vaping system that can evaporates a liquid, without flavour or flavoured and may incorporate nicotine [1]. Several types of research assessed health outcomes of electronic cigarettes (E-cigarettes) that are nonetheless underneath debate, seeing that they emerged in 2007 to US marketplace [2], while there were very minimal studied on E-hookah that debuted in 2014 throughout US marketplaces [3]. Although E-hookah is much like E-cigarettes in terms of applying harmful effects on health isn’t recognized but, to date, studies on E-hookah outcomes is limited. It is important to mention that in spite of the reality that almost all E-hookahs are categorized “nicotine-free,” their safety is yet unknown. It is unfortunate that restrictions of age on the packaging of E-hookah apply simplistic to fifty percent of the products [4], accordingly facilitating their use by way of minors, accordingly enabling their utilize by way minors. Concerning the E-hookah use, that could be accepted to form equal toxicity as E-cigarettes. E-hookah evolving nature supports the belief that examining their prevalence, and harmful health outcomes are essential [3]. While, the experts of public health should inform the people about the possible health hazards of these products that unknown yet, whilst policymakers should limit their access to youth.

Nicotine is the tobacco plant alkaloid derived. The mouth mucosal, nose and airways can absorb the nicotine and then transport to the bloodstream. In the human body, nicotine is absorbed into several organs till it's far ultimately detoxified via the liver [5&6]. The liver consists of cytochrome P450 system it is a microsomal enzyme system which detoxifies and metabolizes most chemical pollutants and drugs [7- 9]. Then those toxins are removed by the kidneys, to prevent dangerous substances accumulation in the human body. Also, nicotine prompts an elevated in reactive oxygen species (ROS) or free radicals levels, which causes oxidative stress, and causes tissue damage [10&11]. Also, the nuclear transcription factor kappaB (NF-kappa B) is activating by nicotin, that’s worried in various biological approaches such as cell death and inflammation [12]. So that in the current study, we aim to examine the oxidative stress induced by the vapours of Electronic- hookah on mice liver tissues.

MATERIAL AND METHODS

Animals and experimental design

A total of twenty albino male mice aged 2-3 months (22-30 g) were assigned. Albino male mice cages were placed in a well-ventilated thermostatically regulated room (21 ± 2°C), relative humidity (40% to 60%), and (12:12) light-dark cycles. Following one week of adaption, mice were divided into two groups -randomly- (n=10): control group, and E-hookah group. Mice of E-hookah group were placed in plastic containers and connected to the exposure apparatus and E-hookah smoking device which designed according to [13], the animals were exposed to vapours of commercial E- hookah (Vape SIDIAB 45w, Pakistan), Cartridges containing 400ul of 3% nicotine solution obtained from local market with fruits flavoring - for one hour exposure period every day for 30 days. During each exposure period, the mice in the E-hookah group were exposed to vapours from E- hookah for 20 min, followed by a 5 minutes rest-time (during which the air of room was delivered to protect from hypoxia). At each 20 min period of smoking the actuate of a machine was set to cycles with hookah suction periods of 15 second and rest periods of 15 second (the total time approximately 60 minutes). The bodies weights of the mice were measured at the beginning of the experiment, and on the last day of the
At the ending of the experiment, the mice were anesthetized with ketamine-xylacine and sacrificed. The internal organs (liver, spleen, and kidney) were removed, washed in by normal saline, then dried and weighed to calculate the relative weights of organ (organ weight/body weight%). The livers after weighing were frizzed in liquid nitrogen for further analyses.

**Chemical analyses**

The samples of Liver homogenate were collected for biochemical tests after the preparation according to the procedure that provided by Elabscience colorimetric assay kits and evaluated by the same assay kits for: malondialdehyde (MA), total protein carbonyl (TC), and the content of nitrite in addition to nitrate (CNO).

**Ethics declarations**

This study was licensed by way of the ‘Central Committee for Bioethics’ in the college of Sciences- University of Kufa, Iraq.

**Statistical analysis**

The data of the present study were statistically analyzed using the SPSS program (version 21, IBM Corporation, Somers, NY). The independent t-test analysis was carried out to evaluate the difference between the studied groups. The p value less than 0.05 was counted statistically significant result.

**RESULTS**

The special effects of the vapors of E-hookah on the final body weight, the relative weight of liver, kidney, and spleen of animals with vapors of E-hookah, and the control group are shown in table 1. The current study results highlight significant differences between the two groups: the relative weight of the liver and relative weight of spleen. No significant differences in total body weight and relative kidney weight were pointed.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control Group N=10</th>
<th>E-hookah Group N=10</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBW (mg)</td>
<td>24.227 ± 1.987</td>
<td>23.321 ± 0.087</td>
<td>0.6542</td>
</tr>
<tr>
<td>RLW (%)</td>
<td>4.932 ± 0.329</td>
<td>6.829 ± 0.501</td>
<td>0.0054</td>
</tr>
<tr>
<td>RKW (%)</td>
<td>1.092 ± 0.621</td>
<td>0.843 ± 0.205</td>
<td>0.7078</td>
</tr>
<tr>
<td>RSW (%)</td>
<td>0.982 ± 0.219</td>
<td>2.931 ± 0.403</td>
<td>0.0005</td>
</tr>
</tbody>
</table>

TBW: Total Body Weight (mg), RLW: Relative Liver Weight (%), RKW: Relative Kidney Weight (%), RSW: Relative Spleen Weight (%), Data are expressed as mean ± Standard Error.

Oxidative stress levels were determined through the assessment of oxidative end products of proteins; MA, TC, and CNO in liver mice tissue of the two studied groups. All of the estimated oxidative damage products (TC, MA, and CNO) were significantly higher (P<0.0001) in the E-hookah group when compared to the control animals as in table 2.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control group</th>
<th>E-hookah group</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MA (ng/mg protein)</td>
<td>2.985 ± 0.501</td>
<td>6.021 ± 0.4.91</td>
<td>0.0004</td>
</tr>
<tr>
<td>TC (μmol/mg protein)</td>
<td>30.182 ± 4.201</td>
<td>60.966 ± 3.201</td>
<td>0.0001</td>
</tr>
<tr>
<td>CNO (μmol/g tissue)</td>
<td>0.098 ± 0.081</td>
<td>0.398 ± 0.027</td>
<td>0.0025</td>
</tr>
</tbody>
</table>

MA: malondialdehyde, TC: Total protein carbonyl, CNO: nitrite + nitrate, Results are expressed as mean ± Slandered Error.
DISCUSSION
ENDS is electronic nicotine delivery structures. They are a brand new quickly growing global epidemic [1]. Recently, E-hookahs, have elevated in recognition in Iraq, with the best uptake via younger male, who recommend marketing claims that these merchandise are more secure alternatives to smoking of traditional hookah. Distinct from other ENDS such as E-hookah and E-cigarettes, bowls are utilized via traditional waterpipes, permitting the vapours-holding flavourings, propylene glycol, aerosolized, nicotine, and, glycerin push over a water-filled basin, prior it is inhaled by the employer’s mouth [14]. Sharing E-hookah popularity is the faith that flavoured smokes of E-hookah are detoxified as it pushes across the water-filled basin, making E-hookah a secure tobacco alternative. Nonetheless, an E-hookah bowl delivers flavoured nicotine by creating a vapour of fine less than 2.5 μm, and particles less than 0.1 μm that might induce vascular toxicity [15].

As the liver is actually tending to oxidative damage, concerning its response to toxic materials, this study evaluated lipid and protein oxidation end products, and antioxidant status in liver tissues of mice exposed to vapours of E-hookah. This is the first study that has compared oxidative stress induced by the vapours of E-hookah. As a pointer of the E-hookah toxicity, we measured the changes in total body weight of mice, after one month. this study results show there are no significant difference in body weight with the respective control group. In addition to that the relative organ weights were calculated. It is an important requirement to indicate the effect of xenobiotics present in E-hookah on certain organs between treated group and control group. So, the fresh weights of livers, kidneys, and spleen of mice were measured for recording of relative organ. The differences were significant in the relative liver weights and spleen of mice between E-hookah group and their respective control group. Organs weight can be the most sensitive indicator of an experimental compound [16]. As a significant difference in the weight of organ between exposed and control groups may occur. Actually, a compound that causes reduction in body weight and significant changes in relative weights of most organs has a specific toxic effect on the organs. So, the results of the current study indicate that E-hookah has a harmful toxic effect.

Generally, the oxidative stress that formed by an equivalent between overproduction of reactive oxygen species and antioxidant defense [17-19]. In the current study, we have shown higher level of MA, TC, and CNO in liver tissue of E-hookah mice as that in control group. The existence of free radicals has some harmful outcomes on macromolecular and cellular components like proteins, and membrane phospholipids [20]. As mentioned above the cell-membrane polyunsaturated fatty acids are the primary targets of reactive oxygen species, that can lead to many damages in the structure and function of the cells [21]. The results of our study, suggest E-hookah vapours used in the present study increases the levels of MA, TC, CNO in liver tissue. These levels in the liver tissue are considered especially sensitive to oxidative stress results from E-hookah.

In our research, we observe a significant change in the liver CNO levels within the group exposuer to E-hookah vapours, possibly due to the variation in the metabolism and/or regulation of CNO in the liver tissue. A reduced release of NO is one of the classical markers of endothelial dysfunction [22].

CONCLUSION
The data of this study suggests that the using of E-hookah may induce oxidative stress in the liver tissue. So, further studies will be necessary to estimated the mechanisms of E-hookah on other body organs.

CONFLICT OF INTEREST
We declare that there is conflict of interest

ETHICAL APPROVAL
The research approved by scientific and ethical committee at our department.

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The research funded by the authors only

REFERENCES


