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RESEARCH ARTICLE

Effects of Phenanthrene and Pyrene on Cytogenetic Stability of Human Dermal Fibroblasts Using Alkaline Comet Assay Technique

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Abstract In the present study, the influence of phenanthrene and pyrene on cytogenetic stability of human dermal fibroblasts using alkaline comet assay was evaluated. The cells were isolated from foreskin samples obtained from healthy male infants and in low passages (P 1–3) were exposed to various concentrations (0.0900, 0.0625, 0.0320, and 0.0066 ppm) of phenanthrene and pyrene. Then, alkaline comet assay was performed to investigate the influence of these compounds on DNA damage and cytogenetic stability of human dermal fibroblasts. In the present work H_2O_2 treatment was used as a positive control of comet assay to create DNA damages. The analysis of alkaline comet assay parameters by CaspLab software showed the tail DNA% in high concentration (0.0900 ppm) of phenanthrene and pyrene in the exposed cells got increased as compared to normal cells, while head DNA% decreased. Also, similar to positive control (H_2O_2), DNA damage with long tail comet was observed in high concentration of these compounds as compared to normal cells. The comparison of comet assay parameters specially head DNA% and tail DNA% between each concentrations of phenanthrene and pyrene with other groups (healthy cells and H_2O_2 treatment) by ANOVA and post hoc Tukey test showed that these parameters were more significantly different ($p < 0.05$). These results indicated that phenanthrene and pyrene even in low dosage are dangerous and can increase the DNA damage and affect on cytogenetic stability of dermal fibroblasts. These findings suggested that phenanthrene and pyrene could increase the related diseases and risk of skin cancer in exposed individuals.

Keywords Phenanthrene · Pyrene · Human dermal fibroblasts · Cytogenetic analysis · Alkaline comet assay

Introduction

Human dermal fibroblasts (HDFs) are the principal cell type found in dermis of skin and all connective tissues of mesenchymal origin [1, 2]. These cells secrete collagen and extracellular matrix (ECM) proteins under cell culture conditions and can be obtained from neonatal foreskin and human adult skin [3, 4]. Dermal fibroblasts have many functions in the body including generating connective tissue, autocrine and paracrine interactions, wound healing, and epithelial-mesenchymal interactions [5, 6]. Environmental agents such as polycyclic aromatic hydrocarbons (PAHs) could affect on cytogenetic stability and DNA permanency of dermal fibroblast in people at risk. PAHs are a group of chemicals that are derived from organic compounds and they are environmental contaminants, found in air, water, and soil [7]. PAHs could be released during natural processes (burning coal and wood or during forest fires) or anthropogenic sources and incomplete

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combustion of organic materials such as incomplete burning of coal, oil and gas, garbage, urban sewage, vehicle exhausts, power plants chemical, cooked foods, aluminium smelters, petrochemical and oil industries as well as asphalt works, and hazardous waste sites [8, 9].

PAHs involve many derivatives such as fluoranthene, chrysene, fluorene, phenanthrene, and pyrene. Phenanthrene and pyrene are a kind of chemicals and isolates from soil, oil, seawater, and sediment samples and could be polluting the groundwater and drinking water sources [8, 10]. In recent decades the occurrence of cancer and other diseases associated with PAHs exposures has been increased. The emission of PAHs in environment could affect on ecosystem and exposure to these compounds including ingestion, inhalation, and dermal contact (especially in people who have close contact with these substances and polluted water used for washing and hygiene purpose) are of serious human health concerns [8, 10, 11].

In recent years, many methods have been introduced for investigating the effects of mutagens on cytogenetic instability and their DNA lesions in cells [12–14] such as single cell gel electrophoresis assay (also known as comet assay). Comet assay is a sensitive and simple toll used for detection of DNA lesions caused by single and double strand breaks (SSBs and DSBs), alkali labile sites, oxidative base damage, and genetic instability of cells [14–17].

The effects of phenanthrene and pyrene on human health are carcinogenic, toxic, mutagenic, and teratogenic. So, investigating the effects of these PAH compounds on cytogenetic instability of cells such as dermal fibroblast special in people at risk is important [6, 10]. In the present study, the effects of phenanthrene and pyrene (PAH compounds) on DNA damages and cytogenetic instability of isolated HDFs from human foreskin samples using alkaline comet assay were investigated for the first time.

Material and Methods

HDFs Preparation

In the present experimental study, foreskin specimens of healthy male infants were obtained from Kashani Hospital (Shahrekord city, Iran) and were transferred in transfer media [Dulbecco's modified Eagle's medium (DMEM) containing 1.5 % penicillin and streptomycin] to the Cellular and Molecular Research Center. Then, human foreskin tissues were digested by a combination of mechanical disaggregation and enzymatic treatment (0.25 % Trypsin–EDTA solution, 100 U/mL Collagenase type IV, and 100 μg/mL DNase). The single-cell suspension was cultured in DMEM with 10 % fetal bovine serum (FBS) and 1 % penicillin/streptomycin antibiotics (all Gibco, NY, USA) and were incubated at 37 °C in a 5 % CO_2 atmosphere. After 1 week the cells were prepared for first passage. Cells were washed in phosphate-buffered saline (PBS) twice and were detached using 0.25 % Trypsin–EDTA solution at 37 °C for 5 min. The enzyme was neutralized by DMEM containing 15 % FBS and cells were centrifugated at 1200 rpm for 5 min and were counted using neubauer slide and microscope. Then, the cells were seeded on 6-well tissue culture treated plates at a concentration of 150×10^3 in 1.5 mL of DMEM containing 10 % FBS per well, and were incubated at 37 °C in a CO_2 incubator for 24 h. After the cells confluency reached to 60–70 % in each well, HDFs were detached as mentioned above and were used for alkaline comet assay.

Cell Growth Rate Measurement

For measuring of HDFs growth rate the cells between passages 1–3 were cultured in 6-well tissue culture treated plate with a concentration of 4×10^4 per well. The medium was changed by fresh DMEM supplemented with 10 % FBS every 3 days. Triplicate wells were counted every 24 h for obtaining cell growth curves.

Preparation of Standard Solutions

The standard concentrations of phenanthrene and pyrene were prepared and used in culture media of cells (Table 1).

Alkaline Comet Assay

In the present study, hydrogen peroxide (H_2O_2) with concentration of 50 μM was used as comet assay positive control. In each well of tissue culture treated plates 150×10^3 cells/mL were seeded in 1.5 mL DMEM (containing 10 % FBS, 2 mM glutamine, and 1 % (100 μg/mL) penicillin/streptomycin antibiotics) and were incubated at 37 °C for 24 h in 5 % CO_2 atmosphere and let cells to attach. For positive control the medium was replaced by DMEM containing 50 μM H_2O_2 and was incubated at 37 °C for 2 h to produce massive single-stranded DNA damage.

Table 1 The concentration of phenanthrene and pyrene treated on HDFs culture

Volume (μL)	Phenanthrene (ppm)	Pyrene (ppm)
1	0.0900	0.0900
2	0.0625	0.0625
3	0.0320	0.0320
4	0.0066	0.0066

Each concentration of phenanthrene and pyrene described above was added to DMEM supplemented with 10 % FBS and were incubated for 4 h at 37 °C in CO_2 incubator. The alkaline comet assay was performed according to modified standard procedure described by McKelvey-Martin et al. [15]. In this assay, all slides were washed by ethanol and methanol, respectively. The slides were covered with 250–300 µL of 1 % normal melting point agarose (Carlsbad, Ca, USA) prepared in PBS at 50 °C and were allowed to be fully frosted. The slides were placed on ice for 30 min and coverslips were picked up and were incubated at 37 °C in incubator for 24 h. Then, 10×10^4 cells were embedded in 100 µL of 1 % low melting point agarose (LMA) at 37 °C. Then, the coverslips were placed on the slides and were left on ice to solidify the agarose. The coverslips were picked up again and the third layer of 1 % normal melting point agarose was put on them and were lysed in fresh cold lysis solution (2.5 M NaCl, 100 mM Na_2EDTA, 10 mM Tris, pH = 10, with 1 % Triton X-100 and DMSO 10 % were added just before use) for 24 h at 4 °C.

Subsequently, the slides were drained and were placed in an alkaline electrophoresis buffer (300 mM NaOH, 1 mM Na_2EDTA, pH > 13) for DNA unwinding. After staining with 2 µg/mL ethidium bromide, the comet tails were visualized using a fluorescence microscope.

Ethical Approval

In the current study, the protocols and informed consent forms were approved by the Regional Research Ethical Committee of Shahrekord University of Medical Sciences and foreskin samples of healthy male newborns were obtained and consent forms were filled by parents of each infant. Male newborns with genetic disorders such as Down's syndrome were excluded from the study.

Statistical Analysis

Each experiment was performed at least three times and all data were collected in Statistics programs for the Social Sciences software, version 20 (SPSS, Inc., Chicago, IL, USA). The parameters of alkaline comet assay including head area, tail area, head DNA, tail DNA, tail length, comet length, and tail moment were evaluated by CaspLab (Comet Assay Software Project) software version 1.0.0. The differences between the alkaline comet assay parameters were analyzed using one-way ANOVA (Analysis of variance) followed by Tukey HSD post hoc test for comparing the significant difference among means of various treatments. The p values less than 0.05 (5 %) were considered statistically significant between groups.

Results and Discussion

HDF Cells Preparation

In this study HDFs in low passages (P 1–3) isolated from human foreskin sample were used for evaluation of cytogenetic stability and DNA damages using alkaline comet assay (Fig. 1).

HDFs Growth Rate

The HDFs in passage 2 were seeded in 6 wells plate (4×10^4 cells per well) and were used to evaluate the cells growth rate. According to the observed curve, in 24 h after harvesting the cells the number of cell population did not increase. From 24 to 120 h, HDFs showed normal growth rate with exponential proliferation and between 120 and 144 h the cells population entered stationary growth phase (Fig. 2).

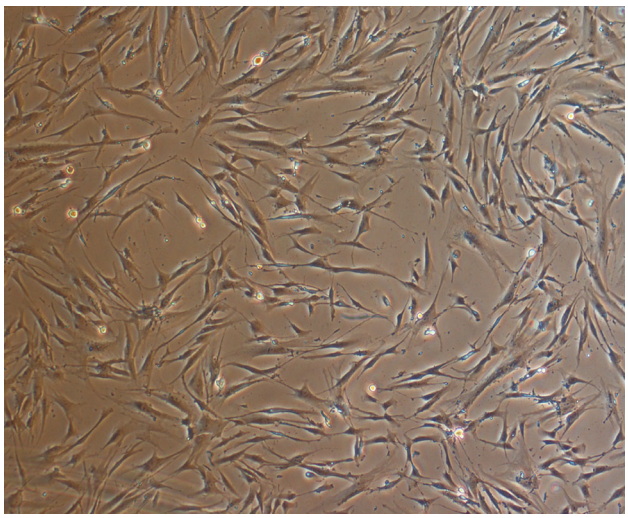

Fig. 1 HDFs in early passage (P 2) isolated from human foreskin specimen

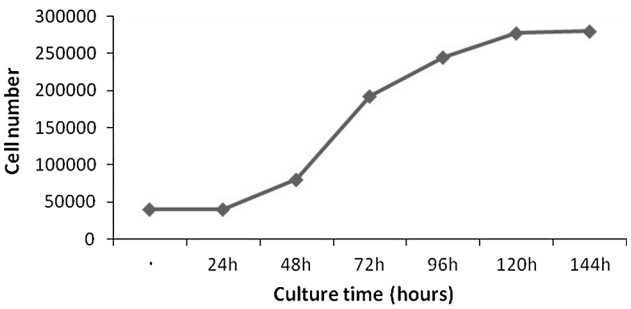

Fig. 2 The growth curve of HDFs through 6 days. The number of cells at 0–24 h had no change and cells show normal exponential curve. Cells at 120–144 h enter to stationary phase

Comet Assay

In this study, alkaline comet assay was performed on healthy HDFs (negative control) and cells exposed by H_2O_2 treatment (positive control) to create DNA damage. The damaged DNA and DNA single-strand breaks were observed in positive control group but in healthy HDFs normal DNA showed no damage (Fig. 3). Also, this test was performed to evaluate the cytogenetic stability of HDFs treated with different concentration (0.0900, 0.0625, 0.0320, and 0.0066 ppm) of phenanthrene and pyrene (Figs. 4, 5).

The comet assay parameters including head area, tail area, head DNA, tail DNA, tail length, comet length, and tail movement in healthy HDFs, positive control, in different concentrations (0.0900, 0.0625, 0.0320, and 0.0066 ppm) of phenanthrene and pyrene were calculated by CaspLab software version 1.0.0 (Tables 2, 3). The relationship and analysis of differences between each concentration and positive control as well as healthy HDFs were evaluated by ANOVA and the Tukey's post hoc test was used for the determination of significant concentration effects (Table 4, 5). The p value less than 0.05 was considered statistically significant.

According to the results presented in Tables 2 and 3 the means of tail DNA% parameter in high concentration (0.0900 ppm) of phenanthrene and pyrene in exposed cells were increased as compared with low concentration and conversely head DNA% decreased. Furthermore, DNA damages and long tail like positive control (H_2O_2) were observed in high concentration of phenanthrene and pyrene as compared with normal HDFs (no DNA damage). The comparison of alkaline comet assay parameters specially head DNA% and tail DNA% between each concentration

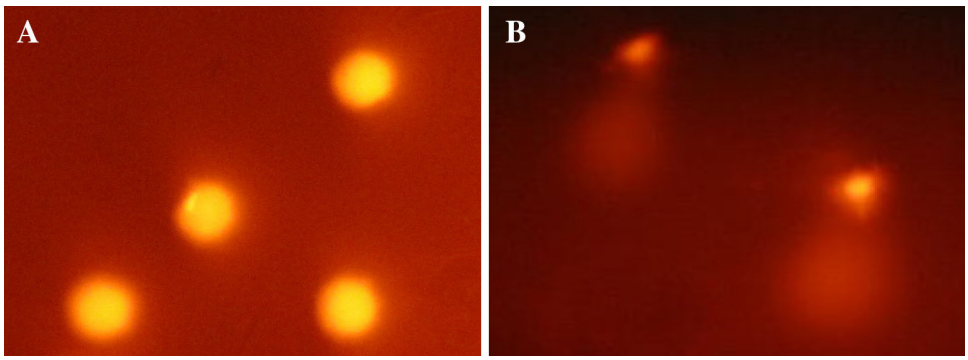

Fig. 3 **a** Alkaline comet assay on healthy HDFs showing normal cells without DNA damage. **b** Cells treated with H_2O_2 (positive control) with DNA lesions

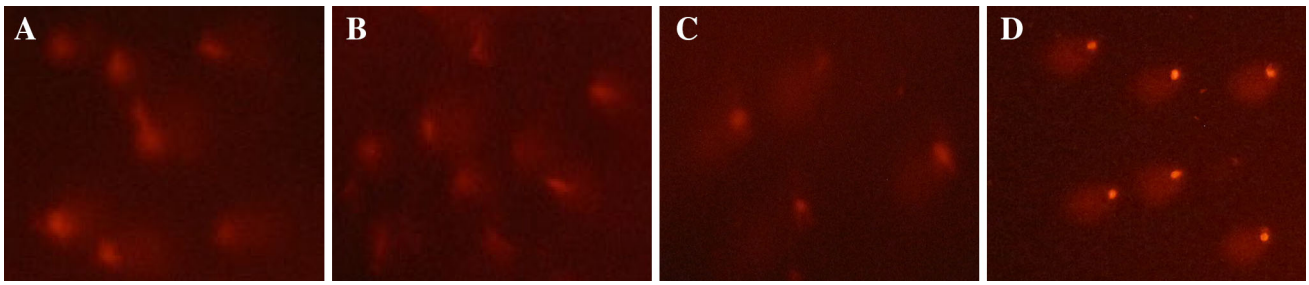

Fig. 4 The alkaline comet assay on HDFs exposed with varied concentrations of phenanthrene **a** 0.0066, **b** 0.0320, **c** 0.0625, and **d** 0.0900 ppm, respectively

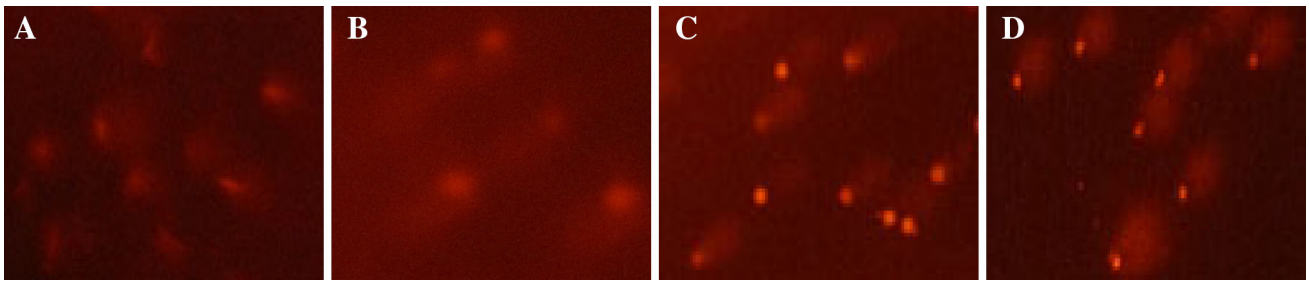

Fig. 5 The alkaline comet assay of HDFs treated by different concentrations of pyrene **a** 0.0066, **b** 0.0320, **c** 0.0625, and **d** 0.0900 ppm, respectively

Table 2 The details of alkaline comet assay analysis on HDFs treated by different concentrations of phenanthrene as compared with positive control (cells exposed by H_2O_2) and healthy cells

Parameters	HDFs treated with 0.0900 ppm Phenanthrene	HDFs treated with 0.0625 ppm Phenanthrene	HDFs treated with 0.0320 ppm Phenanthrene	HDFs treated with 0.0066 ppm Phenanthrene	Positive control (HDFs treated with H_2O_2)	Healthy HDFs
	No. of cells (100) Mean ± SD	No. of cells (100) Mean ± SD	No. of cells (100) Mean ± SD	No. of cells (100) Mean ± SD	No. of cells (100) Mean ± SD	No. of cells (100) Mean ± SD
Head area	124.72 ± 49.942	529.87 ± 108.571	351.16 ± 57.93	364.12 ± 53.18	356.94 ± 207.233	349.52 ± 59.153
Tail area	743.7 ± 174.22	1036.14 ± 240.73	332.64 ± 57.27	297 ± 95.03	1821.38 ± 265.035	41.24 ± 18.05
Head DNA	8.27 ± 4.042	15.8 ± 4.61	14.75 ± 3.532	15.491 ± 3.461	27.12 ± 13.016	35.37 ± 5.26
Tail DNA	28.21 ± 11.73	19.5 ± 5.84	7.36 ± 1.642	7.37 ± 1.65	68.06 ± 17.545	1.03 ± 0.51
Head DNA%	22.4 ± 3.6	44.9 ± 2.13	66.6 ± 2.2	67.71 ± 1.66	27.17 ± 5.695	97.23 ± 1.202
Tail DNA%	77.62 ± 3.6	55.13 ± 2.131	33.41 ± 2.11	31.82 ± 3.02	72.83 ± 5.695	2.77 ± 1.202
Tail length	29.64 ± 3.7	27.62 ± 3.58	13.42 ± 3.7	12.29 ± 3.28	44.86 ± 3.272	4.06 ± 1.162
Comet length	42.72 ± 5.74	54.26 ± 5.48	34.9 ± 4.41	30.87 ± 5.97	67.34 ± 9.812	25.7 ± 2.65
Tail moment	23.1 ± 3.75	15.24 ± 2.125	4.5 ± 1.32	3.532 ± 1.52	32.63 ± 2.1	0.11 ± 0.062

Head area: area of the comet head in pixels, tail area: area of the comet tail in pixels, head DNA: amount of DNA in the comet head, tail DNA: amount of DNA in the comet tail, tail length: length of the comet tail measured from right border of head area to end of tail (in pixels), comet length: length of the entire comet from left border of head area to end of tail (in pixels), tail moment: tail DNA% × tail length [(percent of DNA in the tail) × (tail length)], SD: standard deviation

Table 3 The alkaline comet assay particularity on HDFs after treated by varied concentrations of pyrene as compared with normal HDFs and positive control (HDFs treated by H_2O_2)

Parameters	HDFs treated with 0.0900 ppm Pyrene	HDFs treated with 0.0625 ppm Pyrene	HDFs treated with 0.0320 ppm Pyrene	HDFs treated with 0.0066 ppm Pyrene	Positive control (HDFs treated with H_2O_2)	Healthy HDFs
	No. of cells (100) Mean ± SD	No. of cells (100) Mean ± SD	No. of cells (100) Mean ± SD	No. of cells (100) Mean ± SD	No. of cells (100) Mean ± SD	No. of cells (100) Mean ± SD
Head area	31.2 ± 18.34	86.77 ± 65.104	635.84 ± 175.815	271.12 ± 128.163	356.94 ± 207.233	349.52 ± 59.153
Tail area	228 ± 74.45	252.37 ± 155.41	711.3 ± 187.874	166.52 ± 70.87	1821.38 ± 265.035	41.24 ± 18.05
Head DNA	2.66 ± 1.31	3.03 ± 1.6	15.81 ± 4.064	9.36 ± 4.133	27.12 ± 13.016	35.37 ± 5.26
Tail DNA	8.56 ± 4.005	5.3 ± 3.01	10.94 ± 3.215	2.73 ± 1.182	68.06 ± 17.545	1.03 ± 0.51
Head DNA%	23.45 ± 2.354	37 ± 1.915	59.34 ± 1.891	76.98 ± 4.122	27.17 ± 5.695	97.23 ± 1.202
Tail DNA%	76.55 ± 2.354	63 ± 1.915	40.66 ± 1.891	23.03 ± 4.122	72.83 ± 5.695	2.77 ± 1.202
Tail length	15.16 ± 1.245	16.88 ± 3.97	23.42 ± 4.791	13.66 ± 3.775	44.86 ± 3.272	4.06 ± 1.162
Comet length	22.08 ± 2.55	27.54 ± 7.984	51.86 ± 6.084	32.3 ± 5.223	67.34 ± 9.812	25.7 ± 2.65
Tail moment	11.62 ± 1.211	10.68 ± 2.76	9.55 ± 2.17	3.22 ± 1.265	32.63 ± 2.1	0.11 ± 0.062

Head area: area of the comet head in pixels, tail area: area of the comet tail in pixels, head DNA: amount of DNA in the comet head, tail DNA: amount of DNA in the comet tail, tail length: length of the comet tail measured from right border of head area to end of tail (in pixels), comet length: length of the entire comet from left border of head area to end of tail (in pixels), tail moment: tail DNA% × tail length [(percent of DNA in the tail) × (tail length)], SD: standard deviation

of phenanthrene and pyrene and other groups (positive control and healthy HDFs) by one-way ANOVA followed by post hoc Tukey test showed that these parameters were more significantly different at $p < 0.05$ (Tables 4, 5).

Phenanthrene and pyrene are important environmental pollutants and their health implications could create DNA damages. In the present work, for the first time the cytogenetic affects of these PAH chemicals on HDFs were evaluated by alkaline comet assay. The H_2O_2 treatment was used to induce DNA damage on HDF culture as a positive control of this test. After analyzing the alkaline comet assay parameters, in high concentration of phenanthrene and pyrene the tail DNA% in exposed cells increased, while head DNA% decreased as compared to normal fibroblasts and positive control. Furthermore, the analysis of cytogenetic stability of exposed HDFs with various concentrations of phenanthrene and pyrene as compared with normal HDFs and positive control groups by ANOVA and Tukey test showed alkaline comet assay parameters including tail area, head DNA%, tail DNA%, tail length, and tail moment

Table 4 The relationship of alkaline comet assay parameters between different concentrations of phenanthrene as compared with positive control (cells exposed by H_2O_2) and normal HDFs analyzed by ANOVA and Tukey post hoc test

Parameters	Phenanthrene (0.0066 ppm) compared with positive control (H_2O_2)		Phenanthrene (0.0066 ppm) compared with normal HDFs		Phenanthrene (0.0320 ppm) compared with positive control (H_2O_2)		Phenanthrene (0.0320 ppm) compared with normal HDFs	
	Mean difference ± SE	*p* value**	Mean difference ± SE	*P* value**	Mean difference ± SE	*P* value**	Mean difference ± SE	*P* value**
Head area	7.18 ± 15.415	1.000	14.6 ± 15.415	1.000	−5.78 ± 15.416	1.000	1.64 ± 15.416	1.000
Tail area	−1524.38* ± 21.963	0.000	255.76* ± 21.963	0.000	−1488.74* ± 21.963	0.000	291.4* ± 21.963	0.000
Head DNA%	40.536* ± 0.421	0.000	−29.52* ± 0.421	0.000	39.418* ± 0.421	0.000	−30.64* ± 0.421	0.000
Tail DNA%	−41.004* ± 0.436	0.000	29.05* ± 0.436	0.000	−39.418* ± 0.436	0.000	30.637* ± 0.436	0.000
Tail length	−32.57* ± 0.484	0.000	8.23* ± 0.484	0.000	−31.44* ± 0.484	0.000	9.36* ± 0.484	0.000
Comet length	−36.47* ± 0.844	0.000	5.17* ± 0.844	0.000	−32.44* ± 0.844	0.000	9.2* ± 0.844	0.000
Tail moment	−29.102* ± 0.306	0.000	3.42* ± 0.306	0.000	−28.14* ± 0.31	0.000	4.385* ± 0.31	0.000
Parameters	Phenanthrene (0.0625 ppm) compared with positive control (H_2O_2)		Phenanthrene (0.0625 ppm) compared with normal HDFs		Phenanthrene (0.0900 ppm) compared with positive control (H_2O_2)		Phenanthrene (0.0900 ppm) compared with normal HDFs	
	Mean difference ± SE	*P* value**	Mean difference ± SE	*P* value**	Mean difference ± SE	*P* value**	Mean difference ± SE	*P* value**
Head area	172.92* ± 15.416	0.000	180.34* ± 15.416	0.000	−232.22* ± 15.416	0.000	−224.8* ± 15.416	0.000
Tail area	−785.24* ± 21.963	0.000	994.9* ± 21.963	0.000	−1077.7* ± 21.963	0.000	702.44* ± 21.963	0.000
Head DNA%	17.696* ± 0.421	0.000	−52.36* ± 0.421	0.000	−4.791* ± 0.421	0.000	−74.846* ± 0.421	0.000
Tail DNA%	−17.691* ± 0.436	0.000	52.36* ± 0.436	0.000	4.791* ± 0.436	0.000	74.846* ± 0.436	0.000
Tail length	−17.24* ± 0.484	0.000	23.56* ± 0.484	0.000	−15.22* ± 0.484	0.000	25.58* ± 0.484	0.000
Comet length	−13.08* ± 0.844	0.000	28.56* ± 0.844	0.000	−24.62* ± 0.844	0.000	17.02* ± 0.844	0.000
Tail moment	−17.399* ± 0.306	0.000	15.124* ± 0.306	0.000	−9.53* ± 0.306	0.000	22.992* ± 0.306	0.000

* The mean difference is significant at $p < 0.05$ by one-way ANOVA

** The significant difference among means of various treatments in each groups using post hoc Tukey test ($p < 0.05$)

SE standard error

Table 5 The correlation of alkaline comet assay parameters between different concentrations of pyrene as compared with positive control (cells treated by H_2O_2) and healthy cells analyzed by ANOVA followed by Tukey post hoc test

Parameters	Pyrene (0.0066 ppm) compared with positive control (H_2O_2)		Pyrene (0.0066 ppm) compared with normal HDFs		Pyrene (0.0320 ppm) compared with positive control (H_2O_2)		Pyrene (0.0320 ppm) compared with normal HDFs	
	Mean difference ± SE	*P* value**	Mean difference ± SE	*P* value**	Mean difference ± SE	*P* value**	Mean difference ± SE	*P* value**
Head area	−85.82* ± 15.416	0.000	−78.4* ± 15.416	0.002	278.9* ± 15.416	0.000	286.32* ± 15.416	0.000
Tail area	−1654.86* ± 21.963	0.000	125.28* ± 21.963	0.000	−1110.08* ± 21.963	0.000	670.06* ± 21.963	0.000
Head DNA%	49.799* ± 0.421	0.000	−20.255* ± 0.421	0.000	32.161* ± 0.421	0.000	−37.894* ± 0.421	0.000
Tail DNA%	−49.799* ± 0.436	0.000	20.255* ± 0.436	0.000	−32.161* ± 0.436	0.000	37.894* ± 0.436	0.000
Tail length	−31.2* ± 0.484	0.000	9.6* ± 0.484	0.000	−21.44* ± 0.484	0.000	19.36* ± 0.484	0.000
Comet length	−35.04* ± 0.844	0.000	6.6* ± 0.844	0.000	−15.48* ± 0.844	0.000	26.16* ± 0.844	0.000
Tail moment	−29.418* ± 0.306	0.000	3.104* ± 0.306	0.000	−23.08* ± 0.306	0.000	9.442* ± 0.306	0.000
Parameters	Pyrene (0.0625 ppm) compared with positive control (H_2O_2)		Pyrene (0.0625 ppm) compared with normal HDFs		Pyrene (0.0900 ppm) compared with positive control (H_2O_2)		Pyrene (0.0900 ppm) compared with normal HDFs	
	Mean difference ± SE	*P* value**	Mean difference ± SE	*P* value**	Mean difference ± SE	*P* value**	Mean difference ± SE	*P* value**
Head area	−270.17* ± 15.416	0.000	−262.75* ± 15.416	0.000	−325.74* ± 15.416	0.000	−318.32* ± 15.416	0.000
Tail area	−1569.01* ± 21.963	0.000	211.13* ± 21.963	0.000	−1593.38* ± 21.963	0.000	186.76* ± 21.963	0.000
Head DNA%	9.824* ± 0.421	0.000	−60.23* ± 0.421	0.000	−3.722* ± 0.421	0.000	−73.78* ± 0.421	0.000
Tail DNA%	−9.824* ± 0.436	0.000	60.23* ± 0.436	0.000	3.722* ± 0.436	0.000	73.78* ± 0.436	0.000
Tail length	−27.98* ± 0.484	0.000	12.82* ± 0.484	0.000	−29.7* ± 0.484	0.000	11.1* ± 0.484	0.000
Comet length	−39.8* ± 0.844	0.000	1.84 ± 0.844	0.855	−45.26* ± 0.844	0.000	−3.62* ± 0.844	0.032
Tail moment	−21.949* ± 0.306	0.000	10.573* ± 0.306	0.000	−21.011* ± 0.306	0.000	11.512* ± 0.306	0.000

* The mean difference is significant at $p < 0.05$ by one-way ANOVA

** The significant difference among means of various treatments in each groups using post hoc Tukey test ($p < 0.05$)

SE standard error

significantly different ($p < 0.05$). These results indicated that phenanthrene and pyrene are mutagens and carcinogens which can create DNA damages with long tail comet in dermal fibroblast. These PAH chemicals are capable to induce mutations in the genome and create single and double strand breaks in DNA [17, 18]. Previous studies indicated that PAHs had carcinogenic, teratogenic, and mutagenic properties and exposure by these compounds could induce a DNA lesion [11, 19]. Other studies on oil and petroleum occupational diseases showed that individuals in close contact to PAHs are associated with multiple types of chronic cancers including liver, skin, kidney, bladder, lung, testis, and breast cancer [18–21]. The evaluation of genotoxicity of exposed blood cells to sediments and PAHs in flounder (*Paralichthys olivaceus*) using comet assay performed by Woo et al. [22] showed the significant differences in DNA breakage between exposed cells as compared with non-exposed control. Also, their study demonstrated that the comet assay is a good technique for monitoring of genotoxicity of PAHs and marine sediments in marine organisms. The study of Tannheimer et al. demonstrated that carcinogenic PAHs increased intracellular Ca^{2+} and cell proliferation in primary human mammary epithelial cells [23]. Solhaug et al. [24] showed that PAHs compounds such as pyrene induce both apoptotic and anti-apoptotic signals in Hepa1c1c7 cells using fluorescence microscopy and flow cytometry. Also, PAHs could induce mutagenesis in regulatory key genes such as *p53* and *ras* genes and play important role in DNA repair system and control of cell cycle [10, 25, 26]. Therefore, the mutations in gene regulatory systems disrupt the regulation of cell activities such as proliferation, differentiation, apoptosis, and DNA repair and could lead to severe damages in genes and cells [25, 27]. Denissenko et al. [28] showed that benzo [α] pyrene (BaP) is a chemical carcinogen which induces mutational hot spots in *P53* gene related to lung cancer [28]. PAHs such as 7, 12-dimethylbenz[α]anthracene (DMBA) and BaP have important effects on Ca^{2+} signaling pathways in murine and human lymphoid cells [29].

Future Perspective

In the present study, the influence of phenanthrene and pyrene on cytogenetic stability of HDFs was evaluated. In future researches the effect of these compounds and other PAH materials on various cell lines of skin and other tissues should be investigated.

Conclusions

In conclusion, the results of the present study demonstrated that PAHs compounds like phenanthrene and pyrene even in low dose could influence on cytogenetic stability of dermal fibroblasts and increase the DNA damages by creating SSBs and DSBs same as in positive control (exposed cells with H_2O_2). So, in individuals who have close contact with phenanthrene and pyrene, these compounds can increase the risk of skin cancer. According to the findings of the present study, evaluation of the effects of PAHs on DNA lesions can be helpful to develop better strategies for protection and prevention against different PAH derived compounds and decrease the related diseases in exposed individuals.

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