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TOXICOLOGICAL ASSESSMENT OF MAINSTREAM EMISSIONS FROM TOBACCO HEATING SYSTEM COMPARED TO CONVENTIONAL CIGARETTES AND BEEDIS

Toxicology

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ABSTRACT

This research article evaluates the toxicological impact of mainstream emissions from a Tobacco Heating System (THS) in comparison to conventional cigarettes (CIG) and beedis (BDI). The study highlights the distinct advantage of THS in significantly reducing concentrations of toxicants in its mainstream aerosol due to its low operating temperature, when compared with the mainstream smoke of CIG and BDI. Notably, the reduction rates for carcinogenic toxicants surpass 84% compared to CIG and exceed 69% compared to BDI. The assessment of cytotoxic and genotoxic potencies through NRU and Comet assay methods reveals a substantial decrease in the mainstream aerosol of THS compared to the mainstream smoke of CIG and BDI. These findings underscore the potential harm reduction associated with the use of THS, indicating a marked decrease in the release of harmful substances and a significant improvement in the cytotoxic and genotoxic profiles, thereby contributing valuable insights to public health discourse and regulatory considerations in the context of alternative tobacco products.

KEYWORDS

Cigarette, Cytotoxicity, Comet Assay, Heated Tobacco Products, Toxicity, Toxicants

INTRODUCTION

Tobacco smoking is the leading cause of preventable death and is a major risk factor of cancer, respiratory disease, and cardiovascular disease [1]. Different tobacco products pose varying levels of health risk to users and tobacco emissions are considered carcinogenic to humans (Group 1) by the International Agency for Research on Cancer (IARC) [2]. The chemical composition of the mainstream emissions of tobacco product is complex and is mainly influenced by the type of tobacco, smoking pattern, design of smoking device, presence or absence of filters, types of additives added and also by multiple thermolytic processes including combustion, pyrolysis, and distillation that occur within the confines of the smoking device [3, 4]. As a result, chemical composition, and concentration of individual chemicals in mainstream emissions vary [4]. Though all tobacco products that are smoked deliver considerable concentration of established toxicants to the users, combustible tobacco products such as cigarettes (CIG) and beedis (BDI) that burn tobacco, are the most harmful [5, 6]. Toxicants in mainstream emissions of CIG [3] and BDI [7] such as tobacco-specific nitrosamines, polycyclic aromatic hydrocarbons (PAHCs), volatile organic compounds (VOCs), aldehydes, amines and heavy metals have toxicological properties and can cause health hazards such as cancer, respiratory and cardiovascular diseases [8]. Although quantitative and qualitative relation between reduced exposure to toxicants and reduction in health risks have not been established, reduced exposure to these toxicants has been recommended by various health authorities, including the World Health Organization [9].

The health hazards caused due to smoking have led to the introduction of alternative nicotine delivery products, one of which is tobacco heating systems (THS) or IQOS by Philip Morris International (PMI) [10, 11]. IQOS, a heated tobacco product (HTP), has become popular around the world in recent years as a less harmful alternative to CIG [12]. It is the first HTP authorized through the MRTP framework with “exposure modification order” [13]. THS are new type of tobacco products with limited toxicological data. Unlike conventional CIG that burn tobacco at 850℃, THS heat reconstituted tobacco to about 350℃, instead of burning [14]. Due to this operational difference, THS emissions contains fewer and lower toxicants compared to CIG and BDI emissions, hence, it has established itself as a safer alternative to conventional smoking [15, 16]. To support the health claims of THS, the manufacturers have published several reports [16-20] including few independent, systematic researches [21, 22]. THS are becoming increasingly popular among former and current smokers and among the youth aged 15 to 24 years [23]. Despite increasing number of THS users, the risk assessment and scientific evidence of safety of THS is limited. Thus, the present study evaluates the in vitro toxicity of mainstream emissions from tobacco heating system. Concentration of toxicants in the mainstream emission of THS were chemically analysed using liquid and gas chromatography alone or with mass detector. To further extend the toxicological evaluation, the cytotoxicity and genotoxicity of THS emissions was evaluated by Neutral Red Uptake (NRU) and Comet assay, respectively. The comparison is made with mainstream emissions from CIG and BDI. The purpose of this study was to strengthen our understanding of THS as the potentially reduced risk product.

MATERIALS AND METHODS

Materials

Tobacco products compared were THS, CIG and BDI. IQOS ILUMA and TEREA Smartcore Sticks™ (Philip Morris International, USA) was selected as THS because it is the largest selling and first commercially available THS [10]. TEREA features an electromagnetic core that gently heats tobacco stick from inside [14]. CIG and BDI studied were the most commonly available brand in the country. All the reagents used were of analytical grade and were purchased from Merck (Mumbai, India). Cytotoxicity and genotoxicity tests were outsourced to Life Technologies (India) Pvt Ltd (New Delhi, India).

Sample Preparation Of Tobacco Products

Before commencement of the experiment, the TEREA sticks, CIG and BDI were conditioned at 22°C and 60% relative humidity for 48 h. A modified, handy smoke extraction assembly, IAS 019 Sampler (Ecotech Instruments, New Delhi, India) was used to collect the mainstream emissions from the tobacco products. The emissions are drawn from the end of the cigarette using battery operated controlled vacuum pump either through a glass fiber filter (filter extract) or through a suitable absorption solvent contained in impinger (extract).

To ensure complete absorption and reduce loss of absorbing solutions by evaporation, the impinger tubes were kept in insulated ice tray. Toxicant analysis requires different processing conditions and therefore the emissions from THS, CIG and BDI were extracted and collected separately. The extracts were filtered and suitably concentrated or diluted to desired concentrations prior to analysis. For each analysis, the emission from the whole smoking product was collected and the results were demonstrated as per unit.

Assessment Of Chemical Toxicants

The total particulate matter (2.5 µm) was determined by Temtop M2000 Air Quality Monitor (Elitech Technology Inc, USA). Nicotine, propylene glycol, glycerine, triacetin, menthol, ethylene oxide, polycyclic aromatic hydrocarbons and volatile organic compounds were determined following desorption of glass filter using thermal desorption system (TD 30, Shimadzu, Japan) and analyzed by gas-chromatography mass spectrometry (GC-MS, QP-2010 Plus, Shimadzu, Japan). For aromatic amines, filter was extracted with hydrochloric acid solution and the extract was analyzed by GC-MS. Tobacco specific nitrosamines were analyzed by liquid chromatography-mass spectrometry (LC-MS) following extraction of filter by acetic acid. Carbon dioxide and acrolein was determined by GC using thermal conductivity and nitrogen specific detector, respectively. Oxidants of nitrogen, sulphur dioxide, ammonia, hydrogen sulphide were detected by analysis of absorption solvent using auto analyser (EECL, India). Carbon monoxide was determined by electrochemical monitor and hydrogen cyanide by visible spectrophotometry (Shimadzu Corporation, Japan). Heavy metals such as lead, arsenic and mercury in the mainstream emissions were detected by extraction of glass fiber filter with hydrochloric acid solution and analysed by inductive coupled plasma-atomic emission spectrometer (Perkin Elmer, USA).

Assessment Of Cytotoxicity By Neutral Red Uptake

Chinese hamster (*Cricetulusgriseus*) ovary cell line (CHO-K1) cells were seeded for testing the cytotoxicity effect of extracts of mainstream emissions from THS, CIG and BDI. The CHO-K1 cells were cultured in 10% RPMI media, maintained at 37ºC in 5% CO_2 incubator (humidity 100%, O_2 10-15%, pH~7-7.5). Sub-culturing of cells was done with 0.25% trypsin-EDTA and PBS. For cytotoxicity, the filters obtained after the experimental procedures, were extracted in aqueous medium and this is considered as master stock with 100% concentration. From the master stock lower working concentrations were made by a serial two-fold dilutions (6.25% - 100%). The master stock of standard drug (1mM) was prepared by dissolving 5.43 mg of doxorubicin in 1.0 ml of 1% DMSO solution. For cell viability analysis, exponentially growing cells were trypsinized and seeded in a 96-well plate at required cell density and incubated overnight. After removing the spent medium from 96-well plate, the cells were treated with medium having recommended concentration of extracts and incubated for 24 h. Thereafter, the media was removed and 200 µl of neutral-red media (50 µg/ml) solution was added to each well and incubated for another 2 h. The supernatant was then removed and fixation was done by briefly washing with neutral-red fixative. Following removal of fixative, the incorporated dye was then solubilized using 200 µl neutral-red solubilization solution (destain solution). The cultures were allowed to stand for 10 minutes at room temperature and gentle pipetting was done to enhance mixing. The absorbance was noted at 540 nm using ELISA reader (MPR-404, Alpha Diagnostic Intl., USA). The data was normalized against the appropriate control and expressed as percentage cell viability. Further, cytotoxicity was expressed as IC-50 corresponding to the concentration of test substance for which a decrease of 50% in the uptake of the neutral red dye is observed. IC-50 was determined using logarithmic equation i.e.

$$Y = m(x) + C$$

Where, Y = 50, m is slope and C is intercept derived from the viability graph.

Assessment Of Genotoxicity By Comet Assay

The genotoxicity assessments were also performed on the CHO-K1 cell line. The cells were cultured, subcultured, and incubated with extract as mentioned in the cytotoxicity studies. After incubation, the medium was removed, and the cells were collected by trypsinization followed by pelletization. The cells were then washed once with ice cold PBS, free from Ca^{2+} and Mg^{2+}, and then resuspended in ice cold PBS (Ca^{2}+ and Mg^{2}+ free). The cells were combined with molten LM Agarose (at 37ºC) at a ratio of 1:10 (v/v) and immediately pipetted (75µL) onto the Comet Slides. The slides were placed flat at 4°C in the dark for 30 minutes followed by immersion in pre-chilled lysis solution at 4°C for 60 minutes. The excess buffer was tapped off from the slides and slides were immersed in freshly prepared alkaline solution of pH > 13. The slides were then transferred to horizontal electrophoresis apparatus and electrophoresis was performed for 40 minutes at voltage of about 1 V/cm and current of about 300 mA with each slide. The excess electrophoresis solution was tapped off by dipping several times in deionized water, slides were immersed in 70% ethanol for 5 minutes and were then air dried. The 100 µL of diluted stain (10X) was placed onto each circle of dried agarose and stained for 30 minutes at room temperature in the dark. The excess stain solution was removed and the slides were observed under fluorescence microscope (FITC filter excitation/emission 489/515 nm). The slides were scored using Image J (Open Comet plugin), and later the graphical analysis of the parameters (tail length (pixel), tail DNA %, and tail moment) was done using MS-Excel.

RESULTS

Mainstream Emissions

The fill weight of THS, CIG and BDI was 306.4±0.36 mg, 644.6±0.21 mg, and 245.9±0.12 mg, respectively and the burn time using controlled vacuum was 180 sec, 310 sec and 230 sec, respectively. THS was heated and CIG and BDI sticks were allowed to burn completely and the mainstream emissions were collected on filter. Fig. 1 illustrates the composition of particulate matter generated from THS, CIG and BDI. From the results it was evident that the emissions of THS were quantitatively and qualitatively different from that of CIG and BDI. The single THS stick generated significantly ($P < 0.001$) lower particulate matter (5.1 ± 0.06 mg) compared to BDI (11.2 ± 0.09 mg) and CIG (15.4 ± 0.11 mg). The difference between BDI and CIG was also significant ($P<0.05$) with BDI generating lower particulate matter. The nicotine emitted by THS was 26.0% and 15.0% lower compared to CIG and BDI, respectively. In the particulate matter, the percentage of water, glycerine and triacetin were higher in THS compared to CIG. However, in BDI, the glycerine and triacetin were absent. Further, as expected, the relative yields of other chemical constituents were noticeably lower in the THS and the same is evident visually from the color of the filter after collection of the mainstream emissions from THS, CIG and BDI (Fig. 2). The results observed were in corroboration with the previously published reports [16].

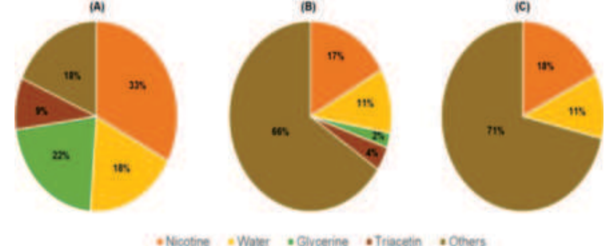

Fig. 1 Representation of composition of total particulate matter generated from mainstream emissions of single unit of (A) THS, (B) CIG and (C) BDI.

Fig. 2 Image of the filters after collection of mainstream emission of single unit of (A) THS, (B) CIG and (C) BDI.

Assessment of Chemical Toxicants

About 49 chemical toxicants in the mainstream emissions of THS, CIG and BDI covering various chemical classes were analyzed. The yield per stick of the chemical toxicants, arranged according to carcinogenic class (IARC) or health hazard (NFPA), in mainstream emission of tobacco products was presented as percent relative difference in Table 1. Four, nineteen, and twenty-three analytes in CIG, THS and BDI which are either below LOQ or not detected, were considered absent and presented as 100% reduced (Table 1). The class 1 toxicants in THS emission were 58% to 100% lower compared to that observed in CIG emission. When compared to BDI, THS showed decrease in all the

class 1 toxicants, sans amino biphenyl, which was not detected in BDI. On average, class 1 toxicants generated in THS emission was nearly 84.4% lower compared to CIG and nearly 69.0% lower compared to BDI. Further, a reduction of 15.3% was observed when emission of BDI was compared to CIG. Likewise, marked decrease in class 2 toxicants in the THS emissions was observed compared to CIG and BDI. The average percent reduction was 79.9% and 48.6% compared to CIG and BDI, respectively. Notably, BDI yielded 23.7% lower class 2 toxicants compared to CIG. Similar pattern was observed for class 3 toxicants, where yield of toxicant was lower in THS emission. Overall results demonstrated that the THS emission liberated nearly 80.1% lower chemical toxicants compared to CIG and nearly 42.1% compared to BDI.

Table 1. Data Representing Relative Percent Change Of Chemical Toxicants Generated In Mainstream Emissions Of THS, CIG and BDI.

S. No	Toxicant	Class	Percentage Reduction		
			THS vs CIG	THS vs BDI	BDI vs CIG
Class 1: Carcinogenic to humans or Severe Health Hazardous					
1	NNK	Nitrosamine	93%	93%	(-)3%
2	NNN	Nitrosamine	93%	85%	76%
3	Acetaldehyde	Aldehyde	77%	32%	67%
4	Formaldehyde	Aldehyde	87%	76%	45%
5	Amino Biphenyl	Amine	100%	0%	100%
6	Butadiene	Volatile	99%	44%	98%
7	Benzene	Volatile	58%	86%	(-)75%
8	Arsenic	Metal	58%	87%	(-)70%
9	Hydrogen Cyanide	Gas	88%	93%	(-)45%
10	Hydrogen Sulphide	Gas	91%	94%	(-)40%
Class 2: Probably / possibly carcinogenic to humans or Serious Health Hazardous					
11	Acrolein	Aldehyde	80%	79%	(-)2%
12	Cresols	Alcohol	100%	100%	92%
13	Styrene	Volatile	100%	100%	(-)80%
14	Benzopyrene	PAHC	100%	0%	100%
15	Amino Naphthalene	Amine	0%	100%	(-)100%
16	Catechol	Alcohol	81%	63%	49%
17	Pyridine	PAHC	50%	50%	0%
18	Quinoline	Volatile	0%	75%	(-)75%
19	Isoprene	Volatile	50%	(-)100%	100%
20	Acrylonitrile	Volatile	67%	77%	(-)33%
21	Diethyl Phthalate	Volatile	100%	0%	100%
22	Diisobutyl Phthalate	Volatile	100%	0%	100%
23	Benz[a]anthracene	PAHC	100%	0%	100%
24	Pyridine carboxylic acid	PAHC	100%	0%	100%
25	Bis-2(-ethyl hexyl) phthalate	Volatile	66%	71%	(-)14%
26	Cyclohexane	PAHC	92%	(-)100%	100%
27	Lead	Metal	100%	100%	(-)67%
28	Ethylene oxide	Gas	98%	96%	46%
29	Sulphur dioxide	Gas	80%	88%	(-)41%
30	Ammonia	Gas	71%	84%	(-)45%
31	Nitrogen Oxides	Gas	78%	91%	98%
32	Carbon monoxide	Gas	96%	96%	(-)6%
Class 3: Not classified as carcinogenic					
33	NAB	Nitrosamine	76%	87%	(-)44%
34	NAT	Nitrosamine	81%	86%	(-)28%
35	Methyl furfural	Aldehyde	77%	96%	100%
36	Toluene	Volatile	50%	78%	(-)56%
37	Phenol	Alcohol	100%	100%	(-)80%
38	Hydroquinone	Alcohol	80%	(-)100%	100%
39	Pyrene	PAHC	86%	69%	55%
40	Methyl Stearate	PAHC	100%	0%	100%
41	Palmitic acid	PAHC	100%	0%	100%
42	Cyclopentanol	Alcohol	100%	0%	100%
43	Squalene	PAHC	41%	31%	15%
44	Butyraldehyde	Aldehyde	82%	(-)100%	100%
45	Nitrobenzene	Volatile	100%	0%	100%
46	3,5-dimethyl pyrazole	PAHC	100%	0%	100%
47	PEG	Alcohol	21%	(-)100	100%
48	Menthol	Alcohol	100%	100%	65%
49	Nicotine	Alkaloid	26%	15%	12%

Classification done according to International Agency for Research on Cancer (IARC) and National Fire Protection Association (NFPA) [Toxicants absent in IARC were classified according to NFPA].

Percent reduction is calculated as: 100 x (Higher yield – Lower yield) / Higher yield. The data is presented as percent reduction however (-) represents percent increase. As an example, NNK: THS vs CIG, - 93% reduced in THS; BDI vs CIG, 3% higher for BDI.

NNK = Nicotine-derived nitrosamine ketone, NNN = N'-Nitrosonornicotine, NAB = Sodium tetraphenylborate, NAT = N′-nitrosoanatabine, PAHC = Poly aromatic hydrocarbons, PEG = Poly Ethylene Glycol.

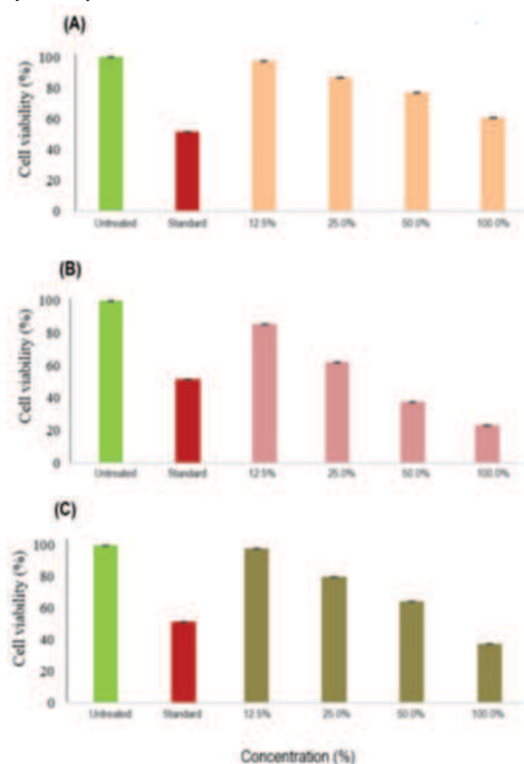

Fig. 3 Plot depicting the cytotoxic effect of mainstream emission of (A) THS, (B) CIG, and (C) BDI against the CHO-K1 cells compared to the control groups

Assessment Of Cytotoxicity By Neutral Red Uptake (NRU) Assay

The cytotoxic effects of mainstream emissions from THS, CIG and BDI on the viability of CHO-K1 cells was analyzed using NRU assay. Dilution ranging from 6.25% to 100% were tested. A concentration-dependent decrease in the number of viable cells was observed for all the three tested tobacco products. At 50% dilution, the cell viability was about 2-fold and 3-fold higher for THS compared to BDI and CIG, respectively. Likewise, the IC-50 value of THS (45.99%) was higher than that of CIG (9.64%) and BDI (17.69%). Accordingly, the in vitro cytotoxicity of the THS emissions was nearly 79.0% lower compared to CIG and nearly 61.5% lower compared to BDI.

Assessment Of Genotoxicity By Comet Assay

For genotoxic assessment, 50% concentration was used for all the tobacco products. The comet assay results (Table 2) showed that the mainstream emissions of all the tobacco products were having DNA damaging effect against the CHO-K1 cell line, when compared to the control (untreated). However, the DNA damaging effect of THS was markedly lower compared to that observed for CIG and BDI. The CIG and BDI extracts were toxic and only few cells (along with more debris) were observed during microscopic image analysis. The representative images are shown in Fig. 4.

Table 2. Data For Tail Length (pixels), Tail DNA (%) And Tail Moment Observed In CHO-K1 Cells Treated With THS, CIG And BDI Extract

Sample	Tail length (pixels)	Tail DNA (%)	Tail moment
Control	3.4	3.7	0.29
THS	96.3	15.1	14.9
CIG	369.2	41.2	190.6
BDI	304.5	30.8	137.7

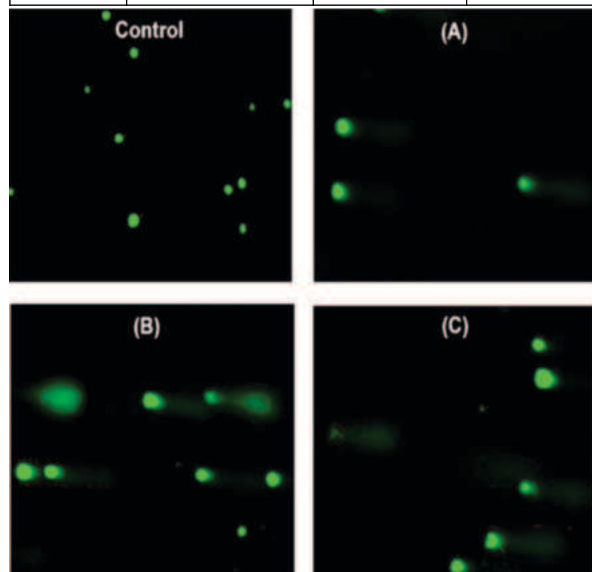

Fig. 4 Image depicting DNA damage observed in CHO-K1 cells treated by extracts from (A) THS, (B) CIG and (C) BDI compared to Control.

DISCUSSION

The objective of this study was to assess the potential for reduced toxicity of mainstream emissions from THS compared with CIG and BDI, based on quantitative analysis of chemical toxicants, in vitro cytotoxicity, and genotoxicity assessments.

To collect mainstream emission of tobacco products for toxicological assessment, we designed an inexpensive and simple assembly. Although, smoking devices have been mentioned in the literature for uniform sampling, but these devices are expensive, require extensive maintenance and creates a smoke that blows into a chamber instead of collecting the extract [24]. The present apparatus not only collects particulate matter via using filter but also allows use of several absorption solvents. This hold benefit since toxicant analysis requires extraction of toxicant in selective solvent system.

Several previous reports quantified toxicants in the mainstream emission of tobacco products with reference to nicotine content [15, 25]. However, we quantified toxicants in emissions generated from either heating or burning of single stick of tobacco. This is because smoking severity is generally measured by the number of tobacco sticks smoked per day and not by amount of nicotine inhaled [26]. Despite marked differences in fill weight and burn time of THS, CIG and BDI, quantification of toxicant on per tobacco stick allows easy comparison. Further, to quantify the exposure reduction, the yields of each toxicant were presented as percent change (reduction or increase) in concentration of chemical toxicant generated in emissions of THS vs CIG, THS vs BDI and BDI vs CIG (Table 1). In addition, instead of chemical classification reported previously [16], we classified toxicants based on their carcinogenic potential (IARC classification) or health hazards potential (NFPA). Our analytical data suggested that THS emissions were quantitatively and qualitatively different from CIG and BDI. Particulate matter in THS emission was 3-fold lower than CIG and 2-fold lower than BDI. Nicotine levels in THS emission was slightly lower, possibly due to lower fill weight of TEREA sticks. Cigarette filter components such as water, glycerine and triacetin were higher in THS compared to CIG. Glycerine and triacetin are often used in THS as humectants to absorb atmospheric water, to decrease smoke irritation in the throat and to produce mainstream emissions. Higher content of water, glycerine and triacetin in particulate matter compositions of HTPs has been demonstrated previously [6]. Notably, glycerine and triacetin were not detected in BDI, which might be due to absence of filters in BDI. Further, as expected, the relative yields of other chemical constituents (toxicants) were noticeably lower in the THS and the same is evident visually from the color of the filter after collection of the mainstream emissions from THS, CIG and BDI (Fig. 2). Class 1 toxicant, amino biphenyl and eight class 2 toxicants (cresol, styrene, benzopyrene, diethyl phthalate, diisobutyl phthalate, benz[a]anthracene, pyridine carboxylic acid and lead) remained undetected in THS emissions. Overall, THS delivered > 80% fewer toxicants than CIG and > 40% fewer toxicants than BDI. Interestingly, for class 1 toxicants the reduction rate was more than 84% (CIG) and 69% (BDI). The reduced toxicants were probably due to unique mechanism of THS, which involves heating the tobacco to ~350°C, unlike burning of tobacco at ~850°C, in conventional cigarettes [14]. This is supported by reduction of volatiles and PAHC (~ 80%), carbon monoxide (~ 96%), and N-nitrosamines (NNN, NNK ~ 93%), produced by high-temperature pyrolysis of carbohydrates and proteins in tobacco, incomplete combustion, and volatilization from tobacco filler to mainstream emissions, respectively. This is consistent with the previously published reports comparing THS products with conventional cigarettes [16]. Low temperature heating in THS and non-combustion of tobacco, contributes to the reduced emission of particulate matter and toxicants and thus THS emissions are referred to as 'aerosols', unlike 'smoke' generated from CIG and BDI [27].

The in vitro toxicity results were in corroboration with the chemical toxicant data. In NRU assay, THS demonstrated 3X and 2X higher cell viability compared with CIG and BDI, respectively. Accordingly, IC-50 for THS was substantially higher than CIG and BDI, indicating its relative lower toxicity on cells. The comet assay results were alike NRU assay results and demonstrated markedly lower DNA damaging effect of THS compared with CIG and BDI. Though, the present data did not suggest any mechanistic conclusion but the overall reduction in the burden of chemical toxicants in the THS emission might be responsible for decreased cytotoxicity and genotoxicity in vitro.

Our study findings indicate that most of the toxic mainstream constituents were markedly reduced in THS, making them safer alternatives to CIG and BDI. Further, independent studies, focussing on safety, efficacy, and concentrations of toxicants in plasma/ urine, following exposure to tobacco product emissions, should be undertaken in laboratory animals and/ or humans to generate further credible scientific evidence.

CONCLUSION

The low operating temperature of THS resulted in markedly reduced concentrations of toxicants in the mainstream aerosol compared with the mainstream smoke of the CIG and BDI. The reduction rate for carcinogenic toxicants was more than 84% compared to CIG and more than 69% compared to BDI. The cytotoxic and genotoxic potencies of the mainstream aerosol of THS, when evaluated by NRU and Comet assay, were reduced significantly compared with the mainstream smoke of CIG and BDI.

REFERENCES

[1]. Samet JM. Tobacco smoking: the leading cause of preventable disease worldwide. Thorac Surg Clin. 2013 May;23(2):103-12.
[2]. Li Y, Hecht SS. Carcinogenic components of tobacco and tobacco smoke: A 2022 update. Food Chem Toxicol. 2022 Jul;165:113179.
[3]. Cai B, Li Z, Wang R, Geng Z, Shi Y, Xie S, Wang Z, Yang Z, Ren X. Emission level of seven mainstream smoke toxicants from cigarette with variable tobacco leaf constituents. Regul Toxicol Pharmacol. 2019 Apr;103:181-188.
[4]. Borgerding M, Klus H. Analysis of complex mixtures--cigarette smoke. Exp Toxicol Pathol. 2005 Jul;57 Suppl 1:43-73.
[5]. Wackowski OA, Bover Manderski MT, Gratale SK, Weiger CV, O'Connor RJ. Perceptions about levels of harmful chemicals in e-cigarettes relative to cigarettes, and associations with relative e-cigarette harm perceptions, e-cigarette use and interest. Addiction. 2023 Oct;118(10):1881-1891.
[6]. Hashizume T, Ishikawa S, Matsumura K, Ito S, Fukushima T. Chemical and in vitro toxicological comparison of emissions from a heated tobacco product and the 1R6F reference cigarette. Toxicol Rep. 2023 Feb 15;10:281-292.
[7]. Oladipupo OA, Dutta D, Chong NS. Analysis of chemical constituents in mainstream bidi smoke. BMC Chem. 2019 Jul 22;13(1):93.
[8]. Hoffmann D, Sanghvi LD, Wynder EL. Comparative chemical analysis of Indian bidi and american cigarette smoke. Int J Cancer. 1974 Jul 15;14(1):49-53.
[9]. Burns DM, Dybing E, Gray N, Hecht S, Anderson C, Sanner T, O'Connor R, Djordjevic M, Dresler C, Hainaut P, Jarvis M, Opperhuizen A, Straif K. Mandated lowering of toxicants in cigarette smoke: a description of the World Health Organization TobReg proposal. Tob Control. 2008 Apr;17(2):132-41.
[10]. Ratajczak A, Jankowski P, Strus P, Feleszko W. Heat Not Burn Tobacco Product-A New Global Trend: Impact of Heat-Not-Burn Tobacco Products on Public Health, a Systematic Review. Int J Environ Res Public Health. 2020 Jan 8;17(2):409.
[11]. E-cigarettes, heat-not-burn and smokeless tobacco products. Breathe (Sheff). 2020 Mar;16(1):161ELF.
[12]. Stoklosa M, Cahn Z, Liber A, Nargis N, Drope J. Effect of IQOS introduction on cigarette sales: evidence of decline and replacement. Tob Control. 2020 Jul;29(4):381-

387.
[13]. Lempert LK, Bialous S, Glantz S. FDA's reduced exposure marketing order for IQOS: why it is not a reliable global model. Tob Control. 2022 Aug;31(e1):e83-e87.
[14]. Smith MR, Clark B, Lüdicke F, Schaller JP, Vanscheeuwijck P, Hoeng J, Peitsch MC. Evaluation of the Tobacco Heating System 2.2. Part 1: Description of the system and the scientific assessment program. Regul Toxicol Pharmacol. 2016 Nov 30;81 Suppl 2:S17-S26.
[15]. Mallock N, Böss L, Burk R, Danziger M, Welsch T, Hahn H, Trieu HL, Hahn J, Pieper E, Henkler-Stephani F, Hutzler C, Luch A. Levels of selected analytes in the emissions of "heat not burn" tobacco products that are relevant to assess human health risks. Arch Toxicol. 2018 Jun;92(6):2145-2149.
[16]. Schaller JP, Keller D, Poget L, Pratte P, Kaelin E, McHugh D, Cudazzo G, Smart D, Tricker AR, Gautier L, Yerly M, Reis Pires R, Le Bouhellec S, Ghosh D, Hofer I, Garcia E, Vanscheeuwijck P, Maeder S. Evaluation of the Tobacco Heating System 2.2. Part 2: Chemical composition, genotoxicity, cytotoxicity, and physical properties of the aerosol. Regul Toxicol Pharmacol. 2016 Nov 30;81
[17]. Schaller JP, Pijnenburg JPM, Ajithkumar A, Tricker AR. Evaluation of the Tobacco Heating System 2.2. Part 3: Influence of the tobacco blend on the formation of harmful and potentially harmful constituents of the Tobacco Heating System 2.2 aerosol. Regul Toxicol Pharmacol. 2016 Nov 30;81 Suppl 2:S48-S58.
[18]. Wong ET, Kogel U, Veljkovic E, Martin F, Xiang Y, Boue S, Vuillaume G, Leroy P, Guedj E, Rodrigo G, Ivanov NV, Hoeng J, Peitsch MC, Vanscheeuwijck P. Evaluation of the Tobacco Heating System 2.2. Part 4: 90-day OECD 413 rat inhalation study with systems toxicology endpoints demonstrates reduced exposure effects compared with cigarette smoke. Regul Toxicol Pharmacol. 2016 Nov 30;81 Suppl 2:S59-S81.
[19]. Oviedo A, Lebrun S, Kogel U, Ho J, Tan WT, Titz B, Leroy P, Vuillaume G, Bera M, Martin F, Rodrigo G, Esposito M, Dempsey R, Ivanov NV, Hoeng J, Peitsch MC, Vanscheeuwijck P. Evaluation of the Tobacco Heating System 2.2. Part 6: 90-day OECD 413 rat inhalation study with systems toxicology endpoints demonstrates reduced exposure effects of a mentholated version compared with mentholated and non-mentholated cigarette smoke. Regul Toxicol Pharmacol. 2016 Nov 30;81 Suppl 2:S93-S122.
[20]. Sewer A, Kogel U, Talikka M, Wong ET, Martin F, Xiang Y, Guedj E, Ivanov NV, Hoeng J, Peitsch MC. Evaluation of the Tobacco Heating System 2.2 (THS2.2). Part 5: microRNA expression from a 90-day rat inhalation study indicates that exposure to THS2.2 aerosol causes reduced effects on lung tissue compared with cigarette smoke. Regul Toxicol Pharmacol. 2016 Nov 30;81 Suppl 2:S82-S92.
[21]. Znyk M, Jurewicz J, Kaleta D. Exposure to Heated Tobacco Products and Adverse Health Effects, a Systematic Review. Int J Environ Res Public Health. 2021 Jun 21;18(12):6651.
[22]. Simonavicius E, McNeill A, Shahab L, Brose LS. Heat-not-burn tobacco products: a systematic literature review. Tob Control. 2019 Sep;28(5):582-594..
[23]. Phan L, Strasser AA, Johnson AC, Villanti AC, Niaura R, Rehberg K, Mays D. Young Adult Correlates of IQOS Curiosity, Interest, and Likelihood of Use. Tob Regul Sci. 2020 Mar;6(2):81-90.
[24]. Thorne D, Adamson J. A review of in vitro cigarette smoke exposure systems. Exp Toxicol Pathol. 2013 Nov;65(7-8):1183-93.
[25]. Upadhyay S, Rahman M, Johanson G, Palmberg L, Ganguly K. Heated Tobacco Products: Insights into Composition and Toxicity. Toxics. 2023 Aug 2;11(8):667.
[26]. Shiffman S. How many cigarettes did you smoke? Assessing cigarette consumption by global report, Time-Line Follow-Back, and ecological momentary assessment. Health Psychol. 2009 Sep;28(5):519-26.
[27]. Sussman RA, Sipala F, Emma R, Ronsisvalle S. Aerosol Emissions from Heated Tobacco Products: A Review Focusing on Carbonyls, Analytical Methods, and Experimental Quality. Toxics. 2023 Nov 21;11(12):947.