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Levels and toxicity of polycyclic aromatic hydrocarbons in black tea varieties consumed in Saudi Arabia

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ABSTRACT

Tea is the most popular beverage consumed in the Kingdom of Saudi Arabia. Keeping in view their prevalence in the environment, eight polycyclic aromatic hydrocarbons (PAHs) were determined in fourteen brands of locally available black tea by using high performance liquid chromatography (HPLC). Benzo(a)anthracene and anthracene were detected in all samples whereas Benzo(k)fluoranthene was not detected in Tetly, Twinings and English Teashop brands. Benzo(a)Pyrene, classified as human carcinogen (class 2A), was found in all samples except English Teashop. The highest levels of Benzo(a)Pyrene were detected in Celestial (5.12 µg/kg), followed by Al Kabous (3.14 µg/kg). Maximum total PAHs were found in Impra tea (52.47 µg/kg) followed by Abu Jabal (45.33 µg/kg). The data were compared with internationally reported values and found to be in agreement with the limits set by world health agencies.

KEYWORDS: toxic PAHs, HPLC, black tea, roasting.

INTRODUCTION

Tea, a product derived from the leaves, internodes, buds and stems of the plant *Camellia Sinesis* is the most popular hot drink in today's world, next to drinking water. Grown on the hill slopes in the tropics, tea is the largest consumed drink (about 3 billion cups a day) in the world, and its global production reached 5.3 million metric tons in 2015 compared to 0.63 million metric tons of coffee [1]. Based on the differences in processing raw leaves, tea can take different forms: White tea (young leaves baked dry), Green tea (leaves steamed, dried and rolled into powder), Oolong tea (leaves dried under intense solar heat to increase oxidation), and Black tea (fully oxidized by withering until the catechins or polyphenols are turned into dark brown tannins or tea color). Being a non-alcoholic beverage, both green and black tea are widely popular brands with geographical variation in terms of their consumptions: European, Southeast Asian, Middle Eastern and American people prefer Black tea while most Chinese and Japanese prefer white and green teas. Green tea, in particular, offers many health benefits to its consumers by increasing lipid and sugar metabolism and insulin resistance in obese and diabetic patients and reducing the serum total cholesterol, thereby reducing the risk of cardiovascular disease and carcinogenic diseases such as breast and prostate cancers [2-5]. Polyphenols, such as epigallocatechin, epicatechin gallate, and epicatechin, the key components of tea and best preserved in green tea, exert most of the anti-oxidative, anti-viral, anti-bacterial, anti-fungal, anti-inflammatory, anti-mutagenic and anti-carcinogenic effects on human health [6-9]. Black tea, in contrast, the most commonly consumed brand contains some toxicants such as heavy minerals,

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fluorides, and polycyclic aromatic hydrocarbons (PAHs) that are carcinogenic to humans, and hence has raised concerns from researchers [10-14]. PAHs released in soils, surface water run-off, and sediments *via* atmospheric fallout, oil and gasoline spill, and urban sewage run-off are consumed by tea plants and they are eventually deposited in tea leaves before finally entering the human food chains. PAHs, released in the air by industrial smoke can be transported over long distance, are also deposited on wide leafy surface area provided by the tea gardens [15-17]. PAHs are identified as being carcinogens with possible genotoxic and mutagenetic properties; and benzo(a)pyrene is a human carcinogen [18]. Possible sources of human exposure to carcinogen PAHs include inhaling PAH-polluted air while working in tea gardens and tea processing factories as well as through daily tea consumption by general population. Compared to green tea, black tea accumulates more PAHs during processing which involves the following sequential steps: withering (air drying of fresh leaves), rolling (withered leaves crushed to powder), fermentation (compression of rolled leaves to achieve desired color and flavor of black tea), drying, and sorting of fermented leaves into different grades. Drying or roasting is done by using hot air and smoke produced from the combustion of pine firewood or turpentine. While repeated roasting using combustion gasses and smoke increases the flavor and color of black tea, and hence, favored by each manufacturer, it also increases the concentration of PAH absorbed by the tea product from combustion of firewood and turpentine [17].

Tea is not a native crop of the Middle East and Saudi Arabia. While Arabic Coffee is a traditional hot beverage in the Arab society, Saudi Arabia relies fully on imported black tea; and it is the most popularly consumed beverage among Saudis and non-Saudi expatriates. Increasing health consciousness among Saudi people has encouraged them to increase their daily tea consumption by moving away from carbonated soft drink. The daily consumption of tea in the Kingdom is now estimated to be over 19 million cups which ranks second in the Arab World after Egypt. Tea import in KSA is valued at over SR 638 million annually. Since black tea contains more toxicants including carcinogenic PAHs, it is important to monitor and identify the nature and quantity of toxicants present in various black tea brands sold and available to Saudi consumers in order to avoid their harmful health impacts. This study purports to examine the nature and quantity of PAHs present in 14 black tea brands sold and available in grocery markets in Saudi Arabia.

MATERIALS AND METHODS

All the reagents used were of high purity grades. HPLC grade ethanol, acetonitrile, n-hexane and dichloromethane were purchased from Merck (Germany). Triply distilled water was obtained from a Thomas Scientific water purification system (QWS4 Water Still). Florisil (a magnesia-loaded silica gel) cartridges, supplied by Agilent, were used as solid-phase extraction columns for purification. Activation of the cartridges was done with 10 mL of dichloromethane and 20 mL of n-hexane before use.

A total of fourteen (14) black tea brands were purchased from the local hyper markets, located in the Eastern Province of Saudi Arabia. There were ten (10) imported and four (4) locally produced tea samples. All the samples were collected in separate polyethylene containers and stored at room temperature till analysis.

PAHs in different tea brands were determined by ultrasonication method. In a typical sample preparation, about 70 g of tea was extracted with 75 ml of dichloromethane (DCM) and acetone (1:1 v/v) under ultrasonication at 30 °C for 30 min. The extract was cleaned by eluting the concentrated extract with methylene chloride from a pre-washed column (I.d 1.5 cm) packed with silica gel and sodium sulfate, 7.5 cm and 2 cm respectively, from top to bottom. The clean extract was evaporated to 0.5 mL under gentle nitrogen flow. Samples were run on an Alliance HPLC system (Waters Associates), equipped with a UV detector (λ = 254 nm) on ODS column (250 x 5 mm). Blank samples were prepared in parallel to detect contamination during the treatment process. During analysis, two injections of a mixture of PAH standards were made every five pairs of tea samples to correct any possible deviation in compound responses. All the samples were analyzed for eight PAH congeners, anthracene, benzo(a)anthracene, benzo(b)fluoranthene, benzo(k) fluoranthene, benzo(a)pyrene, dibenzo(ah)anthracene, benzo(e)pyrene and dibenzo(ghi)anthracene (Table 2). Recoveries of PAHs from the samples were tested by analyzing tea samples spiked at the level of 5 times limits. The PAH standards were spiked into the samples after the homogenization step. Average recoveries of PAHs and limits of quantitation attained by the present methodology are shown in Table 1. Peak identities were confirmed by analyzing samples and standards under duplicate conditions [19, 20].

RESULTS AND DISCUSSION

The levels of different PAHs in selected varieties of tea samples are mentioned in Table 2. The variation of total PAHs content in tea varieties is depicted in Figure 1. These PAH congeners were chosen because of availability of standards and proven carcinogenicity of 5 of them [21]. All samples of tea showed the presence of at least five PAHs out of eight studied. Three PAHs, i.e., Ant, B(a)A and B(e)P were detected in all brands of tea samples. B(b)F was not detected in Lipton, Celestial, Tetly, Twinings and Rabea tea, whereas B(k)F was not detected in Tetly, Twinings and English Teashop brands. B(a)P classified as human carcinogen (class 2A) was found in all samples except English Teashop [18]. Highest levels of B(a)P were detected in Celestial (5.12 µg/kg), followed by Al Kabous (3.14 µg/kg). Maximum total PAHs were found in Impra tea (52.47 µg/kg) followed by Abu Jabal (45.33 µg/kg). Major contributor in both brands was found to be B(a)A, 31.97 µg/kg and 32.71 µg/kg respectively. Satnam *et al.* [7] have reported 16 different PAH levels in tea brands from India. They reported relatively higher values of total PAHs such as 226.1 µg/kg and 464.0 µg/kg of tea for locally produced Red Label and Tata Agni tea. In another study carried out in China, Lin *et al.*, [14] reported different levels of PAHs in different varieties of tea. Black tea contained highest levels of total PAHs (8800 ± 360 µg/kg) followed by Jasmine tea (1220 ± 130 µg/kg), thus proving that roasting process of black tea is mostly responsible for high levels of PAHs. Ziegenhals *et al.* [8] have comprehensively studied the levels of PAHs in various tea types produced and consumed in Germany. They reported highest level of B(a)P in black tea at 14.1 µg/kg as against the present study at 5.12 µg/kg for Celestial tea. They concluded that direct smoldering and roasting of tea leaves is responsible for the higher presence of PAHs in black tea varieties. Table 3 shows the comparison of results obtained within the present study with similar results reported from other parts of the world. To have a good comparison, data reported for the different countries was collected and compared against the sample obtained in this study. Black tea marketed in Saudi Arabia do not show significant difference with the data reported in other studies.

CONCLUSION

Based upon the comparison of data obtained in the present study with internationally available values, it could be safely concluded that tea brands consumed in Saudi Arabia does not contain

Table 1. Average recovery and quantitation limits (ug/kg) of various PAHs.

PAH	Abbreviation	Average recovery (%)	RSD (%)	Quantitation limits
Anthracene	Ant	88	9.4	0.21
Benzo(a)anthracene	B(a)A	95	8.5	0.17
Benzo(e)pyrene	B(e)P	94	10.9	0.09
Benzo(b)fluoranthene	B(b)F	90	6.7	0.83
Benzo(k)fluoranthene	B(k)F	96	7.3	0.72
Benzo(a)pyrene	B(a)P	94	4.6	0.07
Dibenzo(ah)anthracene	D(ah)A	89	6.5	0.15
Benzo(ghi)perylene	B(ghi)P	93	7.9	0.19

Table 2. Concentrations (ug/kg) of various PAHs in tea varieties.

Brand	n	Origin	Ant	B(a)A	B(e)P	B(b)F	B(k)F	B(a)P	D(ah)A	B(ghi)P	ΣPAH
Lipton	4	UAE	8.35±0.38	9.80±1.95	2.90±1.10	ND	1.06±0.90	1.50±1.01	ND	ND	10.48
Royal Club	6	Spain	2.06±0.88	2.26±1.2	2.12±0.78	0.92±0.65	1.34±0.79	2.08±1.56	0.77±0.53	1.03±0.57	12.58
Celestial	3	USA	3.85±1.39	13.96±8.76	1.05±0.70	ND	0.66±0.21	5.12±1.20	1.7±0.75	0.40±0.29	26.74
Tetly	7	UAE	7.10±1.01	12.13±1.75	2.09±1.09	ND	ND	1.01±0.70	1.16±0.66	ND	23.49
Impra Tea	3	Sri Lanka	11.91±3.42	31.97±6.99	1.30±0.66	1.33±1.01	0.95±1.02	2.50±1.99	1.98±0.55	0.59±0.50	52.47
Al kabous	3	Jordon	2.29±1.92	7.49±2.16	2.16±1.19	2.09±1.32	2.05±1.47	3.14±2.89	1.21±0.43	1.19±0.90	21.62
Twinings	4	China	4.35±1.92	11.45±1.45	1.09±0.46	ND	ND	1.27±0.85	0.38±0.13	1.45±0.95	19.99
English Tea shop	3	Sri Lanka	2.45±2.80	11.66±1.99	1.15±0.46	0.98±0.09	ND	ND	0.38±0.23	0.74±0.21	17.36
Al Khair	4	Sri Lanka	3.08±1.87	17.90±3.3	1.26±0.45	0.95±0.42	1.02±0.46	1.68±1.02	0.76±0.07	0.34±0.21	26.99
Tetley	4	Sri Lanka	2.35±2.70	18.99±1.29	1.92±0.33	1.02±0.36	1.05±1.01	1.39±0.33	ND	0.32±0.11	27.04
Rabea	5	Local	4.69±0.14	11.37±1.77	1.03±0.92	ND	0.87±0.65	1.85±0.99	0.33±0.05	0.66±0.36	20.88
Abu Jabal	5	Local	3.80±1.81	32.71±6.21	4.01±2.20	1.05±0.97	1.06±0.20	1.73±0.14	ND	0.97±0.30	45.33
Panda	4	Local	13.55±2.71	8.56±0.32	0.57±0.44	0.97±0.21	0.57±0.34	1.53±0.44	0.95±0.21	0.19±0.07	26.89
Karak Chai	4	Local	7.65±0.83	18.24±2.09	0.68±0.12	1.03±0.76	0.61±0.64	1.99±0.17	1.07±0.87	ND	31.18

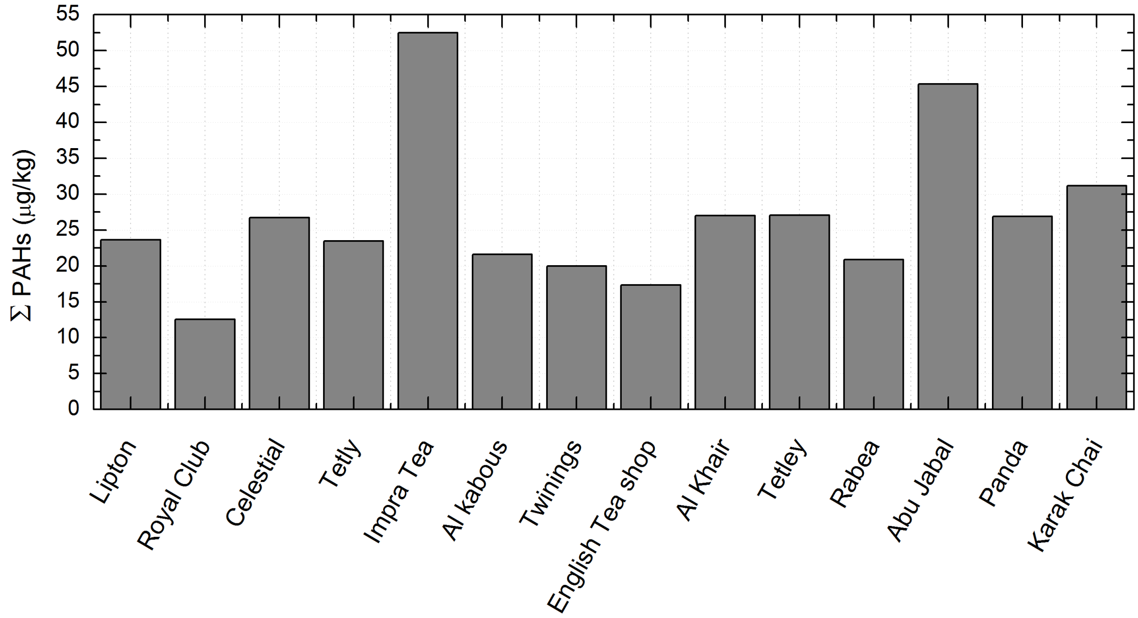

Figure 1. Levels of total PAHs in different varieties of black tea.

Table 3. Comparison of the present data with internationally reported values.

Source Country	n	BaA	D(ah)A	Bbf	BaP	Reference
Argentina	27	0.2-62.8		0.1-67.6	0.2-92.5	[10]
Austria	4	0.7-31.9		1.9-22.0	0.4-5.9	[22]
Nigeria	48	0.82-2.11	6.33-11.9	ND	2.0-6.22	[6]
China	7	175.0		37.6	39.7	[14]
China	9	198.0	8.42	100.0	61.6	[17]
Czech Republic	18	1.4-196.1		0.9-123.2	0.2-151.7	[23]
Germany	11	1.3-13.1	0.1-0.9	1.5-8.1	0.8-14.1	[8]
Japan	4	4.3-44.5		5.2-35.7	5.3-73.2	[24]
Luxemburgo	15	0.6-125.0		0.15c-34.4	0.15c-21.9	[25]
India	3	ND		210.4	1574.1	[26]
Saudi Arabia	14	2.26-32.71	0.33-1.70	0.92-2.09	1.0-5.12	This work

alarming levels of PAHs. However, the detected levels reflect the contributions from withering and roasting stages of black tea processing.

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CONFLICT OF INTEREST STATEMENT

The authors declare that there is no conflict of interest about this work with any other agency.

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