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Molecular mechanism of apoptosis induction in Jurkat E6-1 cells by *Tribulus terrestris* alkaloids extract

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ABSTRACT

The present study demonstrates apoptosis-inducing potential and mechanism of action of *Tribulus terristris* alkaloid extract in Jurkat E6-1 cancer cell line. Liquid Chromatography-Mass Spectrometry and High Resolution-Mass Spectrometry analysis identified the presence of four *N*-feruloyltyramine derivatives, namely trans-*N*-feruloyl-3-hydroxytyramine (**1**), trans-*N*-coumaroyltyramine (**2**), trans-*N*-feruloyltyramine (**3**) and trans-*N*-feruloyl-3-ethoxytyramine (**4**) in the alkaloid extract. Compounds **2** and **3** have not been yet reported in the alkaloid extract of *T. terristris*. *In silico* analysis revealed therapeutic potential of *N*-feruloyltyramine derivatives and strong binding efficiency to both chains of Tumor Necrosis Factor Receptor 1. Treatment of alkaloids extract to Jurkat E6-1 clone induced dose-dependent cytotoxicity (LC_{50} 140.4 μg mL^{-1}). Jurkat cells treated with alkaloids extract at sub-lethal concentration showed DNA fragmentation, enhancement in caspase-3 activity and phosphatidylserine translocation (apoptosis indicator) compared to control cells. Gene expression analysis using Human Apoptosis RT^2 Profiler PCR Array analysis upon alkaloid treatment was found to significantly alter expression of critical genes such as *TNFR1, FADD, AIFM, CASP8, TP53, DFFA* and *NFKB1*. These genes are predicted to mediate apoptotic cell death via both intrinsic and extrinsic apoptosis pathway. In summary, we report the identification of new *N*-feruloyltyramine derivatives from alkaloid extract of *T. terristris* fruit with probable anti-leukemic and pharmacological potential.

1. Introduction

Leukemia has been reported as the prime cause of cancer deaths in United States in 2010 for men aged below 40 years and in women aged between 20 to 59 years.[1] Unlike solid tumours constraints to particular organs, leukemia cells are mobile through blood stream and can infiltrate in other tissues. Therefore, management of leukemia becomes complex. Leukemic cancer can be managed through the employment of stem cell transplantation, chemotherapy and drug therapy. However, these therapies are known to associated with side effects like low blood cell counts, infection, graft versus host diseases and formation of kidney stones. Long term effects include increased risk to heart muscle injury, infertility, thyroid dysfunction, chronic fatigue and secondary cancer.[2] Therefore, worldwide researchers are trying to develop effective anticancer drug analogues with minimal side effects.

Natural compounds are promising candidates for small molecule drugs due to their minimum side effects as compared to synthetic analogues. Natural compounds contributed 64% among all the approved drugs during 1981-2010.[3] The natural bioactive compounds include alkaloids, terpenoids, flavonoids, saponins, tannins, phenolics, etc. Alkaloids have comparatively higher bioactive potential due to their structural peculiarity.[4] Anticancer properties of alkaloids have been reported in various scientific literatures.[5] The alkaloids of *Camptotheca acuminata* alkaloids are marketed as irinotecan (CPT-11, Campto®) and Topotecan (TPT, Hycamtin®) as antitumor agents.[6] Isoquinoline alkaloids, like

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Abbreviations

AIFM	Apoptosis Inducing Factor, Mitochondria Associated
CIDEB	Cell Death-Inducing DFFA-Like Effector B
DFFA	DNA Fragmentation Factor Subunit Alpha
KFKB1	Nuclear Factor Kappa B Subunit 1
NOD1	Nucleotide Binding Oligomerization Domain Containing 1
TRAF2	TNF Receptor Associated Factor 2
TNFR1	Tumor Necrosis Factor Receptor 1
TP53	Tumor Protein 53
BAX	BCL2 Associated X
TP53BP	Tumor Protein P53 Binding Protein

Berberine, Jatrorrhizine and Sanguinarine interact with nucleic acid have importance in antineoplastic research.[7] In bladder cancer, for second line treatment a bifluorinated semisynthetic vinca alkaloid, Vinflunine was approved in September 2009.[8] The fruit extracts of *Tribulus terristris* L. (Sanskrit name: *Gokshura*) has been preliminary researched for aphrodisiac, spermatogenesis, antioxidant, antimicrobial, antidiabetic, anti-inflammatory, analgesic, antihypertensive, and for ameliorative properties.[9] However, the alkaloids of *T. terristris* fruits have not been investigated for apoptosis inducing properties against leukemic cell.

In the present study, we demonstrate isolation and characterization of alkaloids from *T. terristris*. We report trans-*N*-feruloyl-3-hydroxytyramine and trans-*N*-feruloyl-3-ethoxytyramine as newly identified derivatives of *N*-feruloyltyramine in *T. terristris* fruit alkaloids extract. Further we examine their apoptosis induction potential in leukemic cancer cell line. In addition, we catalogue the expression profile of genes associated with apoptosis. Molecular docking studies were performed to investigate interaction and binding efficiency of identified compound in alkaloid extract with critical regulators of apoptotic pathway. *N*-feruloyltyramine derivatives in *T. terristris* fruit alkaloids may presume to be potential anti-leukemic drug molecule. However the results need to be further investigated and validated in order to strengthen the present findings.

2. Material and methods

2.1. Plant sample

T. terristris L. (family Zygopyllaceae) plant fruits were purchased from local traditional medicinal shop in Nagpur, Maharashtra (India). The plant sample was authenticated by Department of Botany, University Campus, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur, Maharashtra. The plant sample was deposited in the Herbarium with voucher specimen no. 9978 dated on 25thMay 2016.

2.2. Preparation of T. terristris fruits alkaloids extract

The fruit alkaloids extract of *Tribulus terrestris* was prepared according to Maldoni[10] with slight modifications in solvent usage. Fruits were dried in an incubator below 50 °C, finely pulverized and defatted with n-hexane in Soxhlet apparatus to get rid of lipophilic contents. The defatted powder was again extracted in soxhlet using methanol until colourless extract was obtained. The extract was concentrated at 100 mbar pressure and 45 °C in a rotary evaporator. Presence of alkaloids was confirmed by Bouchardt, Dragendorff and Mayer's tests.[10] After confirmatory test, 0.5 N HCl (2:1 v/v) was added to the flask containing extract. The flask was kept in an ice bath with continuous stirring for 3 h and then kept overnight at 4 °C to settle down gummy material. Extract was filtered; the gummy material was washed with 0.5 N HCl and the washed solutions were added to the filtrate. The extract was further basified drop wise with 15% NaOH to pH 10 and the liberated alkaloids were extracted in chloroform by liquid-liquid extraction. Finally, chloroformic extracts were combined, concentrated and dried by vacuum and turbo evaporator, successively. Resulting dried extract represented tertiary alkaloids extract form the fruits of *T. terrestris*.

2.3. Ultra-performance liquid chromatography (UPLC)-Electrospray ionization Mass Spectrometry

Liquid Chromatography-Mass Spectrometry (LC-MS) and LC-tandem MS/MS analysis of alkaloids fraction from fruits of *T. terrestris* plant was performed using Waters TQD triple quadrupole mass spectrometer (USA) equipped with H-Class Acquity UPLC system and electrospray ionization (ESI) source. Thermo Betasil C-18 (50 × 2.1 mm, 3 μm) column was used to achieve separation of analytes. One microliter of sample was injected through auto sampler into UPLC. Acetonitrile (**A**) and 5 mM ammonium acetate in 95% water with 5% acetonitrile (**B**) were used as solvents of mobile phase. Elution was performed at the flow rate of 0.25 mL min^{-1} with linear gradients of 90–70% B in 0–3 min, 70-60% B in 3–6 min, 60% B in 6–8 min, 60-30% B in 8–10 min, 30–90% B in 10–13 min, and 90% B up to 16 min. Nitrogen was used as the nebulizing and drying gas at flow rates of 50 and 750 L h^{-1}, respectively. The ESI source parameters were capillary voltage of 3.5 kV and cone potential at 30 V. Source and desolvation temperatures were at 120 and 350 °C, respectively. The mass analyser was scanned between 150 to 1000 Th in 0.6 s. Tandem mass spectra of compounds were measured by precursor ion selection in MS_1 followed by Collision Induced Dissociation (CID) and analysis of the product ions by MS_2. Argon was used as the collision gas and collision energy was ramped between 25 to 15 eV to achieve significant fragmentation. Data acquisition and processing were carried out using MassLynx V4.1 SCN 714 software. The spectra were accumulated from the top of TIC (Total Ion Chromatogram) peak.

2.4. High Resolution Mass Spectrometry (HR-MS)

The accurate mass measurement was recorded on Thermo Scientific Orbitrap Velos Pro hybrid mass spectrometer equipped with Accela UPLC system and electrospray ionization source. The sample was injected into LC and chromatographic conditions were maintained same as above. The capillary voltage and temperature were set to 4 kV and 320 °C, respectively. Full scan mass spectra were recorded from m/z 150–1000 Th and processed with Xcalibur software.

2.5. Cell culture

Acute T cell leukemic (Jurkat E6-1, passage number 29) cell line was procured from National Centre of Cell Science (NCCS), Pune, India. Cells were cultured in Roswell Park Memorial Institute-1640 (RPMI) medium with 10% FBS supplemented with 100 U mL^{-1} penicillin and 50 μg mL^{-1} streptomycin. Cells were incubated at 37 °C in a humidified atmosphere of 5% CO_2.

2.6. Cytotoxicity assays

Cytotoxicity of alkaloids extract in Jurkat E6-1 cells was determined using 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium

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