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Genetic Profile Evaluation of Human Cell Lines Treated with Anastatica hierochuntica Using Forensic DNA Fingerprinting Markers

تقييم السمات الوراثية لخطوط الخلايا البشرية المعالجة بالأدوية باستخدام معلّمات سمات الحمض النووي الجنائي

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Abstract

Cell line authentication using Short Tandem Repeats (STRs) is necessary to ensure the integrity of the cell for its continuous culture and to identify misidentification and cross-contamination issues. This study investigates the changes in the genetic profile of MCF-7 and HepG2 cell lines caused by the methanolic leaf extract of Anastatica hierochuntica (AH) using human identification based STR markers. MCF-7 and HepG2 cell lines were treated with various concentrations of AH extracts for three different periods. The treated and control cells' DNA was extracted using a QIAamp® DNA Micro Kit, quantified using a Quantifiler Duo DNA Quantification Kit, and amplified using an AmpFlSTR Identifiler plus PCR Amplification Kit. The concentrations of the DNA extracted from control and MCF-7 and HepG2 cell lines treated with AH extract at 300 to 2400 µg/ml for 24hr and 150 to 2400 µg/ml

Keywords: Forensic Science, Cell Line Authentication, STRs, MSI, LOH, Insertion, Anastatica Hierochuntic.

المستخلص

يعد توثيق الخط الخلوي باستخدام التكرارات المترادفة القصيرة (STRs) أداة ضرورية لضمان سلامة الخلية خلال عمليات الزراعة المستمرة، بالإضافة للتحقق من عدم وجود خطأ التعرف عليها وحدوث التلوث التداخلي. وتهدف هذه الدراسة إلى التحقق من وجود تغييرات على مستوى النمط الجيني لخلايا MCF-7 وHepG2 التي قد تنتج عن مستخلص الميثانول من أوراق نبتة كف عائشة Anastatica hierochuntica (AH) باستخدام مواقع الـ STR التي تستخدم عادة في الاستعراف. وعولجت خلايا MCF-7 وHepG2 خلال ثلاث فترات زمنية مختلفة بتراكيز متباينة من مستخلص الـAH. وتم استخلاص الحمض النووي من الخلايا الضابطة التي تم معالجتها بمستخلص الميثانول باستخدام أطقم QIAamp® DNA Micro وتقديره الكمي باستخدام أطقم Quantifiler® Duo DNA Quantification ثم تكثيره باعتماد أطقم AmpFlSTR® Identifiler plus PCR Amplification. ولوحظ تباين في تراكيز الحمض النووي لخلايا MCF-7 وHepG2 بين المجموعة الضابطة والمعالجة بمستخلص الـAH إذ تراوحت بين 300 إلى 2400 µg/ml و150 إلى 2400 µg/ml خلال فترات المعالجة (24،

الكلمات المفتاحية: علوم الأدلة الجنائية، مصادقة الخلايا المستزرعة، التكرارات المترادفة القصيرة (STRs)، عدم استقرار المقاطع الوراثية (MSI)، فقدان الزيجوت غير المتماثل (LOH)، إضافة قاعدة نيتروجينية، نبات كف عائشة.

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for 48 and 72hrs were statistically significant ($p<0.05$). Microsatellite instability (MSI), loss of heterozygosity (LOH), insertion/deletions changes in the STRs profile were observed in treated cell lines at 1200 and 2400 μg/ml in MCF-7 cells for 48 and 72hrs and HepG2 cells for 24, 48, and 72hrs. We conclude that the highest concentration of AH extracts affected the genotype of the cell lines leading to misidentification. Therefore, cell line authentication by forensic DNA analysis techniques plays a decisive role for cells tested with a high concentration of chemical compounds and gives the forensic investigator an insight into these changes in the STR genotype of a victim/suspect who has been been under long term chemotherapeutic treatment.

48 و72 ساعة) مع فروقات ذات دلالة إحصائية ($p<0.05$). ولوحظ وجود تغييرات من نوع عدم الاستقرار لـ (MS)، وفقدان الزيجوت غير المتماثل (LOH) بالإضافة إلى عملية الإدخال والحذف في نتائج الـ STR لخلايا MCF-7 وHepG2 المعالجة بتراكيز 1200 و2400 µg/ml خلال الفترات الزمنية 48 و72 ساعة وخلايا HepG2 خلال 24 و48 و72 ساعة من المعالجة. وبالتالي نستنتج أن التراكيز العالية من مستخلصات الـ AH أثرت على الأنماط الوراثية للخطوط الخلوية، ما أدى إلى خطأ بالاستعراف. ولذلك فإن عملية مصادقة الخط الخلوي بواسطة تقنيات تحليل الحمض النووي الجنائي تؤدي دورًا حاسمًا للخلايا التي تم معالجتها بتراكيز عالية من المركبات الكيميائية. كما أنها تساعد المحقق الجنائي في التدقيق والتأكد من التغييرات المحتملة في الأنماط الوراثية للـ STR للضحية أو المشتبه به الخاضع للعلاج الكيماوي لفترات طويلة.

1. Introduction

In-vitro cultured human cell lines are essential and irreplaceable tools in basic and scientific medical research, as they provide models of human disease and offer a greater understanding of developmental biology and genetic evolution [1]. Recent studies and research on human cell lines have been intensified, due to the fast scalability and low production cost [2]. However, this has also exaggerated the chance of cross-contamination and misidentification of cell lines and creates erroneous, misleading, and false-positive data leading to unreliable research discoveries, which wastes time, money, and resources [3].

Numerous studies reported 16 to 35% of misidentification and cross-contamination by the old and well-established cell line [4-8]. For instance, HeLa, the first cervical tumor human cell line established in 1952, was found to contaminate more than 90 cell lines [9, 10]. Similarly, the Deutsche Sammlung von Mikroorganismen und Zelikurturen (DSMZ) German Collection of Microorganisms and Cell Cultures reported the misidentification of ECV304 to be a spontaneously transformed human normal endothelial cell line but was later identified to be T24 bladder cancer cells [11-13]. These disputes greatly jeopardized the quality of journals, and the legitimacy of research outcomes became questionable [14]. This, in turn, forced many journals and granting agencies to mandate the requirement of cell line authentication before manuscript submission [5,6].

Earlier, human cell authentication by isoenzyme and karyotyping was in practice, followed by HLA typing and chromosomal banding. Yet authentication becomes cumbersome in case of similar morphologies or phenotypes [15]. Recently, advances in molecular techniques have led to the discovery of single-nucleotide polymorphism (SNP) typing and forensic STR profiling to identify cell lines to a unique individual-level [16].

STRs profiling is a sensitive technique and popularly used in forensics for human identification. It has been adapted by the Federal Bureau of Investigation Laboratory's Combined DNA Index System [4, 17]. Recently, STR has expanded its application and been employed in cell line authentication for their higher discriminatory power, rapid testing, cost-effectiveness, and the ability to detect human DNA mixtures in human cell lines derived from the individual tissue [18].

The American National Standards Institute (ANSI) and the American Type Culture Collection (ATCC) Standards Development Organization (SDO) assembled a working group and presented a standard ASN-0002 "Authentication of Human Cell Lines: Standardization of STR Profiling" [19]. The ANSI recommends using eight loci for human cells unique identification; CSF1PO, D5S818, D7S820, D13S317, D16S539, TH01, TPOX, and vWA [20]. Also, a slight amount of genetic drift is sometimes accepted for some cell lines. Furthermore, reproducibility of the STR data has supported the development of standard guidelines to be recognized by the ANSI.

Cell line authentication practices are improving and have been followed in many laboratories for cell-based assays studies [21, 22]. However, sometimes research on some chemical compounds, especially chemotherapeutic agents such as cytochalasin B [23], Bleomycin [24], and paclitaxel [25], alter the STR genotypes of the cell line during the experimentation and possibly lead to misidentification of the original cell line. To the best of our knowledge, authentication of chemical compound experimented cell lines has not been conducted elsewhere using DNA-based human identification STR markers.

Anastatica hierochutica (L.) (AH), commonly known as Kaff Maryam, True Rose of Jericho, or Genggam Fatimah, is a monotypic species of the Brassicaceae family and a tumbleweed with resurrection nature [26]. The ethnopharmacological properties of AH were used to ease childbirth during pregnancy and reduce uterine hemorrhage [27] and widely used to treat asthma, dysentery, flu, fevers, headaches, and sterility [28]. The presence of novel bioactive compounds such as Anastatin A and B [29] and hierochins A, B and C [30] has led to the scientific exploration of pharmacologically essential activities, including antimicrobial [31], anti-inflammatory [32], antioxidant [28], hepatoprotective [29], gastroprotective [33] and anticancer [34] properties of AH. In vivo mammalian erythrocyte micronucleus testing of AH demonstrated no significant induction of mutagenicity in rats. Nevertheless, in vitro bacterial reverse mutation assay reported AH to be mutagenic, either through base-pair substitution or frameshift mutation [35].

For that reason, AH has been selected in this study to investigate the potential changes exerted by various concentrations of AH extract in the STR genotype of MCF-7 and HepG2 cell lines in three different time periods (24h, 48h, and 72 h) using a Forensic DNA Amplification kit (AmpFℓSTR® Identifiler® Plus kit).

2. Materials and Methods

2.1. Plant collection and extraction

Anastatica hierochuntica (L.) was collected from the central region of Saudi Arabia from February to April 2021 in a dried condition. Dr. Jacob Thomas authenticated the plant, and voucher specimens were preserved in the Herbarium of the Department of Botany and Microbiology at the College of Science, King Saud University, Riyadh, Saudi Arabia #24083. The leaves were separated from the plant, and about 100 g of leaves was extracted with 300 ml of methanol by percolation at room temperature under continuous shaking for three days [36]. The filtrate was concentrated using a rotary evaporator under reduced pressure and low temperature. The yield of leaf extract was weighed and stored at 4°C until used.

2.2. Cell culture and treatment

MCF-7 and HepG2 cell lines were obtained from ATCC (Manassas, VA, USA) and grown in complete Dulbecco's Modified Eagles Medium (DMEM) with 10% fetal bovine serum (FBS), 2mM of gluta-

mine, and 1% of penicillin (100U) and streptomycin (100μg/ml). Cell culture reagents were procured from Gibco, USA. The cells were seeded at a density of half a million cells/mL in CORING® 6-well plate in triplicates and treated for 24, 48, and 72 hrs with varying concentrations (0, 10, 25, 75, 150, 300, 600, 1200, 2400 μg/ml) of AH. 10 μM Staurosporine (Sigma Aldrich, USA) was used as a positive control along with untreated cells as a negative control. The cells were maintained at 37°C in humidified 5% CO2 incubator.

2.3. DNA extraction and quantification

DNA was extracted from the treated and control MCF-7 and HepG2 cells using a QIAamp® DNA Micro Kit (Qiagen Inc., USA) by following the user-developed purification of genomic DNA from cultured cells protocol. Finally, 50 μL of DNA was eluted in elution buffer and stored at -20°C. Extracted DNA from the treated, control MCF-7 and HepG2 cells was quantified with a Quantifiler Duo DNA Quantification Kit (Thermo Fischer Scientifics, USA) in a 7500 RT-qPCR real-time PCR following the manufacturer protocols.

2.4. Multiplex PCR amplification and fragment analysis

Extracted DNA of MCF-7 and HepG2 cells was amplified for 15 STR loci (D8S1179, D21S11, D7S820, CSF1PO, D3S1358, THO1, D13S317, D16S539, D2S1338, vWA, TPOX, D18S51, D5S818, FGA), and a sex-determining marker, Amelogenin, using the AmpFlSTR Identifiler plus PCR Amplification Kit (Thermo Fischer Scientific, USA). According to the manufacturer's recommended protocol, PCR reactions were prepared and carried out with a Veriti™ 96-Well Thermal Cycler (Thermo Fischer Scientific, USA).

Amplified STR alleles were sized and separated by capillary according to the manufacturer's recommended protocol. GeneScan™ 500 LIZ™ was used as an internal lane size standard electrophoresis (CE) using a 3500 Genetic Analyzer® (Thermo Fischer Scientific, USA). The fragment size of the allele was analyzed using GeneMapper ID-x v.1.4 (Thermo Fischer Scientific, USA). Allele designation was based on comparison with the allelic ladder provided in the kit. Interpretation of MSI is established when one of the heterozygous allele's peak Relative Fluorescence Unit (RFU) is less than 50% of the other allele, for LOH loss of one allele in a heterozygous marker, and insertion/deletion was marked when new allele inserted in marker having a definite allele call and for deletion; deletion of the allele in the marker was reported based on the standard protocols of ANSI/ATCC ASN-0002-2011 [19].

2.5. Statistical analysis

Statistical analysis for Student t-test to compare the DNA concentration of MCF-7 and HepG2 cells treated with AH extracts and STS with their respective untreated control cells was performed using PASW software v 21 (SPSS Inc., Chicago, IL USA). p-value ≤ 0.05 was considered significant.

3. Results

3.1. DNA quantification of MCF-7 and HepG2 cells treated with AH extracts

In this study, the cycle threshold (Ct) of the internal positive control (IPC) for MCF-7 and HepG2 cells treated with AH extracts falls between 28 and 31. Table-1 summarizes the concentration of the DNA extracted from the control (untreated) and treated MCF-7 and HepG2 cells with AH extract and STS for 24, 48, and 72 hrs. We observed that the DNA concentration of MCF-7 cells ranged from 50.36 to 5.47 ng/μL, 44.77 to 1.54 ng/μL, and 36.84 to 0.36 ng/μL during 24, 48, and 72 hrs time periods,

Table 1- *Concentration of the DNA extracted from MCF-7 and HepG2 treated with different concentration of AH extracts for 24, 48 and 72 hrs.*

Cell line	Time (hrs)	Concentration of STS (1μM)	Untreated Control Cells	Concentration of the AH extracts (μg/mL)							
				10	25	75	150	300	600	1200	2400
MCF-7	24	1.89±1.54*	56.15±3.01	50.36± 4.06	42.98±3.54	38.74±2.57	30.25±1.68*	21.34±3.61*	15.02±2.75*	10.86±3.62*	5.47±1.98*
	48	0.17±0.85*	50.58±3.25	44.77±4.28	37.71±3.69	33.45±3.66	25.18±2.81*	16.57±2.15*	9.68±3.46*	5.24±3.20*	1.54±2.17*
	72	0.035±1.7*	48.65±3.96	36.84±3.62	30.96±4.23	26.98±3.21	19.66±4.77*	10.31±3.72*	4.24±1.49*	1.78±2.56*	0.36±1.55*
HepG2	24	0.589±1.87*	32.12±3.25	28.64±1.89	24.83±2.51	22.27±2.65	18.95±3.2*	14.56±2.03*	10.57±3.65*	8.526±1.89*	2.257±3.66*
	48	0.159±2.85*	28.37±2.01	25.48±1.57	20.56±3.29	18.59±3.36	15.48±2.59*	10.89±1.32*	8.01±2.36*	6.278±2.84*	1.648±2.41*
	72	0.015±1.76*	24.85±1.25	20.87±2.14	18.25±1.88	14.88±1.02	10.27±1.96*	8.94±2.88*	4.28±1.24*	2.78±1.06*	0.578±3.15*

Significance (p<0.05)* was calculated using Student t-test

Table 2- *STR genotype of MCF-7 and HepG2 cells treated with different concentration of AH extracts and STS (1μM) for 24 hrs.*

STR loci	MCF 7 treated with AH extracts (μg/mL)											HepG2 7 treated with AH extracts (μg/mL)								
	Con-trol	STS (1μM)	10	25	75	150	300	600	1200	2400	Control	STS (1μM)	10	25	75	150	300	600	1200	2400
D8S1179	10,14	10,14	10,14	10,14	10,14	10,14	10,14	10,14	10,14	10,14	15,16	15,16	15,16	15,16	15,16	15,16	15,16	15,16	15,16	15,16
D21S11	30,30	30,30	30,30	30,30	30,30	30,30	30,30	30,30	30,30	30,30	29,31	29,31	29,31	29,31	29,31	29,31	29,31	29,31	29,31	29,31
D7S820	8,9	8,9	8,9	8,9	8,9	8,9	8,9	8,9	8,9	8,9	10,10	10,10	10,10	10,10	10,10	10,10	10,10	10,10	10,10	10,10
CSF1PO	10,10	10,10	10,10	10,10	10,10	10,10	10,10	10,10	10,10	10,10	10,11	10,11	10,11	10,11	10,11	10,11	10,11	10,11	10,11	10,11*
D3S1358	16,16	16,16	16,16	16,16	16,16	16,16	16,16	16,16	16,16	16,16	15,16	15,16	15,16	15,16	15,16	15,16	15,16	15,16	15,16	15,16
TH01	6,6	6,6	6,6	6,6	6,6	6,6	6,6	6,6	6,6	6,6	9,9	9,9	9,9	9,9	9,9	9,9	9,9	9,9	9,9	9,9
D13S317	11,11	11,11	11,11	11,11	11,11	11,11	11,11	11,11	11,11	11,11	9,13	9,13	9,13	9,13	9,13	9,13	9,13	9,13	9,13	9,13*
D16S539	11,12	11,12	11,12	11,12	11,12	11,12	11,12	11,12	11,12	11,12	12,13	12,13	12,13	12,13	12,13	12,13	12,13	12,13	12,13	12,13
D2S1338	21,23	21,23	21,23	21,23	21,23	21,23	21,23	21,23	21,23	21,23	19,20	19,20	19,20	19,20	19,20	19,20	19,20	19,20	19,20	19,20
D19S433	13,14	13,14	13,14	13,14	13,14	13,14	13,14	13,14	13,14	13,14	15.2,15.2	15.2,15.2	15.2,15.2	15.2,15.2	15.2,15.2	15.2,15.2	15.2,15.2	15.2,15.2	15.2,15.2	15.2,15.2
vWA	14,15	14,15	14,15	14,15	14,15	14,15	14,15	14,15	14,15	14,15	17,17	17,17	17,17	17,17	17,17	17,17	17,17	17,17	17,17	17,17
TPOX	9,12	9,12	9,12	9,12	9,12	9,12	9,12	9,12	9,12	9,12	8,9	8,9	8,9	8,9	8,9	8,9	8,9	8,9	8,9	8,9
D18S51	14,14	14,14	14,14	14,14	14,14	14,14	14,14	14,14	14,14	14,14	13,14	13,14	13,14	13,14	13,14	13,14	13,14	13,14	13,14	13,14
Amel	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	X,Y	X,Y	X,Y	X,Y	X,Y	X,Y	X,Y	X,Y	X,Y	X,Y
D5S818	11,12	11,12	11,12	11,12	11,12	11,12	11,12	11,12	11,12	11,12	11,12	11,12	11,12	11,12	11,12	11,12	11,12	11,12	11,12	11,12*
FGA	23,25	23,25	23,25	23,25	23,25	23,25	23,25	23,25	23,25	23,25	22,25	22,25	22,25	22,25	22,25	22,25	22,25	22,25	22,25	22,25

*MSI,

respectively. Similarly, the DNA concentration of HepG2 cells ranged from 28.64 to 2.57 ng/μL, 25.48 to 1.64 ng/μL, and 20.87 to 0.578 ng/μL during the 24, 48, and 72 hrs time periods, respectively.

In addition, the DNA concentration of HepG2 and MCF-7 cells treated with the positive control (STS) ranged from 1.89 to 0.035 ng/μL and 0.589 to 0.015 ng/μL, respectively, in all the studied periods (Table-1). Statistically significant differences ($p \leq 0.05$) between DNA concentrations of MCF-7 and HepG2 cells treated with AH extracts and STS with their respective untreated control cells were observed for concentration ranging from 300 to 2400 μg/ml and 150 to 2400 μg/mL during 24, 48, and 72 hrs, respectively. Similarly, both MCF-7 and HepG2 cells treated with STS reported a significant difference ($p \leq 0.05$) in their DNA concentration in all the three studied periods.

3.2. STR profiling of MCF-7 and HepG2 cells treated with AH extracts

The genetic profile of MCF-7 and HepG2 cells treated with different concentrations of AH extracts and STS for 24, 48, and 72 hrs were evaluated using AmpFlSTR® Identifiler® Plus kit (Thermoscientific, USA) and the results were analyzed in 3500 genetic analyzers. There were no changes observed in the STR profile of MCF7 cells treated with AH extracts and STS during the 24 hrs (Table-2). However, deviations such as MSI in the peak height of the heterozygous alleles were detected at markers D19S433 and D5S818 in MCF-7 cells treated with AH at 2400 μg/mL concentration during the 48hr time period (Table-3). Similarly, MSI was also detected at D8S1179, D16S539 markers and deletion in the repeat unit of one base pair at D18S51 and D21S11 (Figure-1) in MCF7 cells treated with AH extracts at 1200 and 2400 μg/ml concentration,

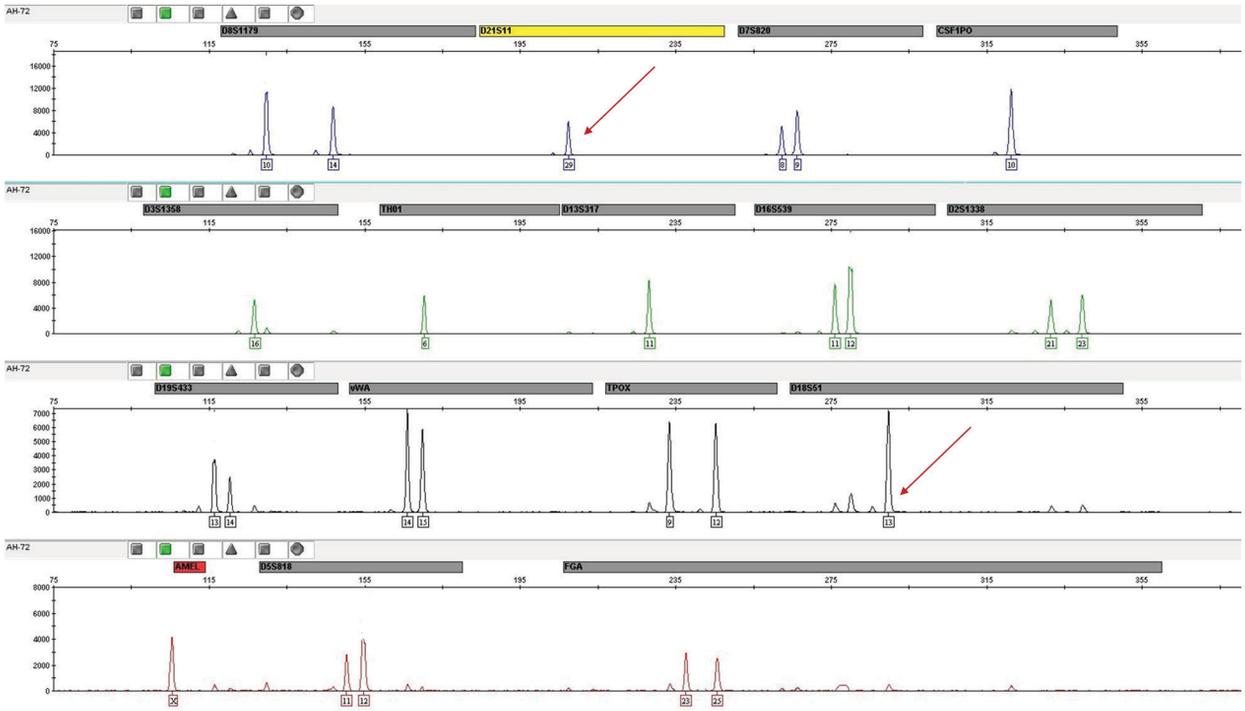

Figure 1- *AH extract treatment in MCF-7 cells during72 hr showed Deletion of repeat unit in D18S51 and D21S11 as indicated in arrow.*

Table 3- STR genotype of MCF-7 and HepG2 cells treated with different concentration of AH extracts and STS (1μM) for 48 hrs.

STR loci	MCF 7 treated with AH extracts (μg/mL)										HepG2 7 treated with AH extracts (μg/mL)									
	Con-trol	STS (1μM)	10	25	75	150	300	600	1200	2400	Control	STS (1μM)	10	25	75	150	300	600	1200	2400
D8S1179	10,14	10,14	10,14	10,14	10,14	10,14	10,14	10,14	10,14	10,14	15,16	15,16	15,16	15,16	15,16	15,16	15,16	15,16	15[§]	15,16
D21S11	30,30	30,30	30,30	30,30	30,30	30,30	30,30	30,30	30,30	30,30	29,31	29,31	29,31	29,31	29,31	29,31	29,31	29,31	29,31	29,31
D7S820	8,9	8,9	8,9	8,9	8,9	8,9	8,9	8,9	8,9	8,9	10,10	10,10	10,10	10,10	10,10	10,10	10,10	10,10	10,10	10,10
CSF1PO	10,10	10,10	10,10	10,10	10,10	10,10	10,10	10,10	10,10	10,10	10,11	10,11	10,11	10,11	10,11	10,11	10,11	10,11	10,11	10,11
D3S1358	16,16	16,16	16,16	16,16	16,16	16,16	16,16	16,16	16,16	16,16	15,16	15,16	15,16	15,16	15,16	15,16	15,16	15,16	15,16	15,16
TH01	6,6	6,6	6,6	6,6	6,6	6,6	6,6	6,6	6,6	6,6	9,9	9,9	9,9	9,9	9,9	9,9	9,9	9,9	9,9	9,9
D13S317	11,11	11,11	11,11	11,11	11,11	11,11	11,11	11,11	11,11	11,11	9,13	9,13	9,13	9,13	9,13	9,13	9,13	9,13	9,13	9,13
D16S539	11,12	11,12	11,12	11,12	11,12	11,12	11,12	11,12	11,12	11,12	12,13	12,13	12,13	12,13	12,13	12,13	12,13	12,13	12,13	12,13
D2S1338	21,23	21,23	21,23	21,23	21,23	21,23	21,23	21,23	21,23	21,23	19,20	19,20	19,20	19,20	19,20	19,20	19,20	19,20	19,20	19[§]
D19S433	13,14	13,14	13,14	13,14	13,14	13,14	13,14	13,14	13,14	13,14*	15.2,15.2	15.2,15.2	15.2,15.2	15.2,15.2	15.2,15.2	15.2,15.2	15.2,15.2	15.2,15.2	15.2,15.2	15.2,15.2
vWA	14,15	14,15	14,15	14,15	14,15	14,15	14,15	14,15	14,15	14,15	17,17	17,17	17,17	17,17	17,17	17,17	17,17	17,17	17,17	17,17
TPOX	9,12	9,12	9,12	9,12	9,12	9,12	9,12	9,12	9,12	9,12	8,9	8,9	8,9	8,9	8,9	8,9	8,9	8,9	8,9	8,9
D18S51	14,14	14,14	14,14	14,14	14,14	14,14	14,14	14,14	14,14	14,14	13,14	13,14	13,14	13,14	13,14	13,14	13,14	13,14	13,14	13[§]
Amel	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	X,Y	X,Y	X,Y	X,Y	X,Y	X,Y	X,Y	X,Y	X,Y	X,Y
D5S818	11,12	11,12	11,12	11,12	11,12	11,12	11,12	11,12	11,12	11,12*	11,12	11,12	11,12	11,12	11,12	11,12	11,12	11,12	11,12	11,12
FGA	23,25	23,25	23,25	23,25	23,25	23,25	23,25	23,25	23,25	23,25	22,25	22,25	22,25	22,25	22,25	22,25	22,25	22,25	22,25	22,25

*MSI , [§]LOH

Table 4- STR genotype of MCF-7 and HepG2 cells treated with different concentration of AH extracts and STS (1μM) for 72 hours.

STR loci	MCF 7 treated with AH extracts (μg/mL)										HepG2 7 treated with AH extracts (μg/mL)									
	Con-trol	STS (1μM)	10	25	75	150	300	600	1200	2400	Control	STS (1μM)	10	25	75	150	300	600	1200	2400
D8S1179	10,14	10,14	10,14	10,14	10,14	10,14	10,14	10,14	10,14*	10,14	15,16	15,16	15,16	15,16	15,16	15,16	15,16	15,16	15,16	15,16
D21S11	30,30	30,30	30,30	30,30	30,30	30,30	30,30	30,30	30,30	29,29+	29,31	29,31	29,31	29,31	29,31	29,31	29,31	29,31	29,31*	29,31
D7S820	8,9	7,8+‡	8,9	8,9	8,9	8,9	8,9	8,9	8,9	8,9	10,10	10,12‡	10,10	10,10	10,10	10,10	10,10	10,10	10,10	10,10
CSF1PO	10,10	10,10	10,10	10,10	10,10	10,10	10,10	10,10	10,10	10,10	10,11	10,11	10,11	10,11	10,11	10,11	10,11	10,11	10,11	10,11*
D3S1358	16,16	16,16	16,16	16,16	16,16	16,16	16,16	16,16	16,16	16,16	15,16	15,16	15,16	15,16	15,16	15,16	15,16	15,16	15,16	15,16
TH01	6,6	6,6	6,6	6,6	6,6	6,6	6,6	6,6	6,6	6,6	9,9	9,9	9,9	9,9	9,9	9,9	9,9	9,9	9,9	9,9
D13S317	11,11	11,11	11,11	11,11	11,11	11,11	11,11	11,11	11,11	11,11	9,13	9,13	9,13	9,13	9,13	9,13	9,13	9,13	9,13	9,13
D16S539	11,12	11,12	11,12	11,12	11,12	11,12	11,12	11,12	11,12*	11,12	12,13	12,13	12,13	12,13	12,13	12,13	12,13	12,13	12,13*	12,13
D2S1338	21,23	21,23	21,23	21,23	21,23	21,23	21,23	21,23	21,23	21,23	19,20	19,20	19,20	19,20	19,20	19,20	19,20	19,20	19,20	19,20
D19S433	13,14	13,14	13,14	13,14	13,14	13,14	13,14	13,14	13,14	13,14	15.2,15.2	15.2,15.2	15.2,15.2	15.2,15.2	15.2,15.2	15.2,15.2	15.2,15.2	15.2,15.2	15.2,15.2	15.2,15.2
vWA	14,15	14,15	14,15	14,15	14,15	14,15	14,15	14,15	14,15	14,15	17,17	17,17	17,17	17,17	17,17	17,17	17,17	17,17	17,18	16,18+‡
TPOX	9,12	8,11+‡	9,12	9,12	9,12	9,12	9,12	9,12	9,12	9,12	8,9	8,12+‡	8,9	8,9	8,9	8,9	8,9	8,9	8,9	8,11+‡
D18S51	14,14	14,14	14,14	14,14	14,14	14,14	14,14	14,14	14,14	13+	13,14	13,14	13,14	13,14	13,14	13,14	13,14	13,14	13,14	13,14
Amel	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	X,Y	X,Y	X,Y	X,Y	X,Y	X,Y	X,Y	X,Y	X,Y	X,Y
D5S818	11,12	11,12	11,12	11,12	11,12	11,12	11,12	11,12	11,12	11,12	11,12	11,12	11,12	11,12	11,12	11,12	11,12	11,12	11,12	11,12
FGA	23,25	23,25	23,25	23,25	23,25	23,25	23,25	23,25	23,25	23,25	22,25	22,25	22,25	22,25	22,25	22,25	22,25	22,25	22,25	22,25

MSI, $LOH, +Deletion, ‡Insertion*

respectively, during the 72 hr period. Insertion/deletions at TPOX and D7S820 were observed in MCF-7 treated STS-treated during the 72 hrs (Table 4).

In HepG2 cells treated with AH extracts, MSI was observed at CSF1PO, D5S818, and D13S317 at 2400 μg/ml for 24 hrs, as displayed in Table 2. LOH were detected at D8S1179, D18S51, and D2S1338 at an AH extract concentration of 1200 and 2400 μg/mL during 48 hrs, respectively (Table-3). MSI at D16S539, D21S11, CSF1PO, and insertion and deletion at TPOX, vWA were observed at 1200 and 2400 μg/ml of AH extracts during the 72 hrs. Insertion and deletion at D7S820 and TPOX were identified in HepG2 cells treated with STS during 72hrs (Table-4).

4. Discussion

In vitro studies using human cell lines are widely encouraged for preliminary screening and have been popularly practiced in biomedical research and technology. Therefore, proper cell line authentication is necessary for the research involving cell cultures to validate and acknowledge the scientific community's findings [14].

In the present study, the forensically used DNA fingerprinting STR markers of MCF-7 and HepG2 cell lines were evaluated for authenticity using the AmpFℓSTR® Identifiler® Plus PCR Amplification Kit with different concentrations of AH for 24, 48, and 72 hrs.

Ct value in RT-qPCR is a fractional cycle number at which the PCR reaction crosses the set threshold value to be detected [37]. Ct value is inversely proportional to the amount of initial template DNA and has been reported higher when it exceeds 32 cycles due to the extracted PCR inhibitors present in the sample [38]. In this study, the outcome of the Ct values between 28 and 31 shows that inhibition was not observed to the DNA of MCF-7 and HepG2 cells treated with AH extracts and STS during all three studied periods.

We observed that DNA concentration decreased for the MCF-7 and HepG2 cells after treatment with AH extracts and STS as the dose and time increased. A statistical significant difference ($p \leq 0.05$) was observed for the concentration of AH extract at 300 to 2400 μg/mL during 24 hrs and 150 to 2400 μg/ml during 48 and 72 hrs in both the cell lines, as shown in Table-1. Our previous study reported that these are the concentrations of AH that were cytotoxic to the MCF-7 cells [34], which is concordant with the present study's results.

STR profiling, a frequently used analysis in DNA-based forensic identification, was considered a gold standard technique for validating and identifying cross-contamination in cell culture compared to other authentication methods [39]. In the present study, both MCF-7 and HepG2 cells treated with AH extracts from 10 to 600 μg/mL showed no changes in the STR profile compared to the respective control cells for all the three studied periods.

Changes such as MSI, LOH, insertion, and deletion were observed in MCF-7 and HepG2 cells treated with AH at 1200 μg/ml during 48 and 72 hrs and 2400 μg/mL for 24, 48, and 72 hrs (Tables-2-4). MCF-7 and HepG2 cells treated with STS showed no changes in the profile during 24 and 48 hr time periods (Table-2,3); conversely, insertion/deletions were detected at TPOX and D7S820 for both the cell lines during the 72 hr treatment (Table-4).

Insertion and deletion could be explained by the fact that multinucleated cells have more than one copy of their chromosomes and are mainly associated with chromosomal instability, oncogenesis, and progression due to mutation or dysfunction of cell division genes [40]. During MSI, amplification of one allele over another occurs due to gene duplication, aneuploidy, or chimeric cell population [19].

LOH happens when the cell lines acquire ad-

ditional genetic changes while in culture or owing to the treatment with chemical compounds. Many studies have reported these variations in cancer cells treated with chemical compounds [1, 41-43]. The higher rate of variations such as LOH and insertion/deletions of repeat units in the STR profile of the MCF-7, and HepG2 cells treated with AH extracts were observed during the 72 hrs treatment. These changes may be attributed to the toxicity of these compounds exerted at these STR markers. In addition, studies have reported that a more extended period of cell growth results in altered DNA fingerprints [4,44].

5. Conclusion

In this research, the STR profile of MCF-7 and HepG2 cells treated with different concentrations of Anastatica hierochuntica leaf extracts for 24, 48, and 72 hrs were evaluated using Forensic DNA testing techniques. MSI was reported at 2400 μg/mL in both the cell lines during 24 and 48 hrs treatment, while LOH, insertion, and deletion were detected at 1200 and 2400 μg/mL concentrations. Therefore, the current study attempts to encourage the essential practice of authenticating the cell line tested with a chemical compound to evaluate and verify any misidentification and cross-contamination issues. The study's outcomes provide forensic investigators with an insight into the changes in STR genotype of a suspect/victim who has been under long-term chemotherapeutic treatment.

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Conflicts of interest

Authors declare no conflicts of interest

References

1. Bian X, Yang Z, Feng H, Sun H, Liu Y. A combination of species identification and STR profiling identifies cross-contaminated cells from 482 human tumor cell lines. Sci.Rep. 2017; 7(1):1-10. DOI :10.1038/s41598-017-09660-w.
2. American Cancer Society. Breast cancer facts & figures 2019–2020. Am. Cancer Soc. 2019; 17:1-44.
3. Hughes P, Marshall D, Reid Y, Parkes H, Gelber C. The costs of using unauthenticated, over-passaged cell lines: how much more data do we need?. Biotechniques. 2007; 43(5):575-86. DOI:10.2144/000112598
4. Qiu J. Scientific publishing: identity crisis. Nat. News. 2008; 451(7180):766-7. DOI: 10.1038/451766a
5. Katsnelson A. Biologists tackle cells' identity crisis. Nat. 2010; 465: 537 DOI: 10.1038/465537a
6. Dolgin E. Venerable brain-cancer cell line faces identity crisis. Nat. news. 2016; 537(7619):149. DOI: 10.1038/nature.2016.20515
7. Parson W, Kirchebner R, Mühlmann R, Renner K, Kofler A, Schmidt S, Kofler R. Cancer cell line identification by short tandem repeat profiling: power and limitations. FASEB. J 2005; 19(3):1-8. DOI: 10.1096/fj.04-3062fje
8. Drexler HG, Dirks WG, MacLeod RA. False human hematopoietic cell lines: cross-contaminations and misinterpretations. Leuk. 1999; 13(10):1601-7.DOI: 10.1038/sj.leu.240 151

9. Nelson-Rees WA, Flandermeyer RR. HeLa cultures defined. Sci. 1976; 191(4222):96-98. DOI: 10.1126/science.1246601
10. Nelson-Rees WA, Daniels DW, Flandermeyer RR. Cross-contamination of cells in culture. Science. 1981; 212(4493):446-52. DOI: 10.1126/science.6451928
11. Dirks WG, MacLeod RA, Drexler HG. ECV304 (endothelial) is really T24 (bladder carcinoma): cell line cross-contamination at source. In vitro Cell. Dev. Biol. Anim. 1999; 35(10):558-9. DOI: 10.1007/s11626-999-0091-8
12. Brown J, Reading SJ, Jones S, Fitchett CJ, Howl J, Martin A, Longland CL, Michelangeli F, Dubrova YE, Brown CA. Critical evaluation of ECV304 as a human endothelial cell model defined by genetic analysis and functional responses: a comparison with the human bladder cancer derived epithelial cell line T24/83. Lab. Invest. 2000; 80(1):37-45. DOI: 10.1038/labinvest.3780006.
13. Suda K, Rothen-Rutishauser B, Günthert M, Wunderli-Allenspach H. Phenotypic characterization of human umbilical vein endothelial (ECV304) and urinary carcinoma (T24) cells: endothelial versus epithelial features. In Vitro Cell. Dev. Biol. Anim. 2001; 37(8):505-514.DOI: 10.1290/1071-2690(2001)037<0505:PCOHUV>2.0.CO;2
14. Dunham JH, Guthmiller P. Doing good science: Authenticating cell line identity. Promega Notes. 2009; 101:15-8.
15. Ayyoob K, Masoud K, Vahideh K, Jahanbakhsh A. Authentication of newly established human esophageal squamous cell carcinoma cell line (YM-1) using short tandem repeat (STR) profiling method. Tumor Biol. 2016; 37(3):3197-204. DOI: 10.1007/s13277-015-4133-4
16. Reid Y, Storts D, Riss T, Minor L. Authentication of human cell lines by STR DNA profiling analysis. Assay Manual.2013. https://pubmed.ncbi.nlm.nih.gov/23805434. Accessed on 15 May 2021
17. Barallon R, Bauer SR, Butler J, Capes-Davis A, Dirks WG, Elmore E, Furtado M, Kline MC, Kohara A, Los GV, MacLeod RA. Recommendation of short tandem repeat profiling for authenticating human cell lines, stem cells, and tissues. In Vitro Cell. Develop. Biol. Anim. 2010; 46(9):727-32. DOI: 10.1007/s11626-010-9333-z
18. Butler JM. Fundamentals of forensic DNA typing. Academic press; 2009.
19. ATCC . Authentication of human cell lines: standardization of STR profiling. ATCC SDO Document ASN-. 2011. https://webstore.ansi.org/standards/atcc/ansiatccasn 00022011. Accessed on 15 May 2021.
20. Dirks PB. Brain tumor stem cells: the cancer stem cell hypothesis writ large. Mol. Oncol. 2010; 4(5):420-30. DOI: 10.1016/j.molonc.2010.08.001.
21. Masters JR. End the scandal of false cell lines. Nature. 2012; 492(7428):186. DOI: 10.1038/492186a
22. Nardone RM. Eradication of cross-contaminated cell lines: a call for action. Cell Biol. Toxicol. 2007; 23(6):367-72. DOI: 10.1007/s10565-007-9019-9.
23. Trendowski M, Wong V, Yu G, Fondy TP. Enlargement and multinucleation of U937 leukemia and MCF7 breast carcinoma cells by antineoplastic agents to enhance sensitivity to low frequency ultrasound and to DNA-directed anticancer agents. Anticancer Res. 2015; 35(1):65-76.
24. Li HR, Shagisultanova EI, Yamashita K, Piao Z, Perucho M, Malkhosyan SR. Hypersensitivity of tumor cell lines with microsatellite instability to DNA double strand break producing che-

motherapeutic agent bleomycin. Cancer Res. 2004; 64(14):4760-7. DOI: 10.1158/0008-5472. CAN-04-0975

25. Park JE, Woo SR, Kang CM, Juhn AH, Ju YJ, Shin HJ, Joo HY, Park ER, Park IC, Hong SH, Hwang SG. Paclitaxel stimulates chromosomal fusion and instability in cells with dysfunctional telomeres: implication in multinucleation and chemosensitization. Biochem.Biophy.Res. Commun.2011;404(2):615-21.DOI: 10.1016/j. bbrc.2010.12. 018.
26. Saleh J, Machado L. Rose of Jericho: a word of caution. Oman Med. J. 2012; 27(4): 338. DOI: 10.5001/omj.2012.86.
27. Mossa JS, Al-Yahya MA, Al-Meshal IA, Medicinal Plants of Saudi Arabia. 1987.
28. AlGamdi N, Mullen W, Crozier A. Tea prepared from Anastatica hirerochuntica seeds contains a diversity of antioxidant flavonoids, chlorogenic acids and phenolic compounds. Phytochem. 2011; 72(2-3): 248-254. DOI:10.1016/j.phytochem.2010.11.0 17.
29. Yoshikawa M, Xu F, Morikawa T, Ninomiya K, Matsuda H. Anastatins A and B, new skeletal flavonoids with hepatoprotective activities from the desert plant Anastatica hierochuntica. Bioorg. Med. Chem. Lett. 2003; 13(6):1045-1049. DOI: 10.1016/S0960-894X (03)00088-X.
30. Yoshikawa M, Morikawa T, Xu F. (7R, 8S) and (7S, 8R) 8-5'Linked Neolignans from Egyptian Herbal Medicine Anastatica hierochuntica and Inhibitory Activities of Lignans on Nitric Oxide Pruduction, Heterocycl. 2003; 60(8):1787-1792.
31. Mohamed AA, Khalil AA, El-Beltagi HE. Antioxidant and antimicrobial properties of kaff maryam (Anastatica hierochuntica) and doum palm (Hyphaene thebaica). Grasas Y Aceites 2010; 61(1): 67-75. DOI: 10.3989/gya.064509
32. Rizk AM, Williamson EM, Evans FJ. Constituents of Plants Growing in Qatar VII An examination of Certain Plants for Anti-Inflammatory Activity. Int J Crude Drug Res 1985; 23(1): 1-4. DOI: 10.3109/13880208509070677.
33. Shah AH, Bhandari MP, Al-Harbi NO, Al-Ashban RM. Kaff-E-Maryam (Anastatica hierochuntica L.): evaluation of gastro-protective activity and toxicity in different experimental models. Biol and Med 2014; 6(1): 1-10. DOI: 10.4172/0974-8369.1000 197.
34. Rameshbabu S, Messaoudi SA, Alehaideb ZI, Ali MS, Venktraman A, Alajmi H, Al-Eidi H, Matou-Nasri S. Anastatica hierochuntica (L.) methanolic and aqueous extracts exert antiproliferative effects through the induction of apoptosis in MCF-7 breast cancer cells. Saudi Pharma. J. 2020; 28(8):985-93.DOI: 10.1016/j. jsps.2020.06.020.
35. Md Zin SR, Mohamed Z, Alshawsh MA, Wong WF, Kassim NM. Mutagenicity evaluation of Anastatica hierochuntica L. aqueous extract in vitro and in vivo. Exp. Biol. Med. 2018; (4):375-85. DOI :10.1177/1535370217748 574
36. Buss AD, Butler MS, editors. Natural product chemistry for drug discovery. London: Royal Society of Chemistry; 2010; 245-247.
37. Kontanis EJ, Reed FA. Evaluation of real-time PCR amplification efficiencies to detect PCR inhibitors. J. Forensic Sci. 2006; 51(4):795-804. DOI: 10.1111/j.1556-4029.2006. 00182.x
38. Kainz P. The PCR plateau phase–towards an understanding of its limitations. Biochim. Biophys. Acta. 2000; 1494(1-2):23-7. DOI: 10.1016/s0167-4781(00)00200-1.
39. Masters JR, American Type Culture Collection Standards Development Organization Workgroup ASN-0002. Cell line misidentification: the beginning of the end. Nat. Rev.Cancer.

2010;10(6):2-6. DOI: 10.1038/nrc2852.

40. Alvarez JV, Pan TC, Ruth J, Feng Y, Zhou A, Pant D, Grimley JS, Wandless TJ, DeMichele A, Chodosh LA, I-SPY 1 TRIAL Investigators. Par-4 downregulation promotes breast cancer recurrence by preventing multinucleation following targeted therapy. Cancer cell. 2013; 24(1):30-44. DOI: 10.1016/j.ccr.2013.05.007
41. Kamat N, Khidhir MA, Hussain S, Alashari MM, Rannug U. Chemotherapy induced microsatellite instability and loss of heterozygosity in chromosomes 2, 5, 10, and 17 in solid tumor patients. Cancer cell Inter. 2014; 14(1):1-9. DOI: 10.1186/s12935-014-0118-4.
42. Pinto JL, Fonseca FL, Marsicano SR, Delgado PO, Sant'Anna AV, Coelho PG, Maeda P, Del Giglio A. Systemic chemotherapy-induced microsatellite instability in the mononuclear cell fraction of women with breast cancer can be reproduced in vitro and abrogated by amifostine. J. Pharm. Pharmacol. 2010; 62(7):931-4. DOI: 10.1211/jpp.62.07.0015.
43. Coelho PG, Marsicano SR, Delgado PO, Pinto JL, Sant'anna AV, Yabiko MY, Del Giglio A, Fonseca FL. Chemotherapy induces genomic instability in oral mucosal cells of women with breast cancer. J. Solid Tumors. 2012; 2(2):10. DOI: 10.5430/jst.v2n2p 10.
44. Masters JR, Thomson JA, Daly-Burns B, Reid YA, Dirks WG, Packer P, Toji LH, Ohno T, Tanabe H, Arlett CF, Kelland LR. Short tandem repeat profiling provides an international reference standard for human cell lines. Proc. Natl. Acad. Sci. USA. 2001; 98(14):8012-7 DOI: 10.1073/pnas.121616198.