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Selective induction of apoptosis by 7-methyljuglone, its derivatives and isolated compounds from *Foeniculum vulgare* Mill. on human cancer cells

by

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List of abbreviations

Acquired Immunodeficiency Syndrome	AIDS
Adenosine diphosphate	ADP
Adenosine triphosphate	ATP
Afrikaans	Afr.
Basal cell carcinoma	BCC
Base pairs	bp
Before Christ	B.C.
Benign prostatic hypertrophy	BPH
Calcium	Ca^{2+}
Carbon dioxide	CO_2
Chloroform	$CHCl_3$
Chronic myelogenous leukaemia	CML
Colon carcinoma	COLO-205
Dalton's lymphoma ascetic	DLA
Death effector domains	DEDS
Deoxyribonucleic acid	DNA
Dysplastic oral keratinocyte	DOK
Ehrlich ascites carcinoma	EAC
English	Eng.
Fas ligand	FasL
Fifty percent inhibitory concentration	IC_{50}
Gas chromatography	GC
Gas chromatography-mass spectrometry	GC-MS
Glutathione (oxidized from)	GSSG
Glutathione (reduced form)	GSH

Hematologic remission	CHR
High-performance liquid chromatography	HPLC
Homoharringtonine	HHT
Human colon carcinoma	Caco-2
Human gastric adenocarcinoma	MK-1
Human immunodeficiency virus	HIV
Human papillomavirus	HPV
Human prostate cancer	LNCaP
Human uterine carcinoma	HeLa
H.G.W.J. Schwelckerdt Herbarium	PRU
Interleukin converting enzyme	ICE
Intraperitoneal	i.p.
Kilo-base pair	kb
Kilograms	kg
Lymphocytic leukaemia	P-388
Mammary gland epithelial, pleural effusion adenocarcinoma	MCF-7
Methanol	MeOH
Murine melanoma	B16F10
Micro mol	µM
Multi Drug Resistance	MDR
Minimal inhibitory concentration	MIC
Murine lymphocytic leukaemia	P-388
Murine melanoma	B16F10
Naphthoquinones	NQ
National Cancer Institute	NCI
Metastases	M
Nicotinamide adenine dinucleotide phosphate	NADPH

Mitochondrial permeability transition pore	MPTP
Node involvement	N
Non-small cell lung cancer	NSCLC
Oesophagus carcinoma	SNO
Peripheral blood mononuclear cells	PBMCs
Prostate epithelial carcinoma	DU-145
Prostate Specific Antigen	PSA
Squamous cell carcinoma	SCC
Sodium 3'-[1-(phenyl amino-carbonyl)-3,4-tetrazolium]-bis-[4-methoxy-6-nitro) benzene sulfonic acid hydrate	XTT
Squamous oesophageal carci- noma	WHCO3
Surveillance Epidemiology and End Results	SEER
Tuberculosis	TB
Tumour	T
Tumour necrosis factor	TNF
Tumour node metastases	TNM
TUNEL	TdT-mediated dUTP nick end labeling
United States	US
United States of America	USA
United States Department of Agriculture	USDA
Vero cells	African green monkey kidney cells
World Health Organization	WHO
Zearalenone	ZEA

Abstract

A naphthoquinone, 7-methyljuglone and some of its 5-hydroxy, 5-acetoxy-, 5-alkoxy- and 1,2,4,5-tetra-*O*-acetate derivatives were tested for their activity in four human cancer cell lines: breast adenocarcinoma, cervical epithelial carcinoma, oesophageal carcinoma and prostate epithelial carcinoma. Compound 2,5-dihydroxy-7-methyl-1,4-naphthoquinone was found to be the most effective one (exhibited a fifty percent inhibitory concentration (IC_{50}) in the range of 5.3 to 14.7 µM), while the parent compound 7-methyljuglone was less active than several of these derivatives. The IC_{50} values of 5-hydroxy-6-methyl-1,4-naphthoquinone were found to be between 19.1 and 15.4 µM on the four cell lines. However this compound showed toxicity on peripheral blood mononuclear cells. Six derivatives were selected for mechanistic studies. Considering the findings from cell cycle analysis, caspase 3/7 activation and annexinV-FITC dual labelling, 5-hydroxy-6-methyl-1,4-naphthoquinone was found to have antitumour effect by inducing apoptosis. Two derivatives namely, '8-fluoro-5-hydroxy-7-methyl-1,4-naphthoquinone' and '2,5-dihydroxy-7-methyl-1,4-naphthoquinone' were found to be not toxic on peripheral blood mononuclear cells suggesting their action is specific for tumour cells. Compound 2,5-dihydroxy-7-methyl-1,4-naphthoquinone was found to induce apoptosis through caspase 3/7 activation. In view of the enhanced potencies associated with these derivatives, these analogues may hold considerable therapeutic potential for the treatment of leukaemia cancers.

The ethanol extracts of seven plant species (ethnobotanically selected) were also tested for their cytotoxicity, assayed by the XTT assay, against four human cancer cell lines at concentrations ranging from 0.78 to 100 µg/ml. Of all the ethanol extracts, *Foeniculum vulgare* was found to have the best activity on HeLa cells, which exhibited an IC_{50} value of 19.97$\pm$ 0.048 µg/ml. Therefore, it was selected for isolation of the bioactive principles. The extract of *Foeniculum vulgare* was fractionated using column chromatography with hexane and ethyl acetate at different ratios as eluent. Two known compounds, '4-methoxycinnamyl alcohol' and 'syringin' were isolated. The IC_{50} values of '4-methoxycinnamyl alcohol' and 'syringin' were found to be 7.82 $\pm$ 0.28 µg/ml and 10.26 $\pm$ 0.18 µg/ml respectively on HeLa cells. Both compounds were tested for their cytotoxicity against U937 cells and also on

peripheral blood mononuclear cells. At the concentrations of 10 and 100 µg/ml '4-methoxycinnamyl alcohol' showed similar cell proliferation as that of the positive control 'cisplatin'. 'Syringin' however, had much lower cytotoxicity on the U937 cells than '4-methoxycinnamyl alcohol'. IC_{50} was found to be 91.14 ± 0.63 µg/ml. Both 'syringin' and '4-methoxycinnamyl alcohol' were not cytotoxic at concentrations of 1 and 10 µg/ml on the PBMCs as compared to cisplatin. '4-Methoxycinnamyl alcohol' was selected based on its activity on the cancer cells, for further investigation with regard to its mechanism of action. On gel electrophoresis it did not show a typical ladder pattern, instead a characteristic smear resulted which indicated necrosis.

Two best derivatives of 7-methyljuglone ('8-fluoro-5-hydroxy-7-methyl-1,4-naphthoquinone' and '2,5-dihydroxy-7-methyl-1,4-naphthoquinone') and the ethanol extract of *F. vulgare* warrant further investigation to be considered for their potential as anticancer agents.