Supplementary

Table 1. Search results from full text studies of articles about opium alkaloids and other opioids

Noscapine

In vitro (mammalian cell assays) studies

	Author (Year)	Type of opium or extraction	Cell line type/ Animal Species	Dosage	Technique	Clinical Index	Result	Conclusion	Risk of Bias
1	Xu G. (2016)	Noscapine	Human hepato-cellular carcinoma HepG2, Huh7, L02: In vitro	1000 μM	Nuclear DNA staining TUNEL Western blotting	• Cell Proliferation • Apoptosis	• Noscapine showed notable inhibition on HCC tumor growth • Noscapine induced apoptosis in HCC cells • Noscapine induced antive-capsase-3, cleavage PARP, and decreased the ratio of Bcl-2/Bax	Protective	4
2	Quisbert-Valenzuela, E. O. (2016)	Noscapine	• A luminal-like adenocarcinoma triple positive cell line MCF-7 breast cancer triple-negative cell line MDA-MB-231	100 mM	MTT assay Western blotting PCR (DD-RT-PCR) DNA fragmentation	• Cell viability • Cell toxicity Apoptosis	• Noscapine effectively had a dose-dependent cytotoxic effect in MCF-10F, MCF-7 and MDA-MB-231 cell lines. • Noscapine significantly increase apoptosis in MCF-7, and MDA-MB-231. • Noscapine-treated MCF-10F cells significantly increased Bax, caspase-8 and IκBα • The IC50 demonstrated that noscapine had specific cytotoxic effect in MCF-7 and MDA-MB-231 breast cancer cell lines requiring higher doses in MCF-10F normal cell line.	Protective	4
3	Jiang, L. (2016)	Noscapine	Osteosarcoma cell lines MG63 and U2OS	10, 20, 30 μM	MTT assay Flow cytometry Reverse transcriptase and quantitative real time PCR Transfection of siRNA Western blotting	• Cell migration and invasion • Cell apoptosis • Kinase glu luminescent assay • Immunoprecipitation and Kinase glu luminescent assay	• Noscapine inhibits the growth and the invasion of MG63 and U2OS cells in dose dependent manner. • Noscapine arrest MG63 cells at the G1 phase of the cell cycle • Noscapine suppresses the kinase acivity of EGFR by inhibiting EGFRp-Tyr1068 in MG63 and U2OS cells • Noscapine inhibited EGFR activity with an IC50 value of 19.26 μ mol/ • Noscapine inhibits the growth of MG63 and U2OS by inhibiting EGFR/Akt/ CDKs and EGFR/Akt/ Bad pathway • Noscapine represses the migratory and invasive potential of MG63 by inhibiting EGFR/Akt/ MMP2 pathway	Protective	4
4	Shen W. (2015)	Noscapine	drug-resistant Ovarian Cancer Cell Line SKOV3/ DDP: In vitro	40 μM of Nos for 48 h	MTT assay Flow cytometry Immunohistochemistry PCR	• Cell Proliferation • Apoptosis	• Noscapine significantly inhibited proliferation • Noscapine in combination with Cisplatin inhibited proliferation and increased the proapoptotic effect • Noscapine combined with Cisplatin reduced both protein and mRNA expression of anti-apoptotic factors XIAP, surviving and NF-kB, and augmented protein and mRNA levels of pro-apoptotic caspase-3	Protective	4
5	Afzali M. (2015)	Noscapine	HT29, T47D and HT1080	4- 17- 75- 120 μM	MTT assay Comet assay	• Viability • Apoptosis • Genotoxicity	• Noscapine and papaverine had a dose-dependent cytotoxic effect on T47D, HT-29 and HT-1080 cell lines, with no cytotoxic effect on noncancerous NIH-3 T3 cells. • Noscapine and papaverine selectively enhanced DNA damage on cancerous cells when compared with noncancerous cells ($p < 0.001$) • Noscapine and papaverine have induced apoptosis on HT-29 and T47D without any significant effect on NIH-3 T3 cell lines • Noscapine and papaverine induced DNA fragmentation in smaller sizes which were distinguishable from negative control group • Noscapine, papaverine did not increase caspase activity	Protective	4
6	Sajadian, S. (2015)	Noscapine hydrochloride	human breast cancer MDA-MB-231 cell line	20 μM	Apoptosis evaluated by Annexin V FITC Apoptosis Assay kit magnet-activated cell sorting (MACS) Flow cytometry MTT assay	• Cell proliferation • Apoptosis	• Early apoptotic + late apoptotic cells were 37%	Protective	4
7	Qi, Q. (2013)	Noscapine	U87MG human glioblastoma cells	10 or 20 μM	Flow cytometry MTT assay	• levels of active caspase-3 and PARP cleavage and Ki67 staining • Cell viability	• Enhance the apoptosis-inducing effect of TMZ, BCNU, and CIS	Protective	4

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8	Yang ,Z. (2012)	Noscapine	HT-29, LoVo and SW480 colon cancer cell lines.	100 μM	Flow cytometry Western blotting Annexin V staining for apoptosis	• Cell proliferation assay	• Noscapine inhibits colon cancer cell proliferation • Noscapine induces G2/M arrest in LoVo cells • Noscapine induces apoptosis and chromatin condensation • Noscapine induces apoptosis through the activation of the mitochondrial pathway	Protective	4
9	Liu, M. (2011)	Noscapine	human gastric cancer cell lines (BGC823, SGC7901, MGC803, HGC27)	0, 50, 100, 150 μM	MTT assay Annexin V staining Flow cytometry Western blotting	• Cell proliferation assay • Detection of apoptotic morphological feature by Hoechst 33258	• noscapine significantly inhibited the proliferation of all cell lines • the number of BGC823 cells was greatly decreased by incubation 100 and 150 μM noscapine • noscapine has a potential not only to prevent gastric cancer cell growth, but also to reduce the number of gastric cancer cells. • noscapine caused cell apoptosis in a dose dependent manner • noscapine induces mitochondrial dysfunction in gastric cancer cells.	Protective	4
10	Sung, B. (2010)	Noscapine	leukemia cells (KBM-5, HL-60, Jurkat, HuT-78, U266, RPMI-	0,10,25 μM	Annexin V staining Flow cytometry Electrophoretic mobility shift assay IKK assay NF-κB–dependent reporter gene expression assay Luciferase assay. MTT assay Live/dead assay Terminal deoxynucleotidyl transferase–mediated dUTP nick end labeling assay	• Cell proliferation • Cell viability	• caspase activation • and PARP cleavage. • Noscapine potentiates apoptosis induced by TNF and chemotherapeutic agents • Noscapine suppresses cell proliferation and enhances apoptosis in cancer cells. • Noscapine represses inducible NF-κB–dependent cell • proliferation proteins.	Protective	4
11	Jackson, T. (2008)	Noscapine	human NSCLC cell lines H460	30, 40 μM	crystal violet assay	• Cell proliferation	• Nos inhibit proliferation of H460 cells in a dose-dependent manner	Protective	4
12	Newcomb, E. (2008)	Noscapine	Glioma cell lines (U87MG, U118MG, LN229, and T98G)	20–150 μmol/l	Flow cytometry Western blot analysis Immunofluorescence microscopy Interfering RNA transfection	• Cell proliferation • Apoptosis • Protein expression	• Noscapine inhibits cell proliferation • Noscapine induces M-phase arrest • Noscapine alters expression of apoptosis and cell cycle-related proteins • Noscapine induces apoptosis associated with release of mitochondrial proteins cytochrome c and apoptosis-inducing factor • Noscapine induces apoptosis-inducing factor-dependent cell death	Protective	4*
13	Aneja, R. (2007)	Noscapine	human colon cancer cell lines HCT116	25 μM	Immunofluorescence microscopy Flow cytometry Immunoblot analysis.	• Cell proliferation	• Sensitivity of human colon cancer cells to noscapine depends on the p53/p21 status. • Noscapine induces G2-M arrest and apoptosis in a p53/p21– dependent manner • Noscapine treatment alters levels of cell cycle and apoptosis • regulatory proteins	Protective	4
14	Heidari, N. (2007)	Noscapine	myelogenous leukemia HL60 cells and apoptosis-resistant myelogenous leukemia K562 cells,	20 μmol/l	MTT assay Assessment of nuclear apoptotic morphology Annexin V staining DNA fragmentation Caspase activity assay Western blotting	• Cell proliferation • Protein expression	• Apoptosis induced by noscapine in HL60 and K562 cells	Protective	4
15	Kirpnick, Z. (2005)	Noscapine	CHO-WBL cell	1000- 5000- 10000 μg/ml	The DEL assay The recombination assay The micronucleus assay	• Determination of the DEL recombination rates	• Noscapine did not lead to a significant induction of • DEL recombination	No affect	4

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	Author (Year)	Type of opium or extraction	Cell line type/ Animal Species	Dosage	Technique	Clinical Index	Result	Conclusion	Risk of Bias
16	Qi, Q. (2013)	Noscapine	Rat C6 glioma cell line	0, 50, 250, 500, or 1000 μM	Cell Density Assay Tubulin, DNA, and Bromodeoxyuridine Staining Flow cytometry In Vitro Bovine Brain Microvessel Endothelial Cell Assay high-performance liquid chromatography (HPLC) Hematoxylin and eosin stain	• Cell proliferation • Cell cycle status	• Noscapine Inhibits Rat C6 Glioma Cell Proliferation • Increased Mitotic Arrest • Noscapine Crosses the Blood-Brain Barrier	Protective	4
17	Zhou, LX. (2003)	Brominated noscapine	HeLa cells MCF-7, DU 145, and Caco-2 cells Ca Ski, 1A9, 1A9/ PTX10, 1A9/ PTX22, 1A9/ A8, and A2780/ AD10 cells SigC and T84 cells SK-OV-3 cells MDA-MB-231 cells	10 or 100 μM	Mass spectrometry Tubulin Binding Assay Tubulin Polymerization Assay Flow Cytometric Analysis Immunofluorescence Microscopy Measurement of Sister Kinetochore Distance In Vitro Cell Proliferation Assay	• Cell proliferation assay	• Mitosis arrest • Inhibit cell more than noscapine • proliferation more than noscapine	Protective	4
18	Zhou, LX. (2002)	Noscapine	The 1A9 cell line human ovarian carcinoma cell line	50 and 100 μM	n Vitro Cell Proliferation Assay Immunofluorescence Microscopy TUNEL Annexin V Staining Assay Immunoprecipitation JNK Activity Assay Western Blot Analysis Antisense Oligonucleotide Treatment Transient Transfections annexin V-FITC staining assay	• Cell proliferation sulforhodamine B assay	• Noscapine Inhibits the Proliferation of Both Paclitaxel-sensitive and Paclitaxel-resistant Human Ovarian Carcinoma Cells • Noscapine Induces Apoptosis in Human Ovarian Carcinoma • Cells • JNK Activation upon Noscapine Treatment	Protective	4
19	Landen, LX. (2002)	Noscapine	Murine B16LS9 melanoma cells	0, 0.1, 1, 2, 10, 50, 100, or 1000 μM for cell viability and 50, 250, 500, or 1000 μM for flow cytometry	Cell Viability Assay Analysis of Microtubule Dynamics Visualization of Microtubules and Chromosomes Flow Cytometric Analysis	• Dynamicity •Cell cycle status	• Noscapine significantly reduced microtubule dynamicity	Protective	4
20	Ke,,Y. (2000)	Noscapine	The T cell lymphoma cell line, EL4	10 μM	XTT assay	• Cell proliferation	• Inhibit cell proliferation • Noscapine inhibits growth of tumor cells in vitro • inhibit proliferation of nontransformed as well as transformed cells	Protective	4
21	Ye, K. (1998)	Noscapine	HeLa cells	20 μM for DNA fragmentation analysis	trypan blue exclusion analysis Immunofluorescence of Microtubules Flow Cytometric Analysis of Cell Cycle Status and Apoptosis DNA Fragmentation Cytochemical Staining of Apoptotic Cells Assays for Microtubule Assembly [3H]Colchicine Binding Assays	• cell viability • Cell cycle status • Dynamicity	• Discovery that Noscapine Arrests Cells at Mitosis • Induces Apoptosis in HeLa and Thymocyte Cells • Noscapine Binding Induces a Conformational Change in Tubulin and Alters Microtubule Assembly	Protective	4*
22	Ye, K. (1998)	Noscapine	*HeLa cells injected to Female C57BL/6 (H-2b) mice, 8 to 12 weeks of age	120 mg/kg	TUNEL xenograft mice	• Tumor size	• Inhibits Growth of Murine and Human Tumors Implanted in Mice by Inducing Apoptosis • Noscapine Shrinks Murine Thymoma Solid Tumor • Noscapine Eliminates Human Tumors	Protective	5

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Noscapine

In vivo studies

	Author (Year)	Type of opium or extraction	Cell line type/ Animal Species	Dosage	Technique	Clinical Index	Result	Conclusion	Risk of Bias
23	Xu G. (2016)	Noscapine	Female athymic BALB/c nude mice: In vivo	300 mg/kg	Nuclear DNA staining TUNEL Western blotting	• Cell Proliferation • Apoptosis	• Noscapine showed notable inhibition on HCC tumor growth • Noscapine induced apoptosis in HCC cells • Noscapine induced antive-capsase-3, cleavage PARP, and decreased the ratio of Bcl-2/Bax	Protective	5
24	Yang ,Z. (2012)	Noscapine	Six-week-old male Balb/c nude mice	1 × 107 MG63 cell were suspended in 200 µ l of PBS and subcutaneously injected into animal Noscapine 5mg/kg inject 16 ays	Nude mouse xenogfraft	• Body weight Mortality	• Noscapine inhibits the migratory and invasive of MG63 cells by inhibiting EGFR pathway.	Protective	4
25	Liu, M. (2011)	Noscapine	Drug-resistant Ovarian Cancer Cell Line SKOV3/DDP: In vivo • Female BALB/c nu/nu mice	40 mg/kg IP	MTT assay Flow cytometry Immunohistochemistry PCR	• Cell Proliferation • Apoptosis	• Noscapine combined with Cisplatin increased the apoptotic rate • Tumor volume decreased from 1.733 ± 0.155 g in mice treated with Noscapine in combination with Cisplatin alone to 1.191 ± 0.106 g in animals treated with Nos or DDP	Protective	5
26	Qi, Q. (2013)	Noscapine	Nude mice (nu/nu) (for tumor growth assay) and C57BL/6J mice (for toxicological revaluation) of 5–6 weeks	10 or 20 µM Intraperitoneal (IP) 3 weeks (3 time in a week)	Nude mouse xenogfraft Western blotting Immunohistochemical analysis	• Tumor volume • Body weight	• Decrease of tumor volume and weight	Protective	5
27	Yang ,Z. (2012)	Noscapine	Male BALB/c-nu/nu nude mice, 4– 6 weeks old	LoVo cells, suspended in 100 lL PBS Control, low-dose group (10 mg/kg), a mid-dose group, (20 mg/kg), and a high-dose group (40 mg/kg) inject Test in 100 mm3 tumor size Intratumoral injection 36 days every 3 days	TUNEL Hematoxylin and eosin stain nude mouse xenogfraft	• Tumor volume	• inhibited tumor growth	Protective	4
28	Liu, M. (2011)	Noscapine	Male BALB/c nude mice, 4–5 weeks of age	Control group, low-dose group (10 mg/kg), mid-dose group (20 mg/kg), and high-dose group (40 mg/kg) inject BGC823 cells, 5.0 9 106, suspended in 100-ll PBS Test in 100-150 mm3 tumor size intratumoral injection 33 days every 3 days	TUNEL Hematoxylin and eosin stain nude mouse xenogfraft	• Histologic analysis • In situ apoptosis	• Noscapine caused obvious cell death in tumor mass via apoptosis	Protective	3
29	Barken, L. (2010)	Noscapine	Pc3 prostate cancer cell line injected to Male, athymic, Sim (NCr) nude mice, 5-6 weeks of age, treatment and pretreatment	Noscapine 300 mg/kg oral 56 ays	Xenograft mice	• Body weight • Tumor volume	• Body weight slightly increase • No significant differences in the incidence of either lymphatic or lung metastasis between the two noscapine groups • Treatment and pretreatment is useful for tumor growth inhibition	Protective	5
30	Jackson, T. (2008)	Noscapine	Female Nu/Nu mice six weeks old	10 mg/kg Oral 24 days	Western blotting xenograft mice TUNEL Immunohistochemistry	• Tumor volume • Body weight	• body weight slightly increase • No significant differences in the incidence of either lymphatic or lung metastasis between the two noscapine groups • Treatment and pretreatment is useful for tumor growth inhibition	No affect	5

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	Author (Year)	Type of opium or extraction	Cell line type/ Animal Species	Dosage	Technique	Clinical Index	Result	Conclusion	Risk of Bias
31	Barken, L. (2008)	Noscapine	100 µl of cell suspension was injected	300 mg/kg Oral 56 days	Haematoxylin and eosin (H&E) staining xenograft mice	• Tumor volume • Body weight	• very signiWcant (P < 0.01) reduction in tumor volume • no body weight loss	Protective	5
32	Landen, J. (2004)	Noscapine	Eight-week-old athymic female mice (nu/nu)	300 mg/kg Oral 15 days	In Vivo Tumorigenicity Assays: tumor xeno-graft in brain Hematoxylin and eosin stain	Determination of Noscapine Concentration in Animal Tissues by High-Performance Liquid Chromatography Image Analysis	• Inhabitation in tumor growth	Protective	4
33	Landen, LX. (2002)	Noscapine	Pathogen-free 8–10-weekold female C57BL/6 mice	300 mg/kg Oral 16 days	xenograft mice Histopathological Analyses Flow Cytometric Analysis CBC Hematoxylin and eosin stain	• Tumor volume	• Inhibits tumor progression but does not arrest it.	Protective	4
34	Ke,,Y. (2000)	Noscapine	The T cell lym-phoma cell line, EL4 injected to Female C57BL/6 (H-2b) mice, 8±12 weeks of age	3 mg/mouse (approx. 120 mg/kg body weight) Intraperito-neal (IP) 3weeks	Xenograft mice TUNEL Flow Cytometric Histopathological Analyses	• Tumor volume	• Noscapine inhibits tumor growth and induces apoptosis of tumor cells	Protective	5
35	Ye, K. (1998)	Noscapine	*HeLa cells in-jected to Female C57BL/6 (H-2b) mice, 8 to 12 weeks of age MCF-7, injected to female BALB/c athymic (nu/nu) nude mice 6–7 weeks of age	120 mg/kg Intraperito-neal (IP) 3weeks	TUNEL xenograft mice	• Tumor size	• Inhibits Growth of Murine and Human Tumors Implanted in Mice by Inducing Apoptosis • Noscapine Shrinks Murine Thymo-ma Solid Tumor • Noscapine Eliminates Human Tumors	Protective	5

Other Opioids (Morphine and Heroine)

	Author (Year)	Type of opium or extraction	Cell line type/ Animal Species	Dosage	Technique	Clinical Index	Result	Conclusion	Risk of Bias
36	Igder,S. (2013)	Morphine	Jurkat cells	2.86 × 10^{-3} g/ml	• Mtt assay • GC-MS spec-trometry • Flow cytometry • Annexin v staining	• Cell viability • Apoptosis	• There was a decrease 32% of in the percent of cell viability compared to control.	Protective	4
37	Dillenburg, C. F. (2008)	Morphine	Wistar rats	5 mg/kg/day for morphine and DEN Oral 23 weeks (4 days in a week)	• Histopathological Analyses • Hematoxylin and eo-sin stain	• Body weight • histologic analysis	• morphine did not promote neoplasia	Protective	-
38	Riberio Pinto, L. (1997)	Morphine	Male Sprague–Dawley rats	143 µg morphine sulphate/ kg body wt in 200 µl H2O and 6, 18 or 30 mg morphine sulphate/kg body wt in 200 µl H2O Injection 8 days	DNA analysis	• Organ weight	• DNA alkylation increase in kidney and no effect on liver	Carcinogen	5
39	Zagon, I. S. (1981)	Heroin	Male syngeneic AlJax mice (6-8 weeks of age)	3 mg/kg Injection 3 weeks	Subcutaneous injection to xenograft mice	• Tumor size	• No differences in 7 days but protec-tive in 14 days of injection	Protective	5

Table 2. Values in opium and its three main derivatives

Comp #	Compound Name	Units	1305	1306	1307	1308
			Tofaleh 1	Sukhteh 4	Shireh 2	Teriak 3
1	Naphtalene	ppb	10.3	24.8	10.9	10.2
2	Acenaphtylen	ppb	N.D	N.D	N.D	N.D
3	Acenaphten	ppb	N.D	N.D	N.D	N.D
4	Florene	ppb	N.D	N.D	N.D	N.D
5	Phenanthrene	ppb	20.6	21.5	N.D	9.5
6	Anthracene	ppb	N.D	N.D	N.D	N.D
7	Fluorantene	ppb	7.1	10.3	N.D	2.3
8	Pyrene	ppb	6.6	9.3	N.D	2.5
9	Benzo(a)ant	ppb	N.D	N.D	N.D	N.D
10	Chrysene	ppb	0.4	0.9	N.D	0.3
11	B(b)F	ppb	N.D	**N.D**	N.D	N.D
12	B(k)F	ppb	N.D	N.D	N.D	N.D
13	B(a)P	ppb	N.D	N.D	N.D	N.D
14	Dibenzo(a,h)Anthracene	ppb	N.D	N.D	N.D	N.D
15	Benzo(g,h,i)Perylene	ppb	N.D	N.D	N.D	N.D
16	Indeno(1,2,3-cd)Pyrene	ppb	N.D	N.D	N.D	N.D

Note: This table is provided to the authors by the Cancer Biology Research Center of Tehran University of Medical Sciences

Table 3. Chemical analysis of opium and its three main derivatives (all components reported by ppm)

Code	Ag	Al	As	B	Ba	Be	Ca	Cd	Ce	Co	Cr	Cu	Fe	Hg	K
1	<1	384	<1	211	25	<1	10518	<1	<1	<1	<1	356	806	<1	6440
2	<1	433	<1	59	8	<1	2521	<1	<1	<1	<1	8	211	<1	7434
3	<1	45	<1	80	11	<1	3770	<1	<1	<1	<1	14	212	<1	5666
4	<1	240	<1	109	12	<1	5118	<1	<1	<1	<1	88	367	<1	12024

La	Mg	Mn	Mo	Na	Ni	P	Pb	S	Sb	Sc	Se	Sn	Sr	V	Zn
<1	1005	32	<1	1838	13	2475	2119	17402	10	<1	<1	<1	112	<1	78
<1	798	10	<1	804	3	1624	213	22286	<2	<1	<1	<1	38	<1	11
<1	496	8	<1	589	7	1095	531	19007	6	<1	<1	<1	37	<1	15
<1	866	28	<1	1802	8	1991	96	25690	<2	<1	<1	<1	81	<1	22

Note: This table is provided to the authors by the Cancer Biology Research Center of Tehran University of Medical Sciences