Original Article

Comparison of Serum Benzo(a)pyrene Diol Epoxide – Protein Adducts Level between Kretek Cigarette Smokers and Nonsmokers and the Related Factors

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Abstract

Background: Benzopyrene is a carcinogenic agent found in cigarette smoke. Benzo(a)pyrene diol epoxide (BPDE) is one of the benzopyrene metabolites. In this study, we investigated the level of serum BPDE in *kretek* cigarette smokers compared to non-smokers. **Methods:** A cross-sectional study which involved 32 "healthy" *kretek* cigarette smokers and 32 "healthy" nonsmokers were conducted. We collected the blood sample and the serum BPDE level was assayed using enzyme-linked immunosorbent assay kit. The BPDE serum level in *kretek* cigarette smokers was compared to the level in nonsmokers. **Results:** A total of 32 *kretek* smokers and 32 controls underwent an examination of the BPDE-protein adducts level. In the *kretek* smokers group, 59.4% were aged over 45 years and 56.3% have a high educational background, while in the control group, 87.5% were aged under 45 years and 75% have high educational backgrounds. The level of BPDE-protein adducts in the *kretek* smokers subject was 12.15 (8.87–33.55) ng/ml and the levels in the control group were 11.4 (3.87–13.27) ng/ml, $P = 0.004$. The factors which influence the levels BDPE-protein adducts in smokers cigarettes, as determined by multivariate analysis, were sucking pattern ($P = 0.002$) and the degree of addiction ($P = 0.047$). **Conclusion:** The serum BPDE-protein adducts level was higher in smokers compared to nonsmokers, and the sucking pattern and degree of addiction are the influencing factors.

Keywords: Benzo(a)pyrene diol epoxide-protein adducts, nonsmokers, smokers

Introduction

Smoking is the main cause of lung cancer in 80%–90% of lung cancer cases. Smokers are 23 times more likely to develop lung cancer than nonsmokers. Cigarettes will burn at high temperatures, produce thousands of chemicals, and cause smokers to inhale a mixture of toxic substances, and most of the substances are carcinogens and toxins.[1-5]

The most widely known components of cigarette smoke are tar, nicotine, and carbon monoxide. Tar contains carcinogenic polycyclic aromatic hydrocarbon (PAH) compounds. One component of PAH, benzo(a)pyrene (BaP), is known as the first chemical carcinogen found. Benzo(a)pyrene diol epoxide (BPDE) is a mutagenic active metabolite of BaP. BaP is declared as carcinogenic to humans (Group 1) in the 2010 International Agency for Research on Cancer (IARC). Cigarettes are one of the sources of high BaP levels in addition to emissions of gas and grilled food. The researchers concluded that BPDE-protein adducts in blood cells can be used as appropriate biological markers of BaP genotoxic exposure and are very promising in assessing human health risks.[1,6,7]

Ludovici *et al*. measured BPDE-protein adducts in 30 subjects divided into three groups including smokers ($n = 10$), former smokers ($n = 5$), and nonsmokers ($n = 15$). The level of BPDE-protein adducts in smokers was 4.46 ± 5.76 ng/ml while

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in nonsmokers was 1.76 ± 1.69 ng/ml.[8] An analytical study conducted by Warouw *et al*., who assessed the levels of BaP in highway road officers, concluded that the average inhalation intake of BaP was 3.9 μg/m^3, and the estimated level of cancer risk was 11.6 new cases per 10,000 population.[9] There has been no study that related to the levels of BPDE-protein adducts in the population of *kretek* smokers and nonsmokers conducted in Indonesia. This study's aim was to compare the levels of BPDE-protein adducts in the population of *kretek* smokers and nonsmokers.

Methods

A cross-sectional study was conducted at the Persahabatan Hospital, Jakarta, Indonesia, from January to March 2016. The sampling was carried out using consecutive sampling. Inclusion criteria including *kretek* smokers and nonsmokers, male, aged 30–60 years who were at the hospital and were willing to voluntarily participate in all research programs by signing the written informed consent. Exclusion criteria were a history of liver disease. Subjects who met the inclusion criteria were then filled out a baseline data questionnaire and Fagerstrom questionnaire underwent physical examination, and blood sampling for the BPDE examination.

The determination of BPDE-protein adducts levels was done by the enzyme-linked immunosorbent assay (ELISA) method using Oxiselect™ BPDE-Protein Adduct ELISA Kit catalog number STA-301 (Cell Biolabs Inc., San Diego, CA). Samples were processed according to the manufacturer's guideline. This research has received ethical approval from the Ethics Committee of the Faculty of Medicine, Universitas Indonesia (Number: 963/UN2.F1/ETIK/2015, date 2 November, 2015).

The data obtained was then be coded, continued by data entry, and verification to be further processed statistically. The descriptive and analytic analysis was carried out. Inferential analysis to determine the differences in BPDE-protein adduct levels between *kretek* smokers and nonsmokers was tested with the unpaired *t*-test or the Mann–Whitney test if it did not meet the parametric test requirements. The Pearson correlation test was used to see the correlation between BPDE-protein adduct levels and CO exhalation and if it did not meet the parametric test requirements then the Spearman correlation test was used. Multivariate analysis was performed to the factors which influence the linear regression test. The statistical analysis was performed using the Statistical Package for Social Science (SPSS) software program version 20 (IBM Corp, Armonk, NY, USA.

Results

The total subjects in this study were 64 respondents, including 32 *kretek* smokers subjects and 32 nonsmoker subjects. Table 1 shows the distribution of sociodemographic characteristics of *kretek* smokers and nonsmokers as a comparison. Most of the age of subjects in the *kretek* smokers group was >45 years while in the nonsmoker group was <45 years. The education levels of respondents in both the groups were mostly highly educated. The types of occupations in the two groups were mostly working with no high risk of exposure to BaP. The neighborhoods in both groups mostly lived in environments with no high risk of BaP exposure. Table 2 shows the characteristic distribution of subjects' smoking habits in *kretek* smokers. Smokers who consume cigarettes >10 cigarettes/day were more common in *kretek* smokers group and shallow vaping was more common in *kretek smokers*. Most smokers had a moderate Brinkman Index. The degree of addiction was evaluated with the Fagerstrom questionnaire and most smokers had a low degree of addiction or dependency. The last time smoking for all smokers was <8 h before sampling.

Table 1: Distribution of kretek smokers and nonsmokers subjects based on sociodemographic characteristics

Variables	Smokers, *n* (%)	Nonsmokers, *n* (%)
Age (years)		
<45	13 (40.6)	28 (87.5)
>45	19 (59.4)	4 (12.5)
Education		
Low educated	14 (43.7)	8 (25)
High educated	18 (56.3)	24 (75)
Occupation		
Not high risk for BaP exposure	21 (65.6)	30 (93.7)
High risk for BaP exposure	11 (34.4)	2 (6.3)
Environment		
Not high risk for BaP exposure	26 (81.2)	29 (90.6)
High risk for BaP exposure	6 (18.8)	3 (9.4)

BaP: Benzo(a)pyrene

The level of BPDE-protein adducts in *kretek* smokers and nonsmokers were determined initially. As shown in Table 3, the levels of BPDE-protein adducts in kretek smokers were significantly higher than the levels of BPDE-protein adducts in the nonsmokers.

Next, we analyzed the BPDE-protein adducts based level on the sociodemographic characteristics. No significant differences of BPDE-protein adduct levels based on age, education level, and environment, of both kretek group and nonsmokers group, were reported [Table 4]. However, in the *kretek* group, subjects with a high risk of BaP exposure occupation has a statistically significant higher of BPDE-protein adducts levels as compared to those who have not high risk of BaP exposure.

We then determined the level of BPDE-protein adducts based on smoking habits [Table 5]. The BPDE-protein adducts levels were not statistically different in subjects based on the number of cigarettes/day, Brinkman Index, and addiction level according to the Fagerstrom questionnaire. However, subjects with deep cigarette sucking pattern have higher BPDE-protein adducts levels as compared to subjects with the vaping pattern. Furthermore, subjects who had last smoke of fewer than 8 h showed significantly higher BPDE-protein adducts levels as compared to those who had lest smoke of 8 h or more.

Table 2: Distribution of kretek smoker subjects based on the smoking habits characteristic

Variables	*Kretek* smokers, *n* (%)
The number of cigarettes/days	
1–10	9 (31.3)
>10	23 (68.7)
Brinkman index	
Mild	8 (25)
Moderate-heavy	24 (75)
Sucking pattern	
Shallow	20 (62.5)
Deep	12 (37.5)
Addiction level	
Low	23 (71.9)
High	9 (28.1)
Last time smoking (h)	
<8	20 (62.5)
≥8	12 (37.5)

Table 3: Levels of benzo(a)pyrene diol epoxide protein adducts in kretek smokers and nonsmokers group

Variables	BPDE levels (ng/ml)	
	Median (min-max)	*P*
Smokers	12.15 (8.87–33.55)	0.004
Nonsmokers	11.4 (3.87–13.27)	

BaP: Benzo(a)pyrene, BPDE: Benzo(a)pyrene diol epoxide

The multivariate analysis was conducted to determine the sociodemographic factors and smoking habits which influence BPDE-protein adducts levels in all *kretek* smoking groups by including factors with a value of <0.25 from the table of bivariate analysis test results. The multivariate analysis was carried out on factors of age, environment, occupation, sucking pattern, degree of addiction, number of cigarettes/day, and the Brinkman Index. In Table 6, the results of multivariate tests with backward regression linear methods found that the most influential factor on BPDE-protein adducts levels in *kretek* smokers was a vaping pattern ($P = 0.002$) and addiction degree ($P = 0.001$).

Discussion

In this study, we studied the level of serum BPDE in *kretek* cigarette smokers compared to nonsmokers. The age distribution of subjects in this study was almost the same as reported in the Indonesian Basic Health Research (*Riset Kesehatan Dasar*, Riskesdas).[10] Christen *et al*.[11] found that male *kretek* smokers averaged between the ages of 19–40 years. Studies by Marie *et al*.[12] and Ngahane *et al*.[13] also found that male *kretek* smokers were dominant at productive age.

The level of education in this study was almost the same as the study by Zhu *et al*.[14] which reported that the average smokers even had a moderate-high level of education but the level of education, either low education or high education, basically did not have a direct relationship with a person's smoking habits. A study by Chatterjee *et al*.[15] in 2011 stated that clusters or types of education have a relationship with smoking status. According to Alexopoulus *et al*.,[16] those who have a health-associated educational background tend not to smoke.

We found that respondents who work in an occupation with no high risk of BaP exposure were more likely to smoke. In contrast, a previous study reported that most of the patients who smoked worked in an industry with a risk of BaP exposure.[17] Furthermore, Rengrajan, *et al*.[18] found that respondents who worked with a high risk of BaP exposure have a habit of smoking. In our study, both the *kretek* smokers and nonsmokers group were mostly lived in environments with no high risk of BaP exposure. However, Wu *et al*.[19] stated that the high risk of diseases, including lung cancer, was because patients were living in a high-risk environment and also had a smoking habit.

In our study, the level of BPDE-protein adducts in *kretek* smokers was significantly higher than the levels of BPDE-protein adducts in nonsmokers. Ding *et al*.[20] stated that the BPDE level in *kretek* smokers was higher than other smokers and nonsmokers. The review conducted by Hecht *et al*.[21] found that the level of BPDE-protein adducts in smokers was 20–40 ng/ml and there was a significant relationship between smokers and increased levels of BPDE which cause lung cancer. Kaiserman *et al*.[22] found the average value of the BPDE level in *kretek* smoker respondents was 24.7 ng with a range of 3.36 ng to 28.39 ng.

A study by Campo *et al*.[23] stated that the range of BPDE-protein adducts levels in nonsmokers was 1.2-1.9 ng/m^3. Neal *et al*.[24] explained that the average level of BPDE-protein adducts in 10 nonsmokers was lower (0.03 ± 0.01 ng/ml). Rojas *et al*.'s[25] study found that in 18 nonsmokers, the levels of BPDE-DNA adducts were lower than smokers and former smokers, although it was not explained the difference in average levels. In our study, BPDE-protein adducts levels of nonsmokers were slightly higher than in previous studies. Our result is different from the previous study where nonsmokers' subjects were included from rural areas which may have less air pollution.

In terms of age, our result was similar to the study by Ledesma *et al*.[26] who found that age was not associated with increased BPDE levels in smokers including *kretek* smokers. A study by Wang *et al*.[27] stated that a person's age was not associated with an increased toxicity effect from *kretek* cigarettes but an increase in toxicity levels including BaP, depending on the number of *kretek* cigarettes per day and the length of time that a person smokes.

The education level is still debated as a variable that affects BPDE-protein adducts levels on smokers' respondents. Koning *et al*.[28] stated that the higher education level increases awareness which reduces the smoking hazards. Zhang *et al*.[29] stated that respondents who had higher education levels were aware not to smoke to maintain quality of life.

In our study, no statistically significant relationship was found between the high-risk environment of BaP exposure

Table 4: Levels of benzo(a)pyrene diol epoxide protein adducts in kretek smokers and nonsmokers group protein adducts in study subjects based on sociodemographic characteristics

Variabels	BPDE-protein adducts levels (ng/ml)			
	Kretek smokers (Mean±SD)	*P*#	Nonsmokers (Mean±SD)	*P*#
Age (years)				
<45	11.94±3.30	0.177#	10.79±5.28	0.512#
≥45	13.76±6.31		11.22±2.69	
Education level				
Low-moderate	13.23±5.05	0.268#	10.74±7.79	0.598#
High	12.31±4.52		10.99±1.57	
Environment				
Not high risk of BaP exposure	11.40±3.85	0.698#	10.38±2.77	0.493#
High risk of BaP exposure	14.11±5.24		11.10±3.89	
Occupation				
Not high risk of BaP exposure	11.70±3.21	0.024#	10.20±2.26	0.397#
High risk of BaP exposure	16.27±7.40		14.31±5.88	

#Mann–Whitney test. BaP: Benzo(a)pyrene, BPDE: Benzo(a)pyrene diol epoxide

Table 5: The benzo(a)pyrene diol epoxide protein adducts level based on smoking habits

Variables	BPDE-protein adducts levels (ng/ml)		
	n	Medium (min-max)	*P*
Cigarettes/day			
1–10	9	11.40 (9.32–18.45)	0.132#
>10	23	12.62 (8.87–33.55)	
Brinkman index			
Light	8	11.99 (9.80–13.95)	0.101#
Moderate-heavy	24	12.95 (8.87–33.55)	
Sucking pattern			
Shallow	20	11.99 (8.87–17.66)	0.002#
Deep	12	16.55 (11.40–33.55)	
Addiction level			
Low-moderate	23	11.99 (8.87–22.44)	0.088#
Heavy	9	12.95 (9.80–33.55)	
Last time smoking (h)			
<8	20	12.95 (8.87–33.55)	0.048#
>8	12	11.99 (9.32–15.38)	

#Mann–Whitney test. BPDE: Benzo(a)pyrene diol epoxide

Table 6: Factors which influence the benzo(a)pyrene diol epoxide-protein adducts levels in kretek smokers

Variables	*B*	SE	*P*
Occupation	3.291	1.630	0.053
Sucking pattern	5.707	1.630	0.002
Addiction level	3.659	1.758	0.047
Constant	10.14		

Multivariate test with backward regression linear method. BPDE: Benzo(a)pyrene diol epoxide, SE: Standard of error

and BPDE-protein adducts levels in both populations. This is probably because the subjects in our study mostly lived in environments that were not at high risk of BaP exposure. The Public Health England review explained that high-risk environments such as industrial zones, household wood burning, coal, motor vehicle exhaust emissions, and all the smoke produced from organic materials burning including cigarette smoke and in grilled foods with charcoal provide increased levels of PAH (benzopyrene).[30]

The sucking patterns of *kretek* smoking in this study were among the most influential factors on the BPDE level of protein adducts statistically. Our results were in line with the study of Ding *et al*.,[31] which stated that the pattern of sucking or behavior of patients in smoking was also one of the factors that increase the levels of BaP. They found that the smoking patterns of smokers that reach the inner volume will increase the levels of BaP and nicotine.

The results of the multivariate analysis found a significant relationship between increasing BPDE levels and nicotine dependence levels. Almost the same as the previous study who found the degree of *kretek* smoking addiction by 46.4% and an increase in nicotine levels would increase the level of toxicity of other ingredients.[13] *Kretek* cigarettes were the main source of exposure to BaP and exposure to nicotine which affects the degree of smoker dependence.[32] Therefore, reducing BaP exposure will help to reduce nicotine levels which certainly affects one's addiction.

In our study, the last time smoking <8 h had a higher mean BPDE level although it did not reach statistical threshold. Robins *et al*.[33] reported that environmental tobacco smoke that deposits from cigarettes in the lung (such as tar and BaP) occurred 8 h after smoking. Meanwhile, the European Food Safety Authority report stated that f BPDE levels of *kretek* smoke exposure were up to 10 ng/m^3 for 5 h per day and BaP levels were higher from additional passive smokers around 40 ng/day.[34]

Conclusion

The BPDE-protein adducts level in *kretek* was different between smokers and nonsmokers. The factors that influence the levels of BPDE-protein adduct in *kretek* smokers were

vaping patterns and the addiction degree can be important for health promotion and therapy intervention. Further research can be explored to investigate the BPDE-protein adducts level as biomarkers for predictive or prognostic factor.

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Conflicts of interest

There are no conflicts of interest.

References

1. Samet JM, Gupta PC, Ray CS, Sauvaget C, Winn DM. Tobacco smoking and smokeless tobacco use. In: Stewart BW, Wild CP, editors. World Cancer Report 2014. France: The International Agency for Research on Cancer; 2014. p. 88-95.
2. World Health Organization. Cancer Fact Sheet. Geneva: World Health Organization; 2015. Available from: http://www.who.int/.mediacentre/factsheets/fs297/en. [Last accessed on 2015 Apr 05].
3. Forman D, Ferlay J, Stewart BW, Wild CP. The global and regional burden of cancer. In: Stewart BW, Wild CP, editors. World Cancer Report 2014. France: The International Agency for Research on Cancer; 2014. p. 16-53.
4. Jemal A, Murray T, Ward E, Samuels A, Tiwari RC, Ghafoor A, *et al*. Cancer statistics, 2005. CA Cancer J Clin 2005;55:10-30.
5. Hoffmann D, Djordjevic MV, Hoffmann I. The changing cigarette. Prev Med 1997;26:427-34.
6. World Health Organization International Agency for Research on Cancer. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. France: International Agency for Research on Cancer; 2004. p. 49-263.
7. Hoffmann D, Hoffmann I, El-Bayoumy K. The less harmful cigarette: A controversial issue. a tribute to Ernst L. Wynder. Chem Res Toxicol 2001;14:767-90.
8. Lodovici M, Akpan V, Giovannini L, Migliani F, Dolara P. Benzo[a] pyrene diol-epoxide DNA adducts and levels of polycyclic aromatic hydrocarbons in autoptic samples from human lungs. Chem Biol Interact 1998;116:199-212.
9. Warouw SP, Kusnoputranto H, Karim F, Musadad A, Anwar A, Sukana B, *et al*. Analysis of benzopiren levels among highway officers [Studi analisis kadar benzopiren pada petugas jalan tol]. Dissertation. Jakarta; 2008.
10. Center for Health Research and Development [Badan Penelitian dan Pengembangan Kesehatan]. Basic Health Research 2010 [Riset Kesehatan Dasar 2010]. Ministry of Health, Republic of Indonesia [Kementerian Kesehatan Republik Indonesia], Jakarta, Indonesia; 2010. Available from: http://kesga.kemkes.go.id/images/pedoman/Riskesdas%202010%20Nasional.pdf. [Last Accessed on 2020 April 04].
11. Christen WG, Glynn RJ, Manson JE, Ajani UA, Buring JE. A prospective study of cigarette smoking and risk of age-related macular degeneration in men. JAMA 1996;276:1147-51.
12. Ng M, Freeman MK, Fleming TD, Robinson M, Dwyer-Lindgren L, Thomson B, *et al*. Smoking prevalence and cigarette consumption in 187 countries, 1980-2012. JAMA 2014;311:183-92.
13. Mbatchou Ngahane BH, Atangana Ekobo H, Kuaban C. Prevalence and determinants of cigarette smoking among college students: A cross-sectional study in Douala, Cameroon. Arch Public Health 2015;73:47.
14. Zhu BP, Giovino GA, Mowery PD, Eriksen MP. The relationship between cigarette smoking and education revisted: Implications for categorizing persons's educational status. Am J Public Health 1996;86:1582-9.
15. Chatterjee T, Haldar D, Malik S, Sarkar GN, Das S, LahiRI SK. A study on habits of tobacco use among medical and non-medical student of Kolkata. Lung India 2011;28:5-10.
16. Alexopoulos EC, Jelastopulu E, Aronis K, Dougenis D. Cigarette smoking among university students in Greece: A comparison between medical and other students. Environ Health Prev Med 2010;15:115-20.
17. The WHO European Centre for Environment and Health. WHO Guidelines for Indoor Air Quality. Selected Pollutants. World Health Organization Regional Office for Europe, Bonn; 2010. Available from: https://www.euro.who.int/__data/assets/pdf_file/0009/128169/e94535.pdf.[Last Accessed on 2020 May 06].
18. Rengrajan T, Rajendran P, Nandakumar N, Lokeshkumar B, Rajendran P, Nishigaki L. Exposure to polycyclic aromatic hydrocarbons with special focus on cancer. Asia Pac J Trop Biomed 2015;5:182-9.
19. Great Lakes Commission. An assessment of Benzo(a)Pyrene air Emissions in the Great Lakes Region. International Emission Inventory Conference; 16 August, 2012. Available from: https://www3.epa.gov/ttnchie1/conference/ei20/session10/asoehl_pres.pdf. [Last Accessed on 2020 May 06].
20. Ding YS, Zhang L, Jain RB, Jain N, Wang RY, Ashley DL, *et al*. Levels of tobacco-specific nitrosamines and polycyclic aromatic hydrocarbons in mainstream smoke from different tobacco varieties. Cancer Epidemiol Biomarkers Prev 2008;17:3366-71.
21. Hecht SS. Tobacco smoke carcinogenesis and lung cancer. J Nation Cancer Inst 1999;91:1994-2000.
22. Kaisermann MJ, Rickerts WS. Carcinogenesis in tobacco smoke: Benzo(a)Pyrene from Canadian cigarette and Cigarette tobacco. Am J Public Health 1992;82:1023-6.
23. Campo L, Vimercati L, Carrus A, Bisceglia L, Pesatori AC, Bertazzi PA, *et al*. Environmental and biological monitoring of PAHs exposure in coke-oven workers at the Taranto plant compared to two groups from the general population of Apulia, Italy. Med Lav 2012;103:347-60.
24. Neal MS, Zhu J, Foster WG. Quantification of benzo[a] pyrene and other PAHs in the serum and follicular fluid of smokers versus non-smokers. Reprod Toxicol 2008;25:100-6.
25. Rojas M, Cascorbi I, Alexandrov K, Kriek E, Auburtin G, Mayer L, *et al*. Modulation of benzo[a] pyrene diolepoxide-DNA adduct levels in human white blood cells by CYP1A1, GSTM1 and GSTT1 polymorphism. Carcinogenesis 2000;21:35-41.
26. Ledesma E, Rendueles M, Díaz M. Benzo(a)pyrene penetration on a smoked meat product during smoking time. Food Addit Contam Part A Chem Anal Control Expo Risk Assess 2014;31:1688-98.
27. Wang SS, Samet JM. Tobacco smoking and cancer: The promise of molecular epidemiology. Salud Publica Mex 1997;39:331-45.
28. Koning P, Webbink D, Martin NG. The Effect of Education on Smoking Behaviour: New Evidance from Smoking Duration of a Sample of Twins. IZA Discussion Papers 4796, Institute of Labor Economics (IZA);2010. https://ideas.repec.org/p/iza/izadps/dp4796.html. [Last Accessed on 2020 April 08].
29. Zhang XY, Liang J, Chen DC, Xiu MH, He J, Cheng W, *et al*. Cigarette smoking in male patients with chronic schizophrenia in a Chinese population: Prevalence and relationship to clinical phenotypes. PLoS One 2012;7:e30937.
30. Public Health England. Polycyclic Aromatic Hydrocarbons (Benzo(a) Pyrene) Toxicology Overview. PHE Centre for Radiation, Chemical and Environmental Hazards, UK; 2018. Available from: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/737017/PAH_TO_PHE_240818.pdf. [Last Accessed on 2020 April 20].
31. Ding YS, Ward J, Hammond D, Watson CH. Mouth-level intake of benzo[a] pyrene from reduced nicotine cigarettes. Int J Environ Res Public Health 2014;11:11898-914.
32. Andrade LF, Alessi SM, Petry NM. The effects of alcohol problems and smoking on delay discounting in individuals with gambling problems. J Psychoactive Drugs 2013;45:241-8.
33. Robins JM, Blevins D, Shneiderman M. The effective number of cigarette inhaled daily by passive smokers: Are epidemiologic and dosinetric estimate consistent. J Hazard Mater 1990;21:215-38.
34. Alexander J, Benford D, Cockburn A, Cravedi JP, Dogliotti E, Domenico AD, *et al*. Scientific opinion of the panel on contaminants in the food chain on a request from the European commission on polycyclic aromatic hydrocarbons in food. EFSA J 2008;724:1-114.