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# Electronic and Conventional Cigarette Exposure Aggravate Metabolic Parameters in High-Fat Diet-Induced Rats

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## Abstract

**BACKGROUND:** The health implications of the perceived use of electronic cigarettes (e-cigarettes) are safer than conventional cigarettes on metabolic parameters are not clearly understood.

**AIM:** The present study evaluates the metabolic parameters as the impact of cigarette and e-cigarette exposure in high-fat diet (HFD)-induced rats.

**METHODS:** Twenty-four male Wistar rats were divided into four groups: (i) NC: normal control group; (ii) HFD Alone; (iii) HFD + Cig, and (iv) HFD + E-Cig, administered HFD followed by cigarette or e-cigarette exposure, respectively. Six cigarettes stick with nicotine 2 mg/stick and 2 ml of e-cigarette liquid with nicotine 6 mg/ml were used for 25 cycles of exposure. In the end, the rats were sacrificed and obtained blood for metabolic parameter analysis, consisting of lipid profile, glucose, uric acid, urea, creatinine, aspartate transaminase (AST), and alanine transaminase (ALT). Statistical analysis with one-way analysis of variance with *post hoc* was used for high-density lipoprotein (HDL), triglyceride, total cholesterol, glucose, uric acid, urea, and creatinine. Furthermore, Kruskal–Wallis with Mann–Whitney U-test was used for non-parametric data such as low-density lipoprotein (LDL), AST, and ALT.

**RESULTS:** Data of all metabolic parameters were shown a significant increase in the group of HFD Alone, HFD + Cig, and HFD + E-Cig, otherwise HDL levels. Furthermore, HFD + Cig followed by HFD + E-Cig groups was significantly higher compared to HFD Alone group.

**CONCLUSION:** E-cigarettes were shown to be less harmful than conventional cigarettes but did not guarantee that it was safe. Both cigarettes and e-cigarettes aggravated metabolic parameters in HFD-induced rats.

## Introduction

Consumption of a high-fat diet (HFD) has been well understood, leading to lipid profile impairment which is a major risk of atherosclerosis but also promotes the development of type 2 diabetes mellitus (T2DM) and other metabolic problems [1], [2]. Most of the fat consumed is contained in food, especially palm oil used in cooking has high saturated fat and fat content in the food itself. The repeated heating process of palm oil during cooking causes changes in properties through chemical reactions that form hydroperoxide, aldehydes, and reactive oxygen species (ROS) compounds that are further absorbed into fried foods. Thus, these compounds enter the systemic circulation, disrupt lipid profiles, and contribute to cardiovascular disease and metabolic syndrome [3]. The previous studies have revealed disrupting lipid profiles, oxidative stress, and dysfunction of liver lipid metabolism from oxidized palm oil [3], [4]. On the other hand, lipid absorption will be increased by the fat content in the food itself, such as quail eggs, which the yolk has a percentage of cholesterol content more than other poultry eggs [5].

Meanwhile, people will usually smoke after eating for pleasure purposes. Smoking has been associated with lipid profile impairment, T2DM, and other metabolic problems through inflammation and oxidative stress [6], [7], [8]. For example, exposure to cigarettes causes a aggravating of lipid profile by a reduced mechanism of expression of low-density lipoprotein receptor (LDLR) in the liver and increased systemic inflammation [6]. Human studies have also reported that smokers have much higher glucose levels and cause insulin resistance than non-smokers through direct activation of glycogenolysis and hepatocyte gluconeogenesis [9]. In addition, studies in humans reported an association between gout and cigarette use [10]. Heart and kidney function is also aggravated by cigarette use [11].

However, cigarettes have been considered harmful by the public due to the existing scientific evidences so that a new product appears, namely, the electronic cigarette (e-cigarette). This situation is shifting from using conventional cigarettes to e-cigarettes [12]. At the same time, e-cigarettes are considered safer than conventional cigarettes and as part of smoking cessation therapy [13]. Nevertheless, e-cigarette

vapor that has gone through the combustion process also contains as many harmful toxins as conventional cigarettes, such as acrolein, acetaldehyde, and formaldehyde [13], [14]. Recent studies have reported that e-cigarettes contribute to memory and cognitive function deficits and increase inflammatory cytokines [15]. Furthermore, e-cigarettes have also aggravated lipid profiles based on population studies [16]. Studies in Korea reported an association between elevated uric acid and high sensitivity of c-reactive protein (hs-CRP), which indicates that inflammation plays a role in its pathogenesis. Meanwhile, animal studies have reported that the use of e-cigarettes has induced hepatotoxicity and nephrotoxicity [17], [18].

The health implications of the perceived use of e-cigarettes were safer than conventional cigarettes on metabolic parameters are not clearly understood. So far, there have been no studies discussing the effect of smoking exposure combined with HFD consumption on metabolic parameters. Due to the potentially harmful effects of HFD consumption in combination with cigarette or e-cigarette use on health, this study aims to evaluate whether the effect of e-cigarette exposure was safer than conventional cigarettes in HFD-induced rats. In addition, this study will also reveals whether the HFD consisting of a mixture of oxidized palm oil and quail egg yolk succeeded in impairing lipid profile and other metabolic parameters.

## Methods

### *Animals and study design*

The study was conducted in the Laboratory of Physiology, Faculty of Medicine of the Universitas Islam Indonesia (FM UII), Yogyakarta, Indonesia. The estimated minimum sample size was determined using the resource equation formula and resulted in the number of samples used 4–6 rats in each group [19]. There were 24 male Wistar rats, aged 4–5 weeks old, weight 135–175 g were purchased from Laboratorium Penelitian dan Pengujian Terpadu, Universitas Gadjah Mada, Yogyakarta, Indonesia. The rats were housed in two rats/cage at a 21–24°C temperature, 40–70% humidity, and a 12-h light/dark cycle. All rats were received free access to tap water and regular feed containing 60% carbohydrate, 16% protein, 21% vitamins and minerals, and 3% fat. Before starting the treatments, the rats were acclimatized for 7 days then randomly divided into four groups as follows:

1. NC: Normal control group (n = 6)
2. HFD Alone: Given administration of HFD (n = 6)
3. HFD + Cig: Exposed to cigarettes immediately after administration of HFD (n = 6)
4. HFD + E-cig: Exposed to e-cigarettes immediately after administration of HFD (n = 6)

After 6 weeks of treatment, the rats were fasted for 12 h before being sacrificed. They were intramuscularly injected with 50–75 mg/kg of Zoletil® 50 (Virbac SA, Carros, France) as general anesthesia and then collected 2 ml of blood from the heart. The blood was put in an EDTA tube and then centrifuged at 4000 rpm for 10 min to obtain plasma for biochemical analysis. All protocols complied with the ethical principle in the International and National Guideline of ethical standards and procedures approved by the Ethics Committee of the FM UII, Yogyakarta, Indonesia

### *HFD administration*

HFD formulated of the mixture of oxidized palm oil and quail egg yolk (60%:40%) was administered by oral gavage at a 2 ml/rat dose and given regular feed. Palm oil (Indofood, Jakarta, Indonesia) was heated for 5 h at 80–100°C to make oxidized palm oil. Quail eggs were purchased from the local market in Sleman, Yogyakarta, Indonesia.

### *Cigarette and e-cigarette equipment*

The cigarettes were used in this study contain 2 mg nicotine and 29 mg tar per stick (Gudang Garam, East Java, Indonesia). DOVPO MVV II Mech Mod e-cigarette (Dovpo, Guangdong, China) with batteries and atomizers (power 280 W, resistance 0.08–3.5-ohm, operation voltage 6.4–8.4 V, and output voltage 1.0–8.0 V) was used in this study. The e-cigarette refill liquid oat milk blueberry flavored contained 12 mg/ml nicotine, 30% propylene glycol (PG), and 70% vegetable glycerin (VG) (Pahlawan Lima Tujuh, Jakarta, Indonesia). The semi-automatic exposure instrument in this study used C++ programmed Arduino Nano (ATmega328, 5 V, 32 kb flash memory, 16 MHz speed) microcontroller (DigiWare, East Java, Indonesia), the improved, and modified version of the previous studies [15,20].

### *Exposure protocol*

The exposure protocol was the improved and modified version of previous studies [15], [20]. Exposure to cigarettes and e-cigarettes was conducted in the exposure instrument chamber containing six rats/groups. Cigarette exposure was carried out using six cigarettes with a nicotine content of 2 mg/stick at once in one exposure. Meanwhile, exposure to e-cigarettes was used with 2 ml of liquid with a 6 mg/ml nicotine content. Both groups received 25 cycles (1 cycle consisting of a 5-sec puff, 30-sec interval, and 30-sec exhaust) per day of exposure for a total dose of nicotine administered was 12 mg/mL/group (Figure 1).

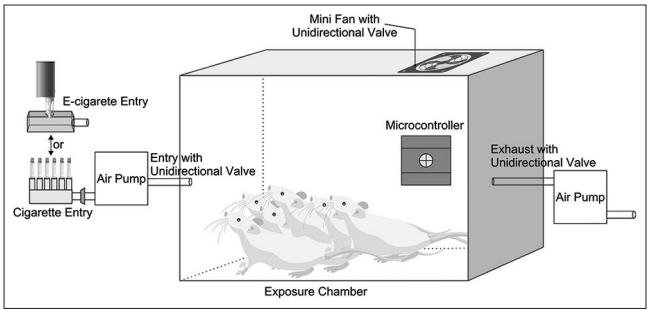


*Figure 1: Exposure instrument chamber. Exposure to cigarettes and e-cigarettes were conducted in the exposure instrument chamber containing six rats/groups. Both groups received 25 cycles (1 cycle = 5-sec puff, 30-sec interval, and 30-sec exhaust) per day of exposure for a total dose of nicotine administered was 12 mg/mL/group*

### *Biochemical analysis*

Plasma lipid profile levels, including high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglyceride, and total cholesterol, were determined using the cholesterol oxidase-peroxidase aminoantypirin (CHOD-PAP) method. Glucose levels in plasma were determined using the glucose oxidase-PAP method. Uric acid levels were determined using the 2,4,5-tribromo-3-hydroxybenzoic acid method. Urea levels were determined using the urease-glutamate dehydrogenase method, and creatinine levels were determined using a kinetic test without deproteinisation according to the Jaffe method. The optimized UV-test method was used to determine aspartate transaminase (AST) and alanine transaminase (ALT) levels. Each parameter was analyzed using commercial reagent kits supplied by DiaSys (Holzheim, Germany) and the procedure followed standard manufacturer's instructions.

### *Statistical analysis*

All data analyses were made using SPSS software version 26 (IBM, Illinois, USA). Shapiro–Wilk test was used for the normality test. Statistical analysis of HDL, total cholesterol, triglyceride, glucose, uric acid, urea, and creatinine levels were determined using one-way analysis of variance followed by Tukey's *post hoc* test for multiple comparisons. Non-parametric data such as LDL, AST, and ALT levels were determined using Kruskal–Wallis followed by the Mann–Whitney U-test. Data were expressed as the mean ± standard deviation of six rats in each group. The data were considered significant if $p < 0.05$.

## Results

### *Neither cigarette nor e-cigarette exposure affected lipid profile in HFD-induced rats*

Cigarette and e-cigarette exposure was aggravating the impairment of lipid profile in rats administered HFD formulated of the mixture of oxidized palm oil and quail egg yolk (Figure 2a-d). After treatment for 6 weeks, HDL, LDL, triglyceride, and total cholesterol levels showed significant results in statistical analysis. Triglyceride and total cholesterol ($p < 0.001$) as well as LDL ($p < 0.01$) levels experienced a significant increase in the HFD + Cig group, followed by the HFD + E-Cig group compared to the NC and HFD Alone groups. They were also increased significantly in the HFD Alone group and compared to the NC group. In contrast, HDL levels were decreased significantly ($p < 0.001$) in HFD + Cig, then HFD + E-Cig groups compared to NC and HFD Alone groups. HDL levels in the HFD Alone group also decreased significantly ($p < 0.05$) compared to the NC group. Compared HDL levels with the HFD + E-Cig group, the HFD + Cig group experienced a significant decrease ($p < 0.001$).

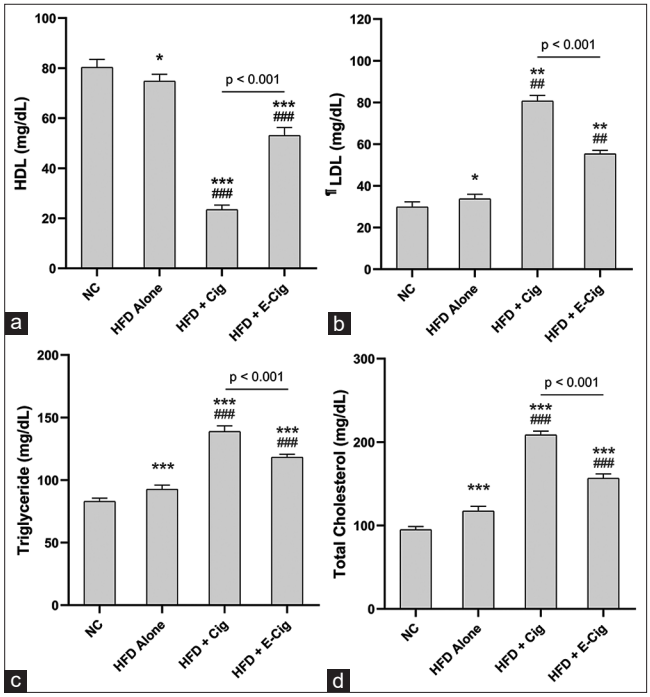


*Figure 2: Lipid profile in normal and treated groups. NC, Normal control; HFD, High-fat diet; HDL, High-density lipoprotein; LDL, Low-density lipoprotein. Data were expressed as the mean ± standard deviation of six rats in each group. Data were analyzed using one-way ANOVA followed by Tukey's post hoc test or non-parametric data using ¶ Kruskal–Wallis followed by Mann–Whitney U-test. *p < 0.05; **p < 0.01; ***p < 0.001 compared with NC group. #p < 0.05; ##p < 0.01; ###p < 0.001 compared with HFD Alone group. (a) HDL, (b) LDL, (c) Triglyceride, (d) Total cholesterol*

### *Aggravating effect of cigarette and e-cigarette exposure on glucose levels of HFD-induced rats*

The administration of HFD plus exposure to cigarettes or e-cigarettes showed increased glucose levels (Figure 3a). The HFD + Cig group rats increased significantly ($p < 0.001$) in glucose levels, followed by HFD + E-Cig group compared to HFD Alone and NC groups. Furthermore, glucose levels were increased significantly ($p < 0.001$) in the HFD Alone group

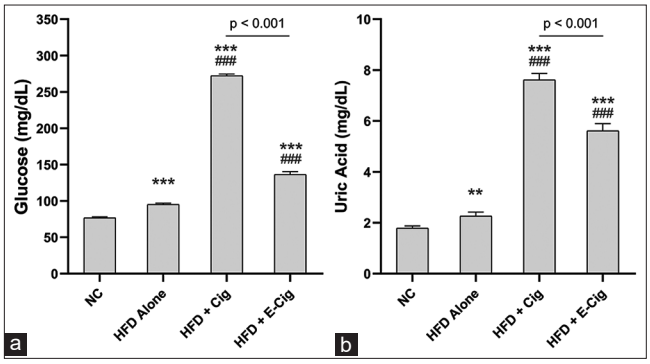


*Figure 3: Glucose and uric acid levels in normal and treated groups. NC, normal control; HFD, high-fat diet. Data were expressed as the mean ± standard deviation of six rats in each group. Data were analyzed using one-way ANOVA followed by Tukey's post hoc test. **p < 0.01; ***p < 0.001 compared with NC group. ##p < 0.01; ###p < 0.001 compared with HFD Alone group. (a) Glucose and (b) uric acid*

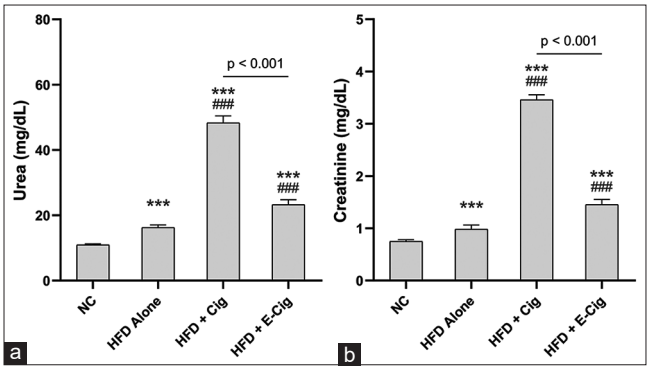


*Figure 4: Urea and creatinine in normal and treated groups. NC, normal control; HFD, high-fat diet; HDL. Data were expressed as the mean ± standard deviation of six rats in each group. Data were analyzed using one-way ANOVA followed by Tukey's post hoc test. ***p < 0.001 compared with NC group. ###p < 0.001 compared with HFD Alone group. (a) Urea and (b) creatinine*

compared to the NC group. Glucose levels were also significantly increased (p < 0.001) in the HFD + Cig group compared to the HFD + E-Cig group.

### *Uric acid was increased after HFD administration and aggravated by cigarette and e-cigarette exposure*

Uric acid levels were increased after exposure to cigarettes or e-cigarettes in HFD-induced rats. A significant increase (p < 0.001) in uric acid level (Figure 3b) was found in the HFD + Cig group, followed by HFD + E-Cig group, compared to NC and HFD Alone groups. The HFD Alone group significantly increased (p < 0.01) uric acid levels compared to the NC group. Furthermore, uric acid levels were significantly increased (p < 0.001) in the HFD + Cig group compared to HFD + E-Cig group.

### *Kidney function parameters were impaired in HFD-induced rats and aggravated by cigarette and e-cigarette exposure*

Urea and creatinine levels in plasma were used to assess kidney function. Exposure to cigarettes or e-cigarettes aggravated the kidney function impairment previously administered by HFD. Urea and creatinine levels were shown similar results (Figure 4a-b). They experienced increased urea and creatinine levels (p < 0.001) in the HFD + Cig group, followed by the HFD + E-Cig group compared to the NC and HFD Alone groups. The HFD group showed a significant increase (p < 0.001) in urea compared to the NC group. Meanwhile, the HFD + Cig group was also significantly increased (p < 0.001) compared to the HFD + E-Cig group.

### *Cigarette and e-cigarette exposure were aggravated liver function in HFD-induced rats*

Liver function was assessed by AST and ALT levels. Exposure to cigarettes or e-cigarettes were able to aggravate the effects of HFD on liver function (Figure 5a-b). A significant increase (p < 0.01) of AST and ALT levels in the HFD + Cig group and the HFD + E-Cig group compared to the HFD Alone and the NC groups. The HFD Alone group showed a significant increase (p < 0.01) in AST compared to the NC group. AST and ALT levels were also significantly increased (p < 0.001) in the HFD + Cig group compared to the HFD + E-Cig group.

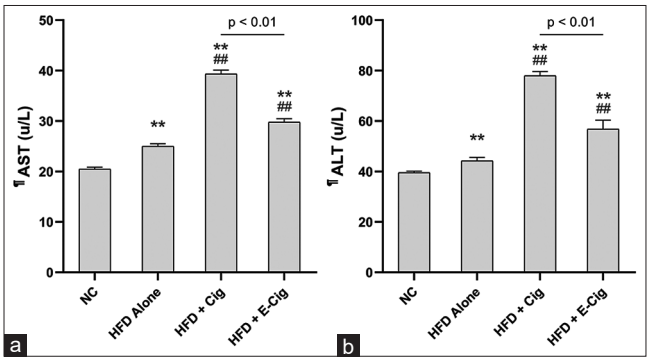


*Figure 5: AST and ALT in normal and treated groups. NC, normal control; AST, aspartate transaminase; ALT, alanine aminotransferase. Data were expressed as the mean ± standard deviation of six rats in each group. Non-parametric data were analyzed using ¶Kruskal–Wallis followed by the Mann–Whitney U-test. **p < 0.01 compared with NC group. ##p < 0.01 compared with HFD Alone group. (a) AST, (b) ALT*

## Discussion

HFD formulated from a mixture of oxidized palm oil and quail egg yolk is revealed to cause profile lipid impairment, increased glucose, uric acid, urea, creatinine, AST, and ALT levels; this finding was in line with previous studies [3], [4], [5]. It is also well known to promote the development of metabolic syndrome,

atherosclerosis, and cardiovascular disease through ROS and inflammation [21], [22]. Meanwhile, cigarettes and e-cigarette exposure aggravated the HFD negative effect. In the present study, rats administered by HFD and continued exposure to cigarettes and e-cigarettes caused impairment of metabolic parameters consisting of lipid profile, glucose, uric acid, liver, and kidney function.

Conventional cigarettes and e-cigarette exposure, which contain nicotine, by inhalation route in this study may differ from the intraperitoneal injection of nicotine as in the previous studies [6], [15], [23]. Especially in the e-cigarette, the liquid combustion can reach high temperatures (>200°C) during the heat-not-burn process that produces low-molecularly toxic aldehydes such as acrolein, acetaldehyde, and formaldehyde [13], [14]. Nevertheless, the present study demonstrates that conventional cigarettes are more harmful than e-cigarettes in HFD-induced rats.

HFD administration is associated with lipid profile impairment and contributes to cardiovascular disease development [21], [22]. In the present study, cigarettes and e-cigarettes exposure for 6 weeks aggravated lipid profile impairment in rats previously administered HFD formulated of the mixture of oxidized palm oil and quail egg yolk. The previous studies support these findings, such as cigarette exposure aggravated lipid metabolism characterized by increased TC, TG, and LDL in treated mice. In accordance, there is a decrease in expression of LDLR in HepG2 cells in the liver and increased inflammation in the endothelial characterized by increased expression of interleukin [IL]-1β and IL-6 in the serum [6]. Studies in humans also report that dual e-cigarette users show increased triglycerides and decreased HDL compared to non-e-cigarette users [16]. These findings indicate that the combined effects of HFD and cigarettes or e-cigarettes exposure promote atherosclerosis and cardiovascular disease development.

Regarding the glucose level, the present study demonstrated that HFD administration and cigarette or e-cigarette exposure are able to increase the glucose level. A consistent result previous study by Liu *et al*. [1] reported that mice given HFD showed increased plasma fasting glucose and insulin levels, lower glucose tolerance, and higher homeostatic model assessment of insulin resistance (HOMA-IR) score. The suggested mechanism of HFD has led to a significant reduction in insulin receptor substrate phosphorylation-1 (IRS-1) in Tyr608 and an increase in Ser307 phosphorylation, indicating IRS-1 inactivation, and caused mitochondrial functional impairment. Furthermore, these changes were accompanied by inflammatory responses in terms of increased expression of nuclear factor kappa-B and inducible nitric oxide synthase and activation of mitogen-activated protein kinase p38 and c-jun n-terminal kinase. Mitochondrial dysfunction and heart damage due to cigarette exposure are associated with insulin resistance and changes in glucose metabolism [24]. Human studies also report that smokers have much higher fasting glucose, insulin, and HOMA-IR index levels than non-smokers [9]. Nicotine content in cigarettes and e-cigarettes was able to induce hyperglycemia directly through activation of glycogenolysis and gluconeogenesis of hepatocytes [25]. The present study's findings reveal that exposure to cigarettes and e-cigarettes in HFD-induced rats causes a more increase in plasma glucose levels and very likely also contributes to insulin resistance and thus the development of the metabolic syndrome and T2DM.

Uric acid was evaluated to increase the HFD and treated group. Lipid profile impairment is associated with increased uric acid. This finding is in accordance with a previous study by Ali et al. [26] reported that increased TG, TC, and LDL levels were positively correlated with increased serum uric acid levels, while HDL decreased. Studies in the adult population in the United States also reported similar results [27]. The use of cigarettes and e-cigarettes also seems to increase uric acid. In accordance, a cross-sectional study in Korea reported that cigarette and e-cigarette use was associated with increased uric acid [10], [28]. The suggested mechanism was that the use of e-cigarettes causes systemic inflammation characterized by an increase in hs-CRP, which contributes to an increase in uric acid levels and a higher risk of hyperuricemia [28]. A Brazilian study also revealed that smokers experienced increased levels of hs-CRP than non-smokers, and smokers with metabolic syndrome had high hs-CRP levels twice [8]. Therefore, cigarette and e-cigarette use in the current study aggravated the uric acid level in rats administered HFD.

When atherosclerosis occurs due to HFD, cigarette or e-cigarette use, it will cause atherosclerosis and reduce the speed of blood flow, affecting kidney function, which is characterized by increased urea and creatinine levels [29]. The findings of this study explain the high levels of urea and creatinine in the group of rats exposed to cigarettes and e-cigarettes previously administered by HFD. In a previous study, a group of rats exposed to six tobacco cigarettes with a nicotine content of 0.8 mg/cigarette for 28 days caused a significant increase in plasma urea and creatinine levels [11]. Cigarette exposure can cause kidney damage both structurally and functionally. The nicotine content in cigarettes can induce kidney damage by increasing intraglomerular pressure, blood pressure, and trigger long-term renal endothelial cell dysfunction [11,30]. The liquid content in e-cigarettes does not drastically affect urea and creatinine levels. This finding is supported by Golli et al. [18], after administration of e-liquid at 18 mg/ml, there was no significant change in urea and

creatinine levels. However, e-liquid can affect structural damage to the kidney. E-liquid consists of PG, VG, and chemical additives flavoring. In a previous study, PG administered intravenously caused acute kidney disease and central nervous system toxicity, while in inhalation, PG caused kidney and liver toxicity. PG can also inhibit renal glucose transport. Moreover, a mixture of PG and VG was able to reduce the survival level of embryonic kidney 293 cells [31]. Both cigarettes and e-cigarettes can affect kidney function, but cigarette does a more significant effect.

The administration of HFD and cigarette or e-cigarette exposure led to fat accumulation in the hepatocyte cytoplasm through mechanisms of increased production of ROS, decreased antioxidants, and increased inflammatory cytokine expression [1], [17], [32]. In the end, liver function was decreased, characterized by the release of AST and ALT in the bloodstream. The release of ALT and AST is also one of the manifestations of non-alcoholic fatty liver disease, non-alcoholic steatohepatitis, and an indicator of liver damage [32]. It was supported by a previous study by El Golli *et al*. [17] that nicotine content in cigarettes and e-cigarettes induced liver damage due to increased oxidative stress increasing ROS and damaging the composition of lipids, proteins, and nucleic acids, thereby causing hepatocyte cell death [18]. In addition, besides nicotine content, e-cigarettes also contain vanillin, ethyl vanillin, ethyl maltol, L-menthol, trans-cinnamaldehyde, isoamyl acetate, PG, and VG, which can reduce the viability of HepG2 cells to accelerate liver fibrosis [33]. In line with the previous elucidation, liver damage caused by HFD administration aggravates cigarette and e-cigarette exposure in the present study.

This study has some limitations. For example, although the number of nicotine cigarettes and e-cigarettes content exposed to rats was the same, the amount of nicotine received by the rats was not evaluated. In addition, we did not convert nicotine levels in humans to rats, so it may be biased into differences in human-rats ratios. Nevertheless, the results of this study are enough to explain the results of the effects of cigarette and e-cigarette exposure in rats previously administered HFD.

## Conclusion

The present study proves that e-cigarettes are less harmful than conventional cigarettes. Nevertheless, being less harmful does not guarantee its safety. Both conventional cigarettes and e-cigarettes contributed to aggravating the evaluated metabolic parameters in HFD-induced rats. Future studies are warranted to evaluate the molecular mechanism of e-cigarette liquid effects on affecting metabolic parameters.

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## Authors' Contributions

NAI conceived and designed the study, collected and organized data, and analyzed and interpreted data. NAI, TN, and ASR conducted research, provided research materials, wrote initial and final draft of article, and provided logistic support. NAI and DNA have critically reviewed and approved the final draft.

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