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Corresponding author:
Iqbal Hussain, Department of Botany, Government College University, Faisalabad-38000, Pakistan.
E.: driqbal@gcuf.edu.pk, T.:+92-41-9201488, F.:+92-41-92006

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Original Research Article

MEASUREMENT OF CYP1A2 PHENOTYPE USING FEMALE VOLUNTEER PLASMA: A FOCUS ON CAFFEINE AND PARAXANTHINE AS A PROBE

Saima Rehman[1], Shagufta Kamal[1¥], Shumaila Kiran[1], Ismat Bibi[2], Iqbal Hussain[3¥*]

1. Department of Applied and Biochemistry, Government College University, Faisalabad-38000, Pakistan
2. Department of Chemistry, Islamia University, Bahawalpur 63100, Pakistan
3. Department of Botany, Government College University, Faisalabad-38000, Pakistan

Abstract

Caffeine neither causing didn't decrease the risk of cancer, yet it used just to note the activity of cytochrome P-4501A2 (CYP1A2) by converting into its metabolite i.e., paraxanthine. The purpose of the present study was to determine the caffeine and its metabolite phenotypes and their relation to cancer risk in healthy female volunteers of local population in Pakistan. The average value of metabolic ratio [(1,7-dimethylxanthine (17X) and Caffeine 1,3,7-trimethylxanthine (137X)] was found to be 1.182995 ± 0.21137. BMI (used to categorize into different groups, i.e., overweight, underweight etc.) of all volunteers were found to be 19.93Kg/m^2. Retention time was 15 and 37 min for 1,7-dimethylxanthine (17DMX) and 1,3,7 trimethylxanthine (137TMX), respectively. The linearity of calibration curve of 137TMX and 17DMX were covered 0–12 µg/mL (R^2 = 0.994). A significant positive correlation was observed between metabolic ratio and cancer risk factors. We could conclude that all the volunteers are fast metabolizers having a greater risk of cancerous diseases.

Key words*:* Cytochrome P-450, caffeine, metabolism, cancer, volunteers

Running Title: Measurement of CYP1A2 using plasma: a focus on caffeine and paraxanthine as a probe

Introduction

Caffeine (137TMX), one of the most commonly ingested compounds throughout the world, is an alkaloid unsurprisingly found in fruit of plants, leaves or seeds such as coffee (Coffea sp.), tea (*Camellia sinensis*), cocoa (*Theobroma cacao*), kola (*Cola acuminate*), Yerba mate (*Ilex paraguariensis*), and Guarana (*Paullinia cupana*)[1]. On the other hand, a variety of common beverages and certain medications, contain caffeine[2-3]. Caffeine acts as an inhibitor of phosphodiesterase, γ-aminobutyric acid (GABA) receptors and decreases anxiety and fatigue [4-5]. Caffeine elevates catecholamine levels, basal metabolic rate (BMR), vigilance, fortitude and neuromuscular coordination, improve mood, cognitive performance and also promotes lipolysis [6]. Complex metabolic pathway of caffeine involves the formation of three principal metabolite i.e theobromine (37DMX: 3,7-dimethylxanthine), theophylline (13DMX: 1,3-dimethylxanthine) and paraxanthine (17DMX) and mainly restricted to the liver which are primarily formed by CYP 1A2. About 80% of the metabolic pathway for caffeine comprises its conversion to 17DMX metabolite. Due to lipophilic properties of caffeine and low plasma protein binding, caffeine easily penetrates all biological membranes, including placental and blood-brain barrier [7] with a half-life of about 4 to 6 h and a total clearance of approximately 2 mL/min/kg[8]. Due to its safe, inexpensive, rapid, complete absorption and short half-life, caffeine is considered an ideal probe drug for estimation of *in vivo*

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Fig. 1. Structures of the isolated compounds

activity of CYP1A2[9]. Drug specific phenotyping of CYP1A2 alongside caffeine have been analyzed by many pharmaceuticals[10]. Efforts to associate the genotype with CYP1A2 metabolic phenotypes have been enduring. However, prediction of the metabolic phenotype based on genotype with CYP1A2 has been inveterate to be challenging and is an area of research that creates continued hullabaloo [11-12].

Phenotyping using plasma and saliva samples to measure the paraxanthine to caffeine (17DMX/137TMX) ratio correlates fit with many measures of CYP1A2 activity. Various urinary metabolic ratios for caffeine phenotyping have been proposed, but shortcomings have been demonstrated for all the proposed urinary metabolic ratios [13]. Nutrition is an ecological factor that greatly affects the activity of P4501A2. In addition to previously mentioned char-grilled meats, Crucifereae vegetables (including broccoli and cauliflower) also have an effect on the metabolic activity of CYP1A2[14]. Apiaceae vegetables (carrot, celery, fennel, parsley and parsnip) exhibited an inhibitory influence, whereas; Allium vegetables (garlic, onions, scallions, leeks, and chives) have also shown effects on CYP1A2 activity when compared with a control diet lacking vegetables [15]. Apart from these environmental factors, age and gender have also been affected the metabolic activity of CYP1A2 with children having higher activity than adults, and men have higher activity than women, although this latter observation is disputed[16].

The CYP Isozymes reduce the half life and duration of exposure to xenobiotics and prevent accumulation of the parent compounds. At the same time, excessive CYP activity without adequate concomitant conjugating activity may also be a risk factor for cancer[17]. Geonatics has a geographical influence on genetics and manifested by dissimilar biochemical, physiological and pharmacological parameters when local populations are compared with foreign counterparts[18]. The differences have been shown to affect the fate of drug, therapeutics standards arid dose regimens on the basis of indigenous investigations[19]. The current study focused on to investigate the activity of CYP1A2 by using the drug i-e Caffeine with the help of HPLC technique, determination of risk factors of cancer related to CYP 1A2 and comparison of the activity of CYP1A2 in the local population and their foreign counterparts.

MATERIALS AND METHODS

General Experimental Details

The study was designed to also elaborate the biotransformation of caffeine, which act as a probe drug to study the activity of CYP1A2 in eleven unrelated healthy female volunteers of age between 17-24 years. All the volunteers were advised to refrain from any form of caffeine intake, including tea, coffee and chocolate, etc. for at least 24 h prior to the study. After completing the washout period, volunteers received a single cup of coffee (Nescafe) which contains 400 mg of coffee. Six hours after caffeine intake, 5mL of venous blood sample was drawn into EDTA (ethylenediaminetetraacetic acid) containing centrifuge tube. Then blood sample was centrifuged at 3000 rpm for 10 min and plasma separated. The plasma was collected in glass tubes with the help of a dropper. Glass tubes containing plasma were stored at -20˚C until analyzed. Volunteer having any type of abnormalities in their history was not included in this study. The demographic data (Age, body weight, height, blood pressure, body temperature and BMI) of eleven healthy female volunteers are given in Table 1.

Chemicals

All the chemicals, i.e., paraxanthine (17DMX), (D5385, Sigma, USA), caffeine (137TMX) (Merck, Darmstadt, Germany) and acetic acid (Sigma Aldrich, Buchs, Switzerland) of analytical grade were used for sample preparation

Preparation of standard solutions

A stock solution of 10 mg of caffeine and paraxanthine in the eluent was used to prepare the standard solutions of caffeine and paraxanthine. All the standard solutions were prepared by diluting this stock solution.

Sample preparation

A simple extraction and quantification procedure were developed that used reverse phase chromatography (RP-HPLC) as described by[20]. The extraction procedure was as follows: the plasma proteins were precipitated by addition of 300µ Lacetonitrile. The mixture was placed in a vortex blender for 10 seconds, followed by centrifugation at 3500g for 10 minutes. The supernatant was evaporated to dryness by using nitrogen evaporation (speed vac plus, savant instruments, Inc, Holbrook, NY) and reconstituted in a 100 µL mobile phase before 20 µL was injected onto a HPLC column.

Table 1. Values of Age, Body weight, Height, Blood Pressure, Body Temperature and BMI of eleven healthy female volunteers

Volunteers ID	Age (year)	Body weight (kg)	Height (m)	Blood Pressure (mm of Hg)		Body Temp (°F)	BMI (kg/m^2)
				Systolic	Diastolic		
1	23	43	1.61	110	70	99	16.8
2	23	48	1.64	100	70	98.8	18.7
3	23	48.5	1.64	120	80	98.6	18.9
4	21	51	1.58	100	70	98.4	20.6
5	24	46.5	1.58	100	65	97.6	19.4
6	20	51	1.58	110	70	95	21.2
7	18	46	1.55	110	70	96	19.8
8	18	43	1.55	100	70	96.6	18.5
9	23	49	1.68	110	70	96.5	18
10	17	52	1.62	120	80	98.3	20.3
11	23	48	1.62	110	70	97.4	19.4
Mean	23.828	49.533	1.60	106.66	70.67	97.49	19.93
SD	± 2.73	± 4.2697	± 0.038	±7.2374	±4.169	± 1.0439	± 1.65
Minimum	17	43	1.55	100	65	94.6	16.8
Maximum	23	52	1.68	120	80	98.8	20.6

Temp: temperature; SD: standard deviation; BMI: body mass index

Analytical procedure

The method of analysis was performed using the following HPLC (Hitachi) system, flow control valve (FCV-10AL), Degasser unit (DGU-12A), System controller (SCL-10A), Spectral detector UV (L-2400) and pump (L-2130). The chromatographic data were collected by using CSW-32 software. Separation was achieved at ambient temperature with a Shim Pack CLC-ODS (150 *4.6 mm, 5 µm pore size) column and analytes were detected at 273 nm. Flow Rate was 1.0 mL/min and run time was 40 min.

Mobile phase

Mobile phase was prepared by using 15% methanol in 25 mmol/L sodium acetate buffer (pH: 4.0). The flow rate was 1.0 mL/min and detection wavelength was 273 nm. Mobile phase was filtered in a vacuum filtration assembly having a cellulose acetate filter, diameter was 47 mm, pore size 0.45 µm, then sonicated (Elma-Sonic E-60) for 15 to 20 min.

Quantification

Peak areas were compared for quantification of caffeine and paraxanthine using acetic acid as an internal standard

Concentration of Caffeine metabolites

Regression equation was used to determine concentration of caffeine metabolites,

X= (Y-b)/a, where X= drug concentration; Y=Peak area

RESULTS AND DISCUSSION

In the present study, paraxanthine was selected as a metabolite of caffeine and its values were used to calculate the activity of CYP 1A2 in female volunteers. Representative chromatogram of standard concentrations of 17X and caffeine is given in Fig.1-2. Figure 3 reveals that the level of 17X is high after 6 h of caffeine administration. Retention time for each analyte is given in Table 2. Representative chromatograms indicated that all the peaks 17X and 137X were not disturbed from each other and solvent peak indicating good performance of each standard determination in the same sample. The calibration curves of 137X and 17X were linear over the concentration range 0-12µg/mL and R_2 was 0.993 and 0.994 respectively, as shown in Fig. 4-5, which is very close to Fig. 1. The concentration of caffeine exhibited various ranges starting from 4.670 to 6.384 with mean 5.570639 ± 0.518101055507µg/mL. Concentration of paraxanthine in the serum of healthy female volunteers ranges from 3.117296 to 6.386429 with a mean of 4.8357 ± 1.055507µg/mL.

Table 2. Retention Time in minutes for each analyte

Analyte	Retention time (min)
17 DMX	15
Caffeine	37

17DMX: 1,7-dimethylxanthine

Table 3. Metabolic ratio for P-450 1A2 activity

Volunteers ID	17DMX	137TMX	17DMX Quantity (µg/mL)	137TMX Quantity (µg/mL)	MR
1	25.12	27.22	5.92	nd	nd
2	24.27	45.99	5.70	6.38	0.89
3	20.19	20.19	4.67	3.11	1.49
4	22.91	26.14	5.36	3.87	1.38
5	21.99	25.29	5.12	3.76	1.36
6	26.93	31.36	6.38	4.53	1.40
7	23.92	41.93	5.61	5.87	0.96
8	25.20	38.19	5.95	5.39	1.10
9	26.13	41.93	6.18	5.87	1.05
10	22.23	34.14	5.18	4.88	1.06
11	22.12	32.36	5.16	4.66	1.107
Average	23.72	33.15	5.57	4.83	1.18
± S.D	2.03	8.144	0.518	1.055	0.21

nd: not detected; SD : standard deviation; 137TMX: 1,3,7 trimethylxanthine; 17DMX: 1,7-dimethylxanthine; MR (metabolic ratio) = paraxanthine/Caffeine

Fig. 1. Representative chromatogram for the standard value of 17X (paraxanthine)

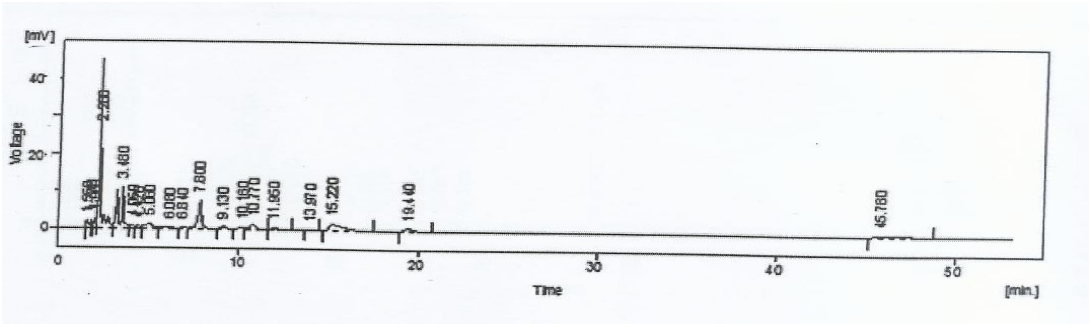


Fig. 2. Representative chromatogram for the standard value of 137 X (Caffeine)

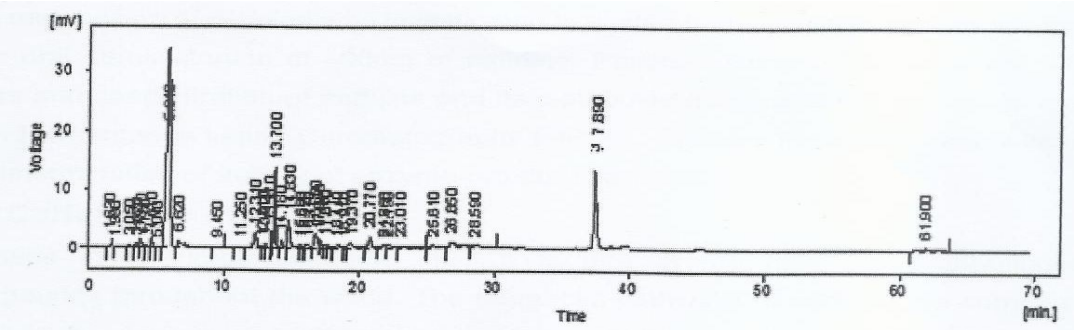


Fig. 3. Representative chromatogram after six hour of caffeine administration

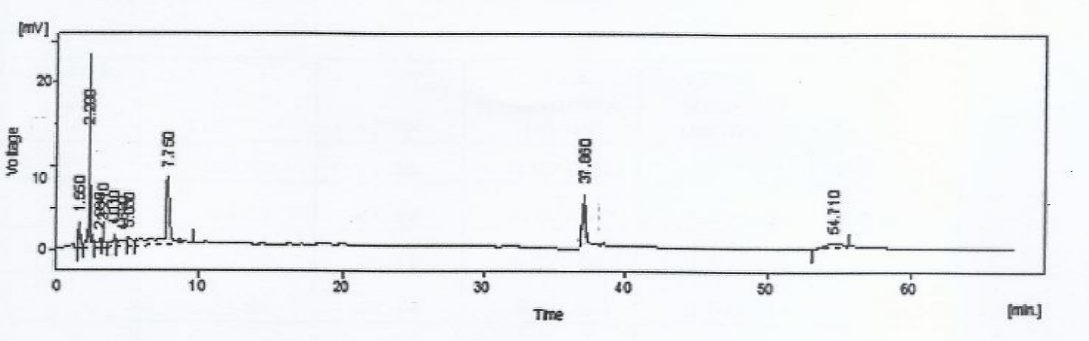
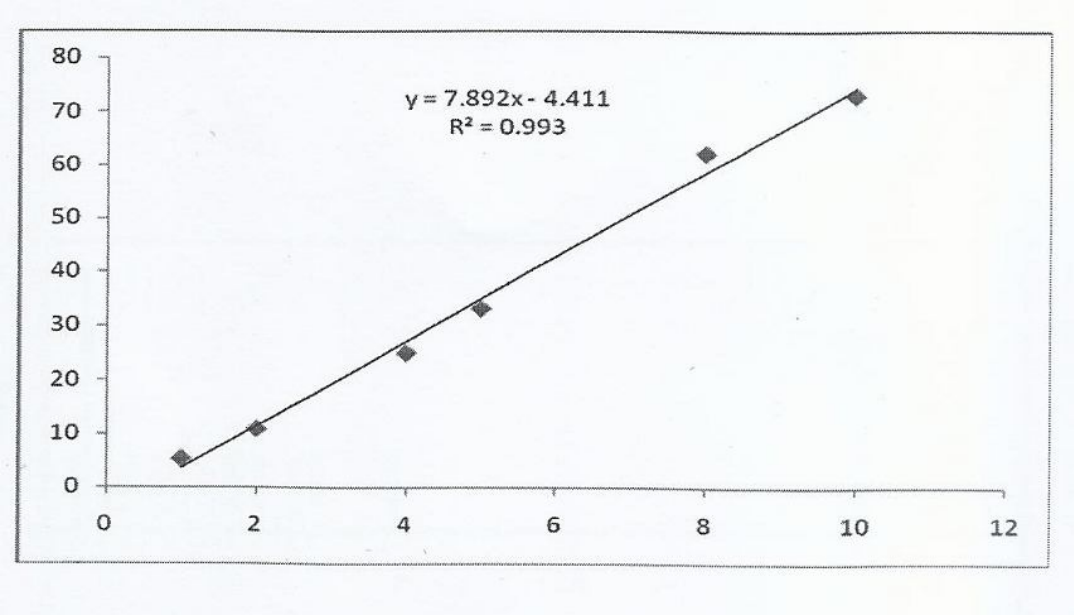


Fig. 4. Calibration curve for caffeine in female volunteer serum



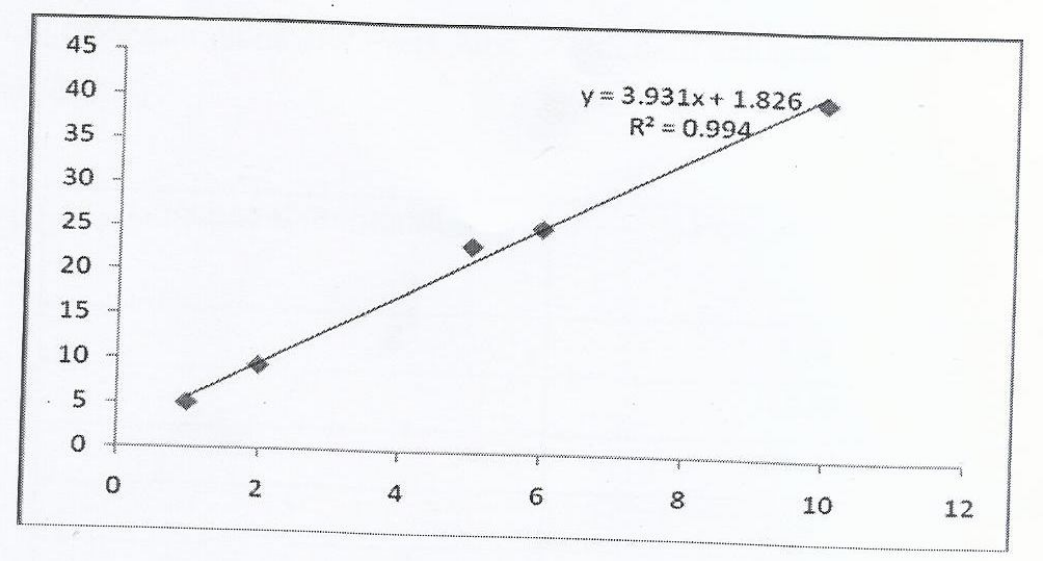


Fig. 5. Calibration curve for dimethylxanthine in female volunteer serum

Results depicted in Table 3 to show the different values of caffeine and 17DMX. These values could be compared with the values reported earlier. The metabolic ratio >0.5 was considered as fast metabolizer and metabolic ratio <0.5 was considered as slow metabolizers [21]. As all the values depicted in table 3 is >0.5 since they all were referred as fast metabolizers. Our results are in consonance with the findings of [22]. These scientists reported that caffeine consumers showed a high metabolic ratio because caffeine has positive relationships with ALT (alanine transaminase) and AST (aspartate transaminase). Several methods of determining an individual's CYP1A2 activity by using caffeine as a probe drug has been developed with various metabolic ratios such as 17DMX×17DMU/137TMX; (17DMU: 1,7-dimethyluric acid)[23].We selected paraxanthine/caffeine (17DMX/137TMX) as a metabolic ratio due to largest amounts (84%) of paraxanthine produced as a result of caffeine biotransformation. There are different risk factors of cancer; one of these is Isozyme of CYP1A2. The MR (metabolic rate) and CYP1A2 activity are directly proportional to each other. The high value of CYP leads to higher chances of cancer to occur.

For sample with paraxanthine concentrations below detection level, no MR value was calculated. Volunteer 1 with lower BMI does not show any metabolic ratio, thus cytochrome P-450 1A2 is lowest and chances of cancer will be less as compared to all other volunteers. Volunteer 3 with BMI values 18.9 show highest metabolic ratio 1.498 thus the activity of CYP 1A2 is high and this Isozyme serve as a risk factor of cancer. The average value of metabolic ratio with ± S.D. 1.182995±0.21137 which is high as compared to the values reported earlier (MR= 0.5). It is reported that Chinese women showed the highest values of CYP1A2 when determined by the caffeine metabolic ratio indicating a higher risk of lung cancer [24]. The rapid cytochrome CYP 1A2 phenotype increases the risk of developing colorectal cancer in humans [25-26].

CONCLUSION

In conclusion, this simplified method can be used to determine CYP1A2 activity and cancer risk factors in the population exposed the CYP1A2 interference agents.

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