Dietary consumption of pre-carcinogenic amines and mutagenicity in humans: An evidence-based study

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Abstract:
Dietary consumption of heterocyclic aromatic amines (HAA) is considered to be a high-risk factor that substantially contributes to the development of mutagenicity and carcinogenicity in humans. This study provides ample evidence for the plausible association between mutagenicity or carcinogenicity development and dietary intake of heterocyclic amines in humans. The current study intends to assess the degree of heterocyclic amine contaminants in high-temperature cooked meats, their subsequent food intake, and potential health risk estimations.

The meat samples were homogenized, filtered, extracted, and eluted. The list of heterocyclic amines to be identified and quantified included PhIP (2-amino-1-methyl-6-phenylimidazo[4,5-b] pyridine), IQ (2-amino-3-methyl-imidazo[4,5-f] quinolone), and MeIQx (2-amino-3,8-dimethylimidazo[4,5-f] quinoxaline). They were simultaneously isolated and studied using the method of high-pressure liquid chromatography (HPLC). The highest heterocyclic amine concentration was found in chicken (2705.99 ± 6.12 ng/g), beef (574.09 ± 1.52 ng/g), and mutton (342.41 ± 3.69 ng/g). PhIP (73%) was the main heterocyclic amine in chicken. The estimated daily dietary intake or exposure in chicken, mutton, and beef was 0.690, 0.050, and 0.144 ng/kg body weight, respectively. The values for margin of exposure were within the range identified by the European Food Safety Authority for mutton (102.06) and chicken (13.250), but not for beef (3.784).

This significantly high prevalence of heterocyclic amines and the associated health risks are sufficient to warn the public about the high dietary intake of meat and its carcinogenic health hazards. The mutational patterns induced by heterocyclic amines resemble those in human tumors, requiring the use of specific biomarkers like HAA-DNA and HAA-protein adducts. Future prospects are high for integrating these biomarkers into epidemiological studies, which could provide a comprehensive assessment of health risks associated with dietary heterocyclic aromatic amines in human cancer.

Keywords: Cytotoxicity, meat, heterocyclic amines, high pressure liquid chromatography, biomarkers, carcinogenicity, health risk

INTRODUCTION

Dietary exposure of humans to heterocyclic amines significantly contributes to the development of mutagenicity and carcinogenicity [1]. The amount and structure of various types of heterocyclic amines depend on the temperature and cooking methods. Heterocyclic amines in varying concentrations were discovered in the deep-fried, roasted, charcoal-grilled (high heat, 20 min), and

barbecued (177–260 and 339–365°C) chicken flesh samples [2]. High-temperature cooking procedures, antioxidant presence, lipid oxidation, meat precursor types, creatine levels, and free amino acids also increase the presence of heterocyclic amines in meat products [3].

PhIP(2-amino-1-methyl-6-phenylimidazo[4,5-b] pyridine), one of the 25 detectable heterocyclic amines, was identified as a carcinogenic heterocyclic amine, abundantly prevalent in high-flame-cooked proteinaceous foods, such as beef, pork, and fish [4].

Heterocyclic compounds are organic compounds with a minimum of one hetero atom [5]. A carbocyclic compound is an organic cyclic molecule with rings, with the most common heterocycles consisting of oxygen, nitrogen, or sulfur atoms [3]. Diverse natural products, physiologically active molecules, functional materials, ligands, and catalysts all comprise heterocyclic compounds with nitrogen, oxygen, and sulfur atoms. These compounds serve as versatile components in synthesizing organic compounds [6]. Heterocyclic moieties are particularly common in commercially available medications and agrochemicals [7]. Significant work has been put into developing synthetic techniques to create heterocyclic molecules [7]. Pyridine, pyrrole, thiophene, and furan are well-known heterocyclic compounds with six-atom rings [8]. Five-membered rings in furan, thiophene, and pyrrole molecules consist of four carbon atoms and one atom of oxygen, sulfur and nitrogen, respectively [9–11].

According to several epidemiological studies and cohort experiments, consumption of processed red meats quadruples the health risk concern of developing colorectal cancer [9]. Additionally, consumption of cooked or processed meat was linked to the development of malignancies in the esophagus, stomach, endometrial lining, pancreas, prostate, and lungs [10]. Busquets *et al.* [11], who conducted a survey based on a food frequency questionnaire, reported that an average daily intake of 13 meat dishes raised heterocyclic amines up to 285.6 ng/g. In addition, the average daily intake of heterocyclic amines could grow by 475.6 ng in the presence of co-mutagens. Kobets *et al.* wrote that a daily dietary intake of heterocyclic amines might bring their level up to 103 and 160 ng, which may trigger the development of various carcinogenic effects [12].

DNA adduct identification and measurement are crucial to understand exposure to genotoxic chemicals and their role in cancer development. Tissues embedded in paraffin and fixed with formalin offer unexplored biosamples for studying cancerogens by adducting DNA biomarkers [13].

Hazardous carcinogenic compounds in the diet and environment can facilitate the formation of genomic DNA adducts, leading to development of cancer and mutations. DNA adduct identification and measurement involve methods like liquid chromatography-mass spectrometry (LC/MS), mass spectrometry, immunoassays, and 32P-postlabeling. New developments in formalin-fixed paraffin-embedded tissues offer untapped biosamples for tumor-causing DNA adduct biomarker research through mass spectrometry-based human biomonitoring (Figure 1) [14].

Annual poultry consumption in Pakistan is 16 kg per person [15]. In 2020, the consumption of sheep, beef, and veal in Pakistan amounted to 6.62, 2.02, and 6.42 kg/per person, respectively [16]. Sohaib and Jamil reported the per capita meat consumption as 11.7, 13.8, and 14.7 kg in 2000, 2006, and 2009, respectively; the authors expected it to surge and reach 47 kg/per capita by 2020 [17]. The mean values for annual per capita consumption of beef were 2.7 (total), 2.4 (rural), and 3.0 (urban) kg, as reported in 2018 by the Household Integrated Economic Survey. However, a field survey
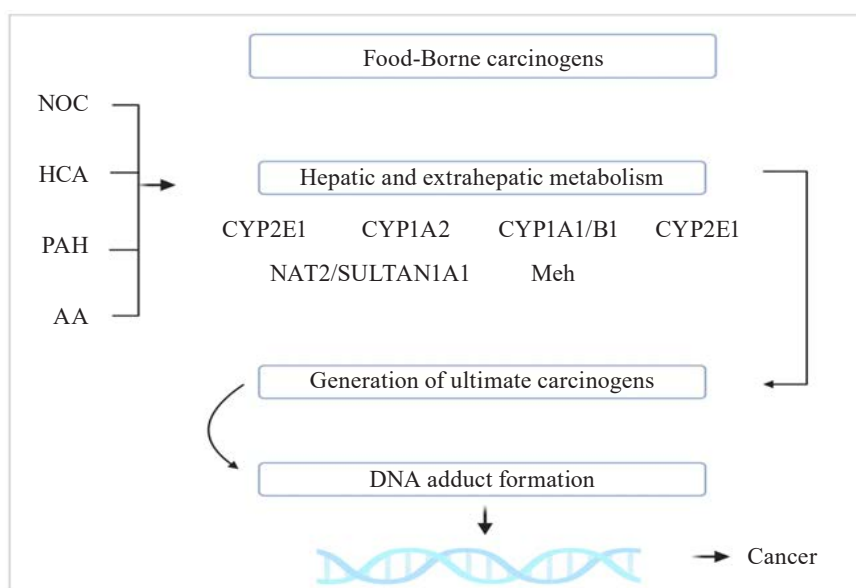

Figure 1 Mutations and cancer as a result of carcinogenic substances in food and environment
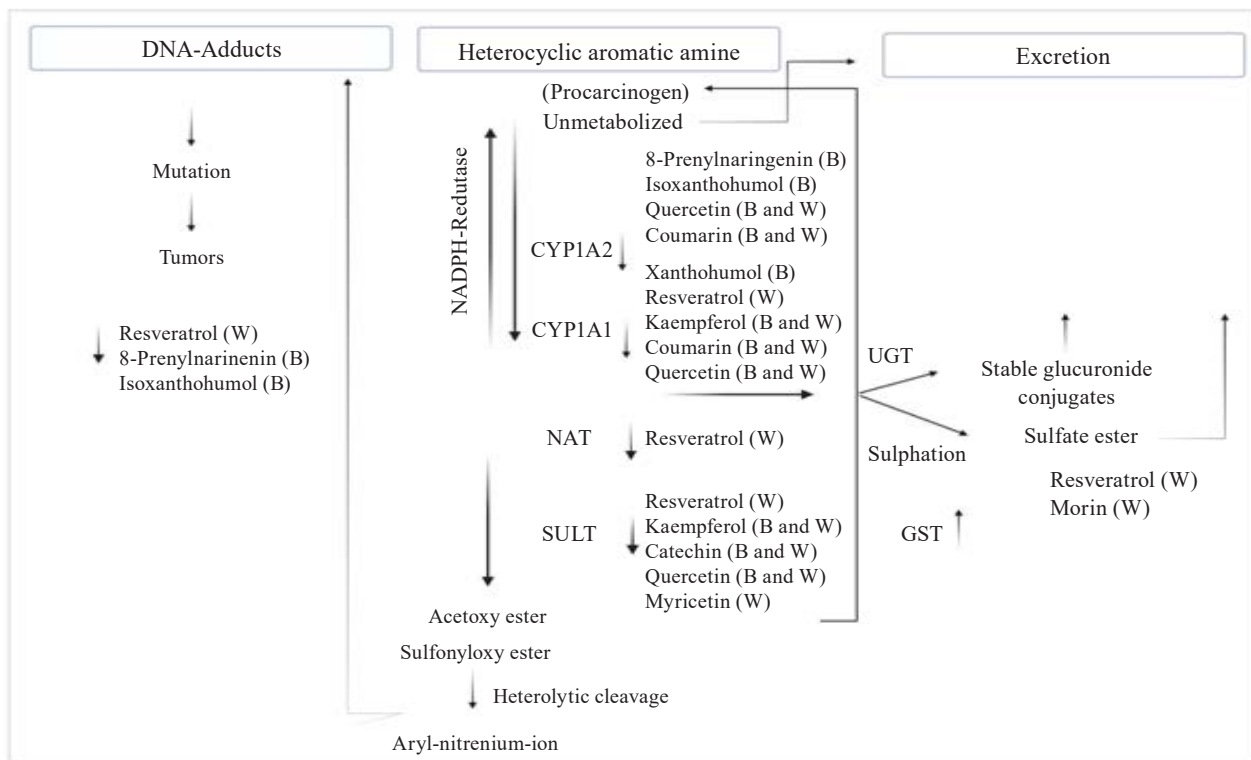
Figure 2 Biomarkers and metabolism of heterocyclic amines in human beings [19, 20]

performed that yielded different mean values, i.e., 3.8 (total), 4.2 (rural), and 3.4 (urban) kg [18].

N-oxidation of exocyclic amine groups results in the production of HONH-HAAs, i.e., genotoxic metabolites of heterocyclic aromatic amines. In rodents, the liver exhibits the highest metabolic activity, especially following enzyme induction with polychlorinated biphenyl or 3-methylcholanthrene. HONH-HAAs form mutation-prone DNA adducts by covalently attaching to DNA. Hepatic cytochrome CYP1A2, extrahepatic CYP1A1, and CYP1B1 are the main cytochromes that catalyze N-oxidation of heterocyclic aromatic amines in humans. CYP1A2 is responsible for 70% of the total metabolism of PhIP and 91% metabolism of MeIQx. The interspecies variations in heterocyclic aromatic amine metabolism occur by cytochromes in nonhuman primates, humans, and rodents. The primary causes are attributed to the cytochrome expression levels, catalytic activity variations, and cytochrome regioselectivities towards heterocyclic aromatic amines. MeIQx is mainly produced in human hepatocytes as the detoxicated metabolite IQx-8-COOH, which is primarily formed in the urine of meat consumers. This metabolite is catalyzed by human CYP1A2. Compared to rat CYP1A2, recombinant human CYP1A2 exhibits catalytic effectiveness of PhIP and MeIQx N-oxidation that are 10–19 times higher. These interspecies differences must be considered when determining the risk of heterocyclic aromatic amines to human health using experimental animal toxicity data because they have an impact on the biological effects of heterocyclic aromatic amines [20].

Food contains such toxic substances as polycyclic aromatic hydrocarbons and mycotoxins, which can pose serious problems for the modern world. Mycotoxin exposure to agricultural products is spreading around the world, and eating processed red meat raises risks of developing cancer, especially colorectal cancer. The International Agency for Research on Cancer has categorized processed meat as a human carcinogen (Group 1) and red meat as a potential human carcinogen (Group 2A). Meat is often subjected to high asparagine levels, baking, frying, drying, combustion products from fossil fuels, heating fats or oils to high temperatures, fermenting, salting, pickling, and rotting. These processing techniques can result in the formation of carcinogenic compounds. Many genotoxic substances found in food undergo biotransformation within the body, which turns them from initial mutagens or carcinogens into ultimate ones. Food procarcinogens are metabolized by Phase I functioning reactions (Phase I) and conjugation reactions (Phase II), which are catalyzed by xenobiotic, or drug-metabolizing enzymes, preferentially (XMEs/DMEs) [12].

As food-genotoxic substances undergo biotransformation, turning into carcinogens or mutagens, they are metabolized through phase I and II reactions, being catalyzed by xenobiotic enzymes. Metabolism (Phase I) of the abovementioned carcinogens in food is affected by cytochrome P450. DNA sites that are vulnerable to electrophilic compounds include: adenine N_3, N_1, and N_7 atoms, guanine O_6, N_7, and N_3 atoms, thymine N_3, O_4, and O_2 atoms, cytosine N_3 and O_2 atoms, phosphodiester
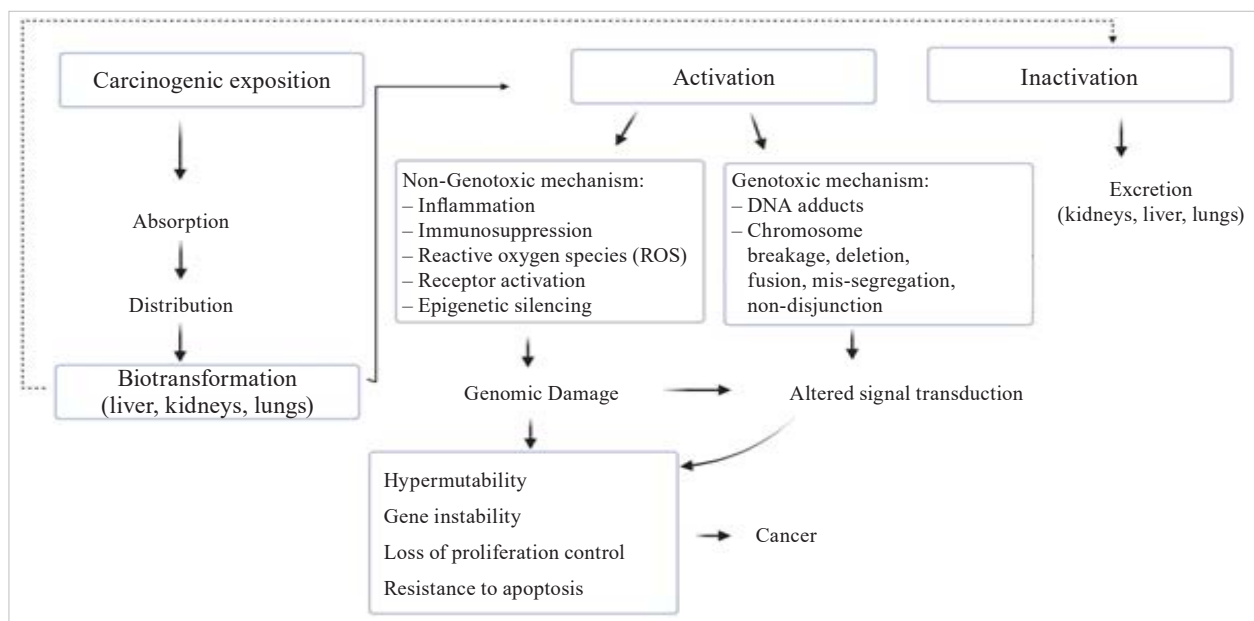
Figure 3 Food-borne carcinogens, metabolism, and development of mutagenicity in humans [21]

skeleton in the phosphate group, etc. When guanine or adenine is alkylated at the N_7 position, they trigger such reactions as spontaneous depurination with the formation of purine sites (AP) or conversion of alkylated bases into a stable formamidopyrimidone (FAPy) form [22].

The O6 positional modifications of pyrimidine and purine bases typically result in mismatched base pairing and mutations in daughter cells. Chemicals with electrophilic properties that bind to DNA covalently have the potential to cause carcinogenesis, which could lead to neoplastic transformation. These chemicals could change into electrophilic forms that bind to nucleophilic sites in DNA, making them mutagenic [14].

Food contains harmful compounds that can pose significant health risks, e.g., mycotoxins, processed red meat, and contaminated grains. The food industry must address these issues and develop effective strategies to prevent and treat these harmful substances in food production. At home and in restaurants, cooking techniques often include grilling, broiling, deep frying, and pan-frying. All these high-temperature treatments, cooking time, meat type, and some cooking utensils lead to high contents of carcinogenic heterocyclic amines in food. High-temperature cooking of meats was found associated with the development of powerfully carcinogenic heterocyclic amines in commercially available ready-to-eat and ready-to-cook meat products [23].

The current study investigated and verified the presence, formation, and quantification of carcinogenic heterocyclic amines and their subsequent health risks in high-temperature cooked chicken, mutton, and beef.

STUDY OBJECTS AND METHODS

Chemicals. Heterocyclic amines from high proteinaceous muscle foods, such as beef, poultry, and mutton, were isolated, identified, and quantified. The methods were in line with those described in [24, 25], with some changes connected with affordability, effective compatibility, and economic viability [23]. In this study, we used three heterocyclic amine standards imported from Toronto Chemical Company, Canada. They included PhIP (2-amino-1-methyl-6-phenylimidazo[4,5-b] pyridine), IQ (2-amino-3-methyl-imidazo[4,5-f] quinolone), and MeIQx (2-amino-3,8-dimethylimidazo[4,5-f] quinoxaline). Sodium hydroxide (NaOH), methanol (CH_3OH), ethyl acetate ($CH_3COOC_2H_5$), and ACN (acetonitrile) were purchased from Sigma-Aldrich, USA. All chemicals and solvents involved in the mobile phase preparation and extraction process were of HPLC-grade. The syringe filters (0.22 μm, 25 mm), diatomaceous earth, and filter papers (0.22 μm, 47 mm) were acquired from Integrated Biosciences, Pakistan. The purity values of MeIQx, IQ, and PhIP were 99.78, 98, and 99%, respectively. The internal standard of 4,7,8-TriMeIQx was added at a constant concentration to the purified samples and standards. Figure 4 illustrates the chemical structures of the four heterocyclic amine standards.

Stock standard solutions and their preparations. We prepared a stock solution (200 μg/mL) for three heterocyclic amine standards, i.e., IQ, MeIQx, and PhIP, in methanol to be used for spiking standards. The calibration curves with three heterocyclic amine standard mixes were obtained between 0.0000001 to 1 μg of heterocyclic amine per 1 mL to establish a linearity range. Figure 2 demonstrates the linearity range for the calibration curves of PhIP, IQ, and MeIQx standards. Prior to the injection of sample extracts and standards in high-performance liquid chromatography (HPLC), we filtered them by using syringe filters (0.22 μm, 25 mm).

Preparing meat samples. Meat samples were barbecued, homogenized, and stored in a refrigerator at –20°C until further use. Then we mixed 1 g of defrosted
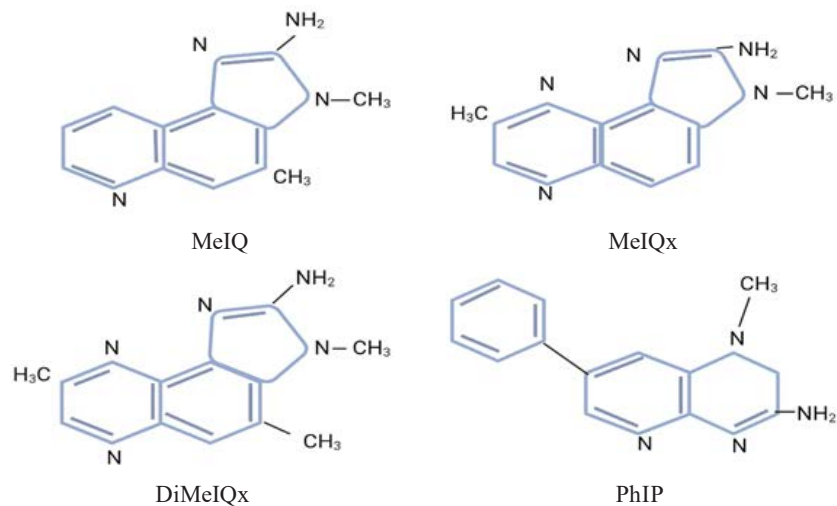
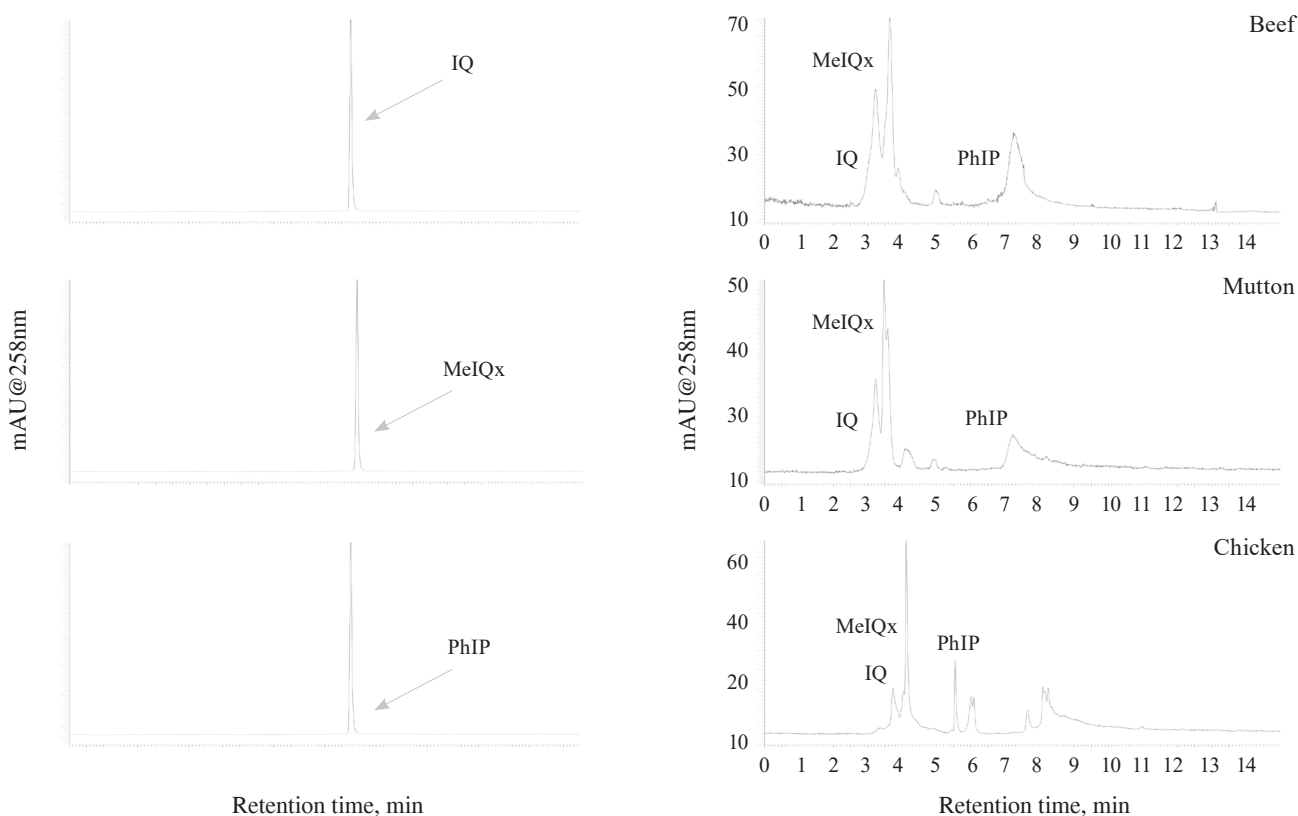**Figure 4** Structural depiction of heterocyclic amines [20]

Figure 5 Chromatograms of heterocyclic amines, i.e., PhIP, IQ, and MeIQx standards (on the left) in beef, mutton, and chicken (on the right)

meat with 12 mL of 1N NaOH and turned it into a homogenous suspension by using magnetic stirring at 600–700 rpm for 1 h. After the mix passed through the EXtrelut columns packed with diatomaceous earth, we added ethyl acetate $CH_3COOC_2H_5$ (20 mL), 0.1 N HCl (2 mL), and MeOH (2 mL). The resultant analyte was eluted by using 2 mL of 25% NH_4OH (ammonium hydroxide) and MeOH (1:19, v/v).

Identification and subsequent quantification of heterocyclic amines. We identified and quantified heterocyclic amines by using a PerkinElmer HPLC sys-

tem. The procedure involved an Ultra-Violet/Visible detector and C18-Column (4.6 mm, 250 L, 100Aº). The flow rates of mobile phase A (H_2O/MeOH/CH_3COOH/ ACN 76/8/2/14 mL) and mobile phase B (ACN) were at 0.7 mL/min; the analyte injection volume was 10 µL. Figure 5 illustrates the chromatograms for PhIP, IQ, and MeIQx standards.

Method validation. We validated this analytical method by various experimental studies. The limits of quantitation and detection (LOQ and LOD) values along with the linearity range were established and calculated

for each of the three heterocyclic amines. Linearity was calculated by spiking the heterocyclic amine standards at six different concentration levels. Graphs were plotted between a peak area and the concentration of the heterocyclic amines to produce calibration curves. We also calculated the linear regression to define the correlation coefficient, intercept, and slope of each calibration line. Figure 6 shows the calibration curves of MeIQx, IQ, and PhIP standards (µg/mL) with the corresponding linear regression and R^2 value. The detection and quantitation limits for MeIQx, PhIP, and IQ were in the ranges of 10–1000, 0.1–100, and 0.1–10 ng/g.

Dietary exposure and health risks estimation. The daily dietary exposure data for heterocyclic amine contamination in meats came from various sources. As it was mentioned above, the annual poultry consumption in Pakistan is 16 kg per person [15]. In 2020, the annual consumption of poultry, sheep, and beef reached 6.62, 2.02, and 6.42 kg/per person, respectively [16]. The Food and Agriculture Organization offers different statistics, according to which the per capita annual meat consumption in Pakistan mainly consists of buffalo (1.7 kg),

beef (6.3 kg), chicken (4.5 kg), goat (1.6 kg), and sheep (2.1 kg) [26]. In this research, we accepted 70 kg as the average body weight of an adult meat consumer. To calculate the estimated daily meat intake, we used the formula proposed by Bogdanović *et al.* [27]:

$$EDI = \frac{C_c \times C_i}{BW} \tag{1}$$

where C_c is the stands for the average daily meat consumption by person, g; C_i is the designates the content of the heterocyclic amine, ng/g; BW is the average body weight of consumers, kg; EDI is the means the estimated daily meat intake expressed as 1 ng/kg body weight/day.

Calculating margin of exposure. Margin of exposure (MOE) calculation made it possible to estimate the health risks. This experiment followed the recommendations of the European Food Safety Authority Panel [32] and the procedure for polycyclic aromatic hydrocarbons (PAH) [28]. The benchmark dose limit ($BMDL_{10}$) value for PhIP was reported as 2.71, 0.74, and 0.4 mg per 1 kg of body weight per day for their association with the
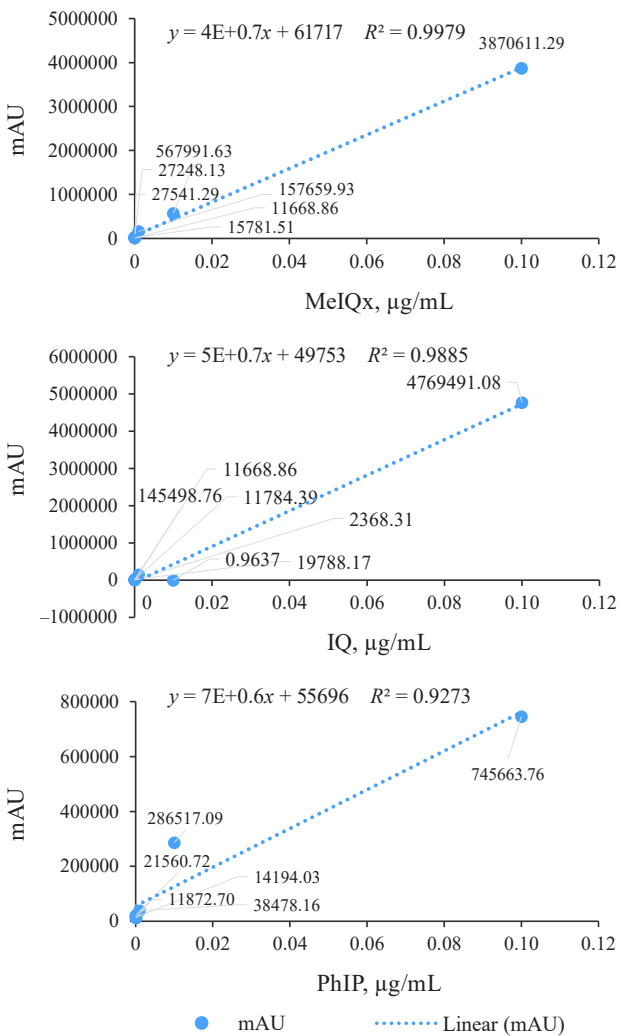
MeIQx				
Concentration, µg/mL	mAU	R^2 value	LOQ, ng/g	LOD, ng/g
0.1	3870611.29	0.9979	100	0.1
0.01	567991.63			
0.001	157659.93			
0.0001	27541.29			
0.0001	15781.51			
0.000001	27248.13			
0.0000001	11668.86			

IQ				
Concentration, µg/mL	mAU	R^2 value	LOQ, ng/g	LOD, ng/g
0.1	4769491.08	0.9637	10	0.1
0.01	1395448.11			
0.001	145498.76			
0.0001	19788.17			
0.00001	11784.39			
0.0000001	2368.31			
0.0000001	11668.86			

PhIP				
Concentration, µg/mL	mAU	R^2 value	LOQ, ng/g	LOD, ng/g
0.1	745663.76	0.9278	1000	10
0.01	286517.09			
0.001	38478.16			
0.0001	11872.70			
0.00001	14194.03			
0.000001	21560.72			

LOQ = Signal: Noise (1:9) ; LOD = Signal: Noise (1:3)

Figure 6 Calibration curves of MeIQx, IQ, and PhIP standards with corresponding linear regression, R^2 values, limits of quantitation (LOQ), and limits of detection (LOD)

colon, mammary, and prostate tumors, respectively [29]. The benchmark dose limit for MeIQx was 8.8 μM (0.001876 mg/kg) [30]. The benchmark dose limit for IQ came from a study conducted on GST-P positive foci, which indicated a significant increase in its numbers at $BMDL_{10}$ = 1.44 ppm (1.44 mg/kg) [31]. These values for $BMDL_{10}$ were used as references in the following formula:

$$MOE = \frac{BMDL_{10}}{EDI} \qquad (2)$$

where EDI is the dietary intake of heterocyclic amines, mg/kg body weight/day; $BMDL_{10}$ is the stands for the benchmark lower dose limit for heterocyclic amine per 1 kg of body weight per day at 10% and measurable response.

Statistical analysis. We used a complete block experiment in a randomized pattern with repeated measurements. Each experimental unit was repeated four times. For statistical analysis, the average of two measurements was made on the same experimental unit. The data were processed using SAS 9.1 to examine all statistical significance. The analysis involved a multiple comparison test and a variance analysis (ANOVA). Means at $p \leq 0.05$ served to assess the significance of the findings [32].

RESULTS AND DISCUSSION

Contamination of barbecued meats with heterocyclic amines. The total heterocyclic amine concentrations in barbecued meat, i.e., mutton, beef, and chicken, ranged between 342.41 ± 3.69 and 2705.99 ± 6.12 ng/g. Table 1 gives the quantitative analysis data of PhIP (2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine), IQ (2-amino-3-methyl-imidazo[4,5-f]quinolone), and MeIQx (2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline) in charcoal-barbecued mutton, beef, and chicken. The resulting heterocyclic amine contents in barbecued chicken (2705.99 ± 6.12 ng/g), mutton (342.41 ± 3.69 ng/g), and beef (574.09 ± 1.52 ng/g) appeared to be considerably higher.

As a rule, heterocyclic amine formation depends on the cooking temperature and time [33]. The PhIP contents for pan and deep-fried samples ranged from 18.33 to 70.00 ng/g and from 21.3 to 22.21 ng/g, respectively. In contrast, microwave treatment at different treatment times were reported to increase the heterocyclic amine level by 0.70–35.00 μg/g [23].

Barbecuing is universally regarded as one of the most prevalent cooking methods. Duck and chicken breasts cooked by grilling were found to contain 112 and 32 ng/g of total heterocyclic amines, respectively [34]. Our findings were consistent with the high PhIP (270 ng/g) and AαC (180.4 ng/g) in chicken breasts grilled at high temperatures [33]. The respective production of mutagens in commercially processed chicken products, i.e., chicken nuggets, olive oil sardines, and tomato sauce sardines, was 166.44, 61.10, and 47.33 ng/g [33].

Predominant concentrations of individual heterocyclic amines. In this study, the predominant PhIP content in beef, mutton, and chicken ranged between 212.10 ± 1.35 and 1987.18 ± 5.73 ng/g. Our results support those published by Perveen *et al.*, who found high heterocyclic amine quantities in high-temperature processed pan-fried (1.11–8.60 g/g), deep-fried (1.6–22.3 g/g), microwaved (0.7–35.0 μg/g), and ready-to-eat chicken kababs [23]. Thus, cooking food at different temperatures may result in various concentrations of PhIP in chicken flesh.

The extremely high PhIP content (1987.18 ± 5.73 ng/g) in this study was in line with the high PhIP contents in barbecued (480 ng/g) or grilled (270 ng/g) chicken [35]. Also, previous studies also detected the highest PhIP concentrations of 420, 320, and 92 ng/g in beef, chicken, and turkey meat, respectively [12].

Kobets *et al.* grilled chicken breast samples at 177–260°C and obtained 480 ng/g PhIP while the chicken samples barbecued at 339–365°C produced a total PhIP level of 330 ng/g [12]. However, Olalekan Adeyeye and Ashaolu [4] reported high MeIQx concentrations of > 100 ng/g.

IQ contents in barbecued meats. The grilled beef samples revealed no IQ quantified for heterocyclic amines. However, the barbecued mutton and chicken samples demonstrated IQ concentrations as high as 116.39 ± 2.20 and 692.33 ± 0.58 ng/g, respectively (Table 1).

Some other studies also verified no IQ content in processed meat products [36]. These findings were, however, found in contrast to the lower IQ contents ranging between 0.44 and 13.98 ng/g [37]. These high IQ quantifications, as detected in the chicken samples, were also in correspondence with our previous findings of IQ, which ranged from 0.25 to 4.97 μg/g [38].

MeIQx and PhIP in beef steaks were reported to be up to a respective level of 2.3 and 16.8 ng/g. A significant correlation occurred between the heterocyclic amine content and the drippings gravy intake. A quarter cup of gravy contained up to 62 ng/g DiMeIQx,

Table 1 Contamination level of heterocyclic amines MeIQx, IQ, and PhIP in various types of barbecued and/or charcoal grilled beef, mutton, and chicken

Meat	MeIQx, ng/g	IQ, ng/g	PhIP, ng/g	Σ, ng/g	*F*-value
Beef	48.34 ± 1.00[c]	0.00 ± 0.00[d]	525.75 ± 0.54[b]	574.09 ± 1.52[a]	308999.19
Mutton	13.91 ± 0.38[d]	116.39 ± 2.20[c]	212.10 ± 1.35[b]	342.41 ± 3.69[a]	11516.19
Chicken	26.48 ± 0.56[d]	692.33 ± 0.58[c]	1987.18 ± 5.73[b]	2705.99 ± 6.12[a]	249743.96

Means that do not share the same superscript are significantly different ($p \leq 0.05$); *P*-value was 0.000 for all samples.

230 ng/g PhIP, and 398 ng/g MeIQx [4]. According to Omofuma *et al.*, pan-fried hamburgers contained twice as much MeIQx as barbecued or grilled steaks. Figure 7 illustrates the process involved in the heterocyclic amine formation [39].

Higher PhIP contents in grilled/barbecued samples. In this study, the grilled beef samples revealed no IQ. Significant concentrations of PhIP (73%) followed by MeIQx (1%), and IQ (26%) were, however, present in barbecued chicken. The charcoal-grilled chicken samples demonstrated higher PhIP percentages than the mutton samples: 73 vs. 61.86%. The barbecued beef samples contained more PhIP (91.43%) and MeIQx (8.57%) than IQ. However, the MeIQx concentration in the barbecued mutton sample was substantially greater (4.18%) than that in the chicken sample (1%).

Our findings agreed with those reported by Zeng *et al.*, who detected more PhIP (38.2–48.54 ng/g) than MeIQx in mutton, chicken, and beef [40]. Barbecued meat (270℃, > 6 h) was reported to result in a tenfold rise in PhIP contents (0–73 ng/g) as compared to MeIQx [41]. However, deep frying produced higher PhIP (31.97 ng/g) concentrations than MeIQx (293.86 ng/g). Thus, pan-fried fish had high PhIP (69.20 ng/g) contents, followed by MeIQx (6.44 ng/g) [39].

The high PhIP content reported by the abovementioned studies was consistent with the results of our investigation. However, Olalekan Adeyeye and Ashaolu found significant MeIQx (22.02 ng/g) and PhIP (19.77 ng/g) contents in fried mutton [4]. In our samples of grilled mutton, the high PhIP (61.86%) levels were followed by IQ (33.95%). These findings were in
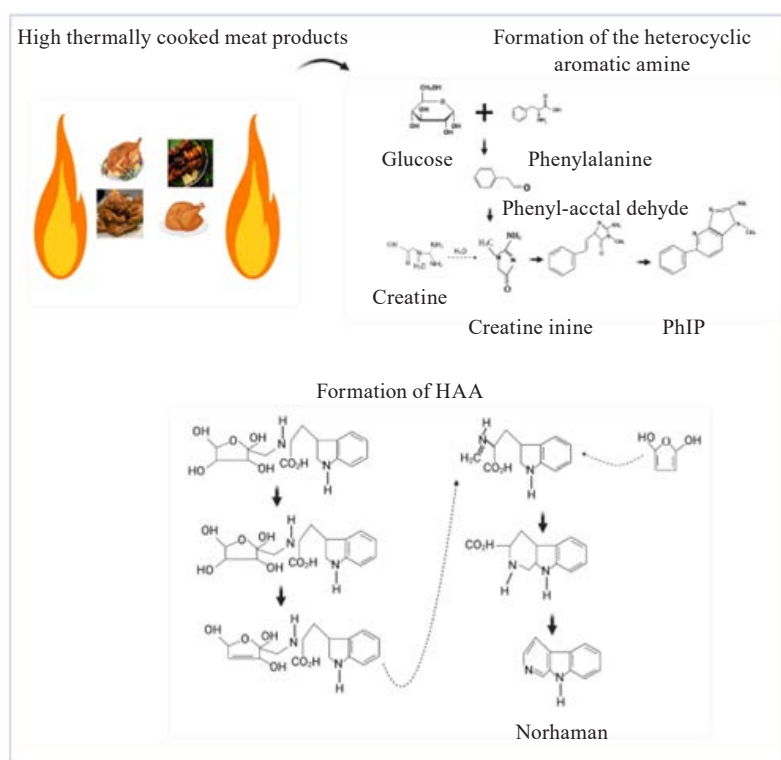

Figure 7 Mechanism involved in heterocyclic aromatic amine formation

Table 2 Estimated daily dietary intake, ng/kg body weight, and risk assessment (margin of exposure) in barbecued beef, mutton, and chicken

Meat	MeIQx		IQ		PhIP		Σ_3	
	Estimated daily dietary intake	Margin of exposure	Estimated daily dietary intake	Margin of exposure	Estimated daily dietary intake	Margin of exposure	Estimated daily dietary intake	Margin of exposure
Beef	0.0121	0.154	0	0	0.132	3.63	0.144	3.784
Mutton	0.0020	0.930	0.017	85.49	0.031	15.64	0.05	102.06
Chicken	0.0068	0.275	0.178	8.088	0.510	4.89	0.69	13.25

Estimated daily dietary intake = Average daily meat consumption by person, g × Heterocyclic amine, ng/g /body weight.

Margin of exposure = Benchmark dose limit / Estimated daily dietary intake.

Average daily meat consumption: beef = 6.42/365 = 0.0176, chicken = 6.62/365 = 0.018 [16], and mutton = 3.7/365 = 0.01013 [26]. Average body weight = 70 kg. Benchmark dose limit for PhIP = 0.48 mg/kg/body weight/day [29], MeIQx = 8.8 µM (0.001876 mg/kg) [29], IQ = 1.44 ppm (1.44 mg/kg) [31].

correspondence with Jahurul *et al.* [38], who reported total heterocyclic amines up to 38.7 ng/g, which was mainly comprised of PhIP followed by MeIQx and IQ.

Table 1 demonstrates the current heterocyclic amine quantifications. ng/g, in various types of barbecued and charcoal-grilled beef, mutton, and chicken detected by UV-Vis HPLC. Our results were in correspondence with those reported by other scientists. The resulting variation in concentrations as reported by different studies might be accredited to different varieties of meat, cooking time, and temperatures.

Estimating dietary exposure. Table 2 shows the daily dietary intake of IQ, PhIP, MeIQx, and Σ_3 heterocyclic amines in adult humans from barbecued or grilled beef, mutton, and chicken.

Dietary ingestion of respective heterocyclic amines in chicken, mutton, and beef was found as 0.69, 0.05, and 0.144 ng/kg body weight per day. A similar study [28] on polycyclic aromatic hydrocarbons (PAHs) reported 0.13–0.72 ng/kg body weight per day for meat doners and 0.03–0.24 ng/kg body weight per day for grilled fish.

The daily dietary intake of heterocyclic amines depends on the eating habits and/or patterns. Additionally, consumers' exposure to heterocyclic amines grows together with the consumption rates.

The estimated dietary exposure to heterocyclic amines in this research was in line the previous studies. According to a preliminary estimation, the mean daily dietary exposure to total heterocyclic amines was 4.43 ng/kg body weight, which fits better within the reported range [42]. Another Malaysian study stated the major contribution of grilled and fried chicken to the high average daily dietary ingestion of heterocyclic amines as up to 553.7 ng per person [38]. Similarly, Pouzou *et al.* [43] found an almost similar concentration of daily exposure to heterocyclic amines (PhIP and MeIQx) from meat and various kinds of bread in the United States as 565.3 ng per person. Sabally *et al.* [44] observed that the daily dietary exposure to MeIQx (0.93 ng/kg body weight) and PhIP (2.34 ng/kg body weight) was within the previously reported ranges for heterocyclic amines.

Dietary exposure to heterocyclic amines and risk assessment. The margin of exposure made it possible to estimate the health risk. If the margin of exposure is below 10.000 for any contamination level, human health may be at high risk [45]. Table 2 gives the estimated daily intake and the margin of exposure for heterocyclic amines in grilled or charcoal chicken, mutton, and beef. In this study, the margin of exposure indicated as lowest vs. highest values was found as 3.784 and 102.06, respectively, in beef and chicken samples. The lowest respective margins of exposure for PhIP as reported by Carthew and co-workers were 20 000, 40 000, and 150 000 for prostate, breast, and colon tumors [29].

Little information is available to estimate the margin of exposure for heterocyclic amines. Our findings of margin of exposure calculation were relatable to the prevalence and exposure to polycyclic aromatic hydrocar-

bons (PAH). Rozentale *et al.* [46] studied high-temperature produced carcinogenic polyaromatic compounds in foods and reported a respective margin of exposure for PAH4 and Benzo[a]pyrene (BaP) as 8.486 and 11.602, respectively, which suggests that middle-aged (39–50 y.o.) consumers may be at high risk. Duedahl-Olesen *et al.* [47] studied the daily consumption of extensively contaminated barbeque and home-cooked meat in Denmark and reported it to pose a health risk when the margin of exposure values exceeded 7.080 and 8.450, respectively. Sahin *et al.* [28], however, reported safe margin of exposure intervals for PAH4 in fish/shellfish (485.437), meat (25.634), and smoked food products (265.957).

CONCLUSION

Variable contents of heterocyclic amines in different meats can be attributed to varying cooking methods, surface heat transfer, cooking temperature, and cooking time. The current study considerably signifies high heterocyclic amine levels in barbecued or charcoal-grilled beef, mutton, and chicken as 574.09 ± 1.52, 342.41 ± 3.69, and 2705.99 ± 6.12 ng/g, respectively. High contents of PhIP (2-amino-1-methyl-6-phenylimidazo[4,5-b] pyridine) were quantified in beef, mutton, and chicken samples as 525.75 ± 0.54, 212.10 ± 1.35, and 1987.18 ± 5.73 ng/g, respectively. The estimated daily dietary consumption of heterocyclic amines, however, was 0.69, 0.05, and 0.144 ng per 1 kg of body weight in chicken, mutton, and beef, respectively. The margin of exposure values calculated for chicken, mutton, and beef proved to be 13.250, 102.060, and 3.784, respectively. The margin of exposure value for mutton and chicken exceeded the critical limit of 10.000 published by the European Food Safety Authority and hence remained within the reliable range. However, the margin of exposure for beef appeared to be far below the critical allowance limit and range, which is a matter of great health risk concern. High heterocyclic amine levels were found in barbecued and/or charcoal-grilled chicken, which necessitates immediate action by public health authorities. The food science needs to explore a variety of strategies to mitigate, reduce, prevent, and/or inhibit the heterocyclic amine formation at high temperatures in meats. The high consumption of proteinaceous foods containing such carcinogenic compounds has, thus, further quadrupled the health risk concerns for human beings.

High-temperature cooked meats and poultry produce harmful heterocyclic aromatic amines (HAA), which can induce cell mutations and contribute to cancers, especially in regions with high meat consumption. These mutational patterns resemble those found in human tumors, highlighting the need for specific biomarkers to understand their role in meat-related cancers. PhIP, a prominent heterocyclic aromatic amine in well-done meats, was identified as a carcinogen. However, biomarkers indicating the biologically effective dose, such as HAA-DNA and HAA-protein adducts, should be incorporated into epidemiological studies for a compre-

hensive assessment of health risks. Future studies integrating specific heterocyclic aromatic amine biomarkers and genetic factors could advance our understanding of the health risks associated with dietary heterocyclic aromatic amines in human cancer.

CONTRIBUTION

Conceptualization, Ishrat Perveen, and Yasar Saleem; methodology, Ishrat Perveen and Yasar Saleem; software, Shaista Nawaz, and Nida Saleem; validation, Yasar Saleem, and Ishrat Perveen; formal analysis, Ishrat Perveen, and Yasar Saleem; investigation, Ishrat Perveen, Tallat Anwar Faridi, and Rozina Shahadat Khan; resources, Maryam Maqsood; Ishrat Perveen; Rozina Shahadat Khan, data curation, Farhat Naseem Alvi, Syed Muhammad Aun, Maria Fareed Siddiqui, Hafiz Muhammad Abrar Awan and Ishrat Perveen; writing of original draft, Ishrat Perveen, Tallat Anwar Faridi,

Maryam Maqsood, and Rozina Shahadat Khan; editing, and review Quratulain Syed, Syed Hussain Imam Abidi supervision and project administration Yasar Saleem, and Ishrat Perveen. The authors have reviewed and approved of the final version of manuscript.

CONFLICT OF INTEREST

The authors declared no conflict of interest regarding the publication of this article.

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