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Antitumor effects of oleandrin in different types of cancers: Systematic review☆

Cristiane Raquel Dias Francischini [a], Carolina Rodrigues Mendonça [b], Kênia Alves Barcelos [a], Marco Augusto Machado Silva [a], Ana Flávia Machado Botelho [a,*]

[a] Postgraduate Program of Animal Science, Escola de Veterinária e Zootecnia, Federal University of Goiás, Brazil
[b] Graduate Program in Health Sciences, Escola de Medicina, Federal University of Goiás, Brazil

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ABSTRACT

Oleandrin, a cardiac glycoside isolated from the leaves of *Nerium oleander*, has known effects on the heart. Evidence from recent studies have highlighted its potential for anticancer properties. Therefore, we aimed to investigate the effects of oleandrin on cancer cell proliferation, viability and apoptosis *in vitro* and *in vivo*. We performed a systematic search in six electronic databases up to Jan 2022. We extracted information about the effects of oleandrin on cell proliferation, cell viability, apoptosis and/or cell cycle arrest in *in vitro* studies, and the effects on tumor size and volume in animal experimental models. We have retrieved 775 scientific studies. 14 studies met the inclusion criteria. They investigated the effects of oleandrin on breast, lung, pancreatic, colon, prostate, colorectal, oral, ovarian, glioma, melanoma, glioblastoma, osteosarcoma, and histiocytic lymphoma cancers. Overall, *in vitro* studies demonstrated that oleandrin was able to inhibit cell proliferation, decrease cell viability, and induce apoptosis and/or cell cycle arrest. In addition, oleandrin had an effect on reducing mean tumor size and volume in animal studies. Oleandrin, as a cytotoxic agent, demonstrated antitumor effects in different types of cancers, however important clinical limitations remain a concern. These results encourage future studies to verify the applicability of oleandrin in antineoplastic therapeutic protocols human and veterinary medicine, the investigation of antimetastatic properties, as well as the potential increase in patient survival and the decrease of tumor markers.

1. Introduction

Cancer develops when abnormal cells grow uncontrollably, beyond their usual limits and invade adjacent parts of the body and/or metastasize to other organs (WHO, 2021). GLOBOCAN estimates indicate that worldwide, about 19.3 million new cancer cases and nearly 10.0 million cancer deaths occurred in 2020, and it is a major cause of morbidity and mortality worldwide (Sung et al., 2021). In this sense, early diagnosis and treatment are critical for cure and increased survival. Besides, finding effective and economically feasible interventions for patients in subdeveloped countries is crucial (Mansouri et al., 2020).

Cardiac glycosides, extracted from the plant *Nerium oleander*, are commonly used in folk medicine for the treatment of heart failure and exhibit analgesic and anti-inflammatory effects (Li et al., 2021). In recent years, research has also shown that the cardiac glycoside oleandrin (OLE) may be a potential antitumor agent. These researches have explored the effects of OLE on lung (Bao et al., 2016), breast (Ko et al., 2018; Li et al., 2020), pancreatic (Pan et al., 2015), prostate (Pathak et al., 2000; Smith et al., 2001) and glioma cancer (Garofalo et al., 2017). However, the low therapeutic index due to its high cardiovascular toxicity limits its clinical use (Carfora et al., 2021). The controversial results found in some studies ratify the importance of a consistent systematic analysis to aggregate information and reach a macro conclusion about the effect of OLE on cancer cells, both on proliferation, viability, apoptosis and/or cell cycle arrest and reduction in tumor size, considering *in vitro* and animal experimental models (Bao et al., 2016; Ko et al., 2018; Li et al., 2020; Pathak et al., 2000; Smith et al., 2001).

In vitro and *in vivo* animal models are important tools in cancer research, allowing identification of carcinogens, development of cancer

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* Corresponding author. Laboratory Toxicology Veterinary, Veterinary Hospital, Escola de Veterinária e Zootecnia/UFG, Avenue Esperança s/nº, Campus Samambaia, CEP 74690-900, Goiânia, Goiás, Brazil.
E-mail address: anafmb@ufg.br (A.F.M. Botelho).

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therapies, and drug screening (Katt et al., 2016). Therefore, our aim was to perform a systematic review to provide an overview of the evidence on the effects of OLE on different types of cancers in both *in vitro* studies and experimental animal models.

2. Materials and methods

This study was elaborated based on the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses - PRISMA (Page et al., 2021) e Systematic Review Center for Laboratory animal Experimentation (SYRCLE) (De Vries et al., 2015).

Protocol was published to the database of the International Prospective Register of Systematic Reviews (PROSPERO) – Review of animal data from experimental studies (CRD CRD42021257696).

2.1. Research strategy

An electronic search of the PubMed, Embase, Science Direct, Scopus, Web of Science and Scielo databases was conducted until 25 Jan 2022. The search terms were: *"Oleandrin"; "Oleander"; "Nerium Oleander";"-Anvirzel"; "Foliandrin"; "Folinerin"; "PBI-05204", "Neriolin"; "Anti-Tumor"; "Effects Anticancer"; "Anti-Cancer"; "Anti-Proliferative"; "Neoplasms"; "Tumors"; "Cancer"; "Lung Neoplasms"; "Pulmonary Neoplasms"; "Colon Cancer"; "Prostate Cancer"; "Cervical Cancer"; "Melanoma Cancer"; "Pancreatic Cancer"; "Osteosarcoma"; "Thoracic Neoplasms"; "Lung Cancer"; "Pulmonary Cancer"; "Lung Cancer"; "Breast Cancer"; "Breast Neoplasms"; "Triple Negative Breast Neoplasms"; "Breast Tumor"; "Inflammatory Breast Neoplasms"; "Carcinoma, Ductal, Breast"; "Carcinoma, Lobular"; "HER-2 Positive Breast Cancer"*. The search strategies for each database are presented in Table 1. Primary study reference list searches were also conducted to identify additional studies.

2.2. Eligibility criteria

The inclusion criteria for this systematic review are based on the preclinical PICO methodology:

- *Patient*: laboratory animals (all species) with any type of cancer.
- *Intervention(s)*: oleandrin (OLE).
- *Comparator(s)*: control group or comparison with no treatment, treatment with other drugs and/or known cancer treatments with radiotherapy or chemotherapy.
- *Outcomes*: antitumor activity (i) cell proliferation, cell viability, apoptosis and/or cell cycle arrest *in vitro*, (ii) tumor volume and size *in vivo*.

Inclusion criteria were: 1) studies that investigated the effects of OLE on different types of cancer using *in vitro* and animal models; 2) original peer-reviewed research articles; 3) articles in English language; 4) no restriction on year of publication. In addition, all studies that investigated the effects of OLE against cancer, regardless of dosage and time of intervention, were included. For *in vitro* studies cancer cell lines from animals and humans will be considered.

The exclusion criteria for the studies were: human clinical trials and epidemiological studies, theses, dissertations, case studies, editorials, letters to the editor, duplicate studies found in more than one database, non-laboratory studies, articles with the full version unavailable, articles in a foreign language other than English, and removed articles. Studies that used OLE in combination with other drugs and those that did not investigate the antitumor effects of OLE in cancer were also excluded.

Cell proliferation: refers to the increase in cell number resulting from cell division (Yang et al., 2014a). Cell proliferation is tightly controlled in normal cells, whereas cancer cells have excessive cell proliferation due to sustained proliferative signaling and evaded growth suppressors (Peng et al., 2016).

Cell viability is the quantification of the number of live cells and is used to estimate cytotoxicity (Fang and Trewyn, 2012). It is a measure of cellular activity and overall cell health (Khan, 2019).

Apoptosis: is known as programmed cell death that occurs in physiological and pathological conditions (Brown et al., 2014). In cancer, there is a loss of balance between cell division and cell death, resulting in more resistant malignant cells (Wong and Research, 2011).

Table 1
Search strategies presented in the databases.

Databases	Search Strategy
Medline/PubMed (May 25th, 2021)	*Search: (oleandrin OR oleander OR Nerium oleander OR Anvirzel OR Foliandrin OR Folinerin OR Neriolin) AND (Anti-tumour OR effects anticancer OR anti-cancer OR anti-proliferative OR Neoplasms OR Tumors OR Cancer OR Lung Neoplasms OR Pulmonary Neoplasms OR Colon Cancer OR Prostate Cancer OR Cervical Cancer OR Melanoma Cancer OR Pancreatic Cancer OR Osteosarcoma OR Thoracic Neoplasms OR Breast cancer OR Breast Neoplasms OR Triple Negative Breast Neoplasms OR Breast Tumor OR Inflammatory Breast Neoplasms OR Carcinoma, Ductal, Breast OR Carcinoma, Lobular OR HER-2 Positive Breast Cancer)* ***Total: 133***
EMBASE (May 25th, 2021)	*1(oleandrin or oleander or Nerium oleander or Anvirzel or foliandrin OR folinerin OR neriolin).mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word] 1236* *2 Neoplasms.mp. or neoplasm/759616* *3 Cancer.mp. or malignant neoplasm/3942153* *4 neoplasm/514761* *5 Anti-tumour.mp.10480* *6 effects anticancer.mp. 31* *7 Lung Neoplasms.mp. or lung tumor/82853* *8 Prostate Cancer.mp. or prostate cancer/244418* *9 Cervical Cancer.mp. or uterine cervix cancer/96562* *10 melanoma/or Melanoma Cancer.mp.148716* *11 Pancreatic Cancer.mp. or pancreas cancer/96936* *12 Osteosarcoma.mp. or osteosarcoma/49296* *13 Thoracic Neoplasms.mp. or thorax tumor/5204* *14 Breast Neoplasms.mp. or breast tumor/109288* *15 Triple Negative Breast Neoplasms.mp. or triple negative breast cancer/24891* *16 breast cancer/or HER-2 Positive Breast Cancer.mp. 391884* *17 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 4349000* *18 1 and 17* ***Total: 193***
Scopus (May 25th, 2021)	*TITLE-ABS-KEY ((oleandrin OR oleander OR nerium AND oleander OR anvirzel OR Foliandrin OR Folinerin OR Neriolin) AND (cancer)) AND (LIMIT-TO (DOCTYPE, "article"))* ***Total: 79***
Science Direct (May 25th, 2021)	(oleandrin OR oleander OR nerium AND oleander OR anvirzel OR *Foliandrin OR Folinerin OR Neriolin*) AND (cancer) ***Total: 220***
Web of Science (May 25th, 2021)	*(oleandrin OR oleander OR Nerium oleander OR Anvirzel OR Foliandrin OR Folinerin OR Neriolin) AND (Anti-tumour OR effects anticancer OR anti-cancer OR anti-proliferative OR Neoplasms OR Tumors OR Cancer OR Lung Neoplasms OR Pulmonary Neoplasms OR Colon Cancer OR Prostate Cancer OR Cervical Cancer OR Melanoma Cancer OR Pancreatic Cancer OR Osteosarcoma OR Thoracic Neoplasms OR Breast cancer OR Breast Neoplasms OR Triple Negative Breast Neoplasms OR Breast Tumor OR Inflammatory Breast Neoplasms OR Carcinoma, Ductal, Breast OR Carcinoma, Lobular OR HER-2 Positive Breast Cancer)* **Total: 149**
Scielo (May 25th, 2021)	*oleandrin OR oleander OR Nerium oleander OR Anvirzel AND Anti-tumour OR effects anticancer OR anti-cancer OR anti-proliferative OR Neoplasms OR Tumors OR Cancer* ***Total: 1***

2.3. Study selection

Two researchers performed title and abstract reading (CRDF and CRM) using *Rayyan software*. The studies identified in the search were jointly reviewed by the authors and critically evaluated based on

knowledge about the antitumor effects of OLE in cancer. Then, the selected articles were read in full by two researchers and the inclusion and exclusion criteria (CRDF and CRM) were applied. Questions and/or disagreements about the articles were discussed by the research team (AFMB, CRDF and CRM).

The following data were extracted: author, year of publication, country, cancer type, model cell type, intervention (concentration and exposure time), outcome (antitumor activity - cell proliferation, cell viability, apoptosis and/or cell cycle arrest for *in vitro* studies and tumor size for animal studies) and adverse effects.

2.4. Risk of bias and quality assessment

The GRADE (*Grading of Recommendations, Assessment, Development and Evaluation*) was used for the quality assessment of pre-clinical studies (Pavan et al., 2015). The items analyzed were: study design, study limitation, inconsistency, imprecision, publication bias, moderate/large effect size, dose effect, and overall quality. The overall quality is indicated as: "high", "moderate", "low" or "very low" (Pavan et al., 2015).

The SYRCLE ROB tool to assess the risk of bias was used for the *in vivo* (animal) studies (Wei et al., 2016). These are classified as "high risk of bias" (+, if one or more criteria were not met), "low risk of bias" (-, if all criteria were met), or "unclear" (? one or more criteria were partially met).

3. Results

3.1. Selection of studies

The systematic search identified 775 articles (Fig. 1). After removing duplicates, 474 articles were selected for reading of titles and abstracts, of which 409 were excluded. Of the 65 articles assessed for eligibility by reading the full text, 14 articles were included in the final analysis. The main reasons for study exclusions are presented in Fig. 1 and Table 2.

3.2. Characteristics of the studies

Tables 3 and 4 present the characteristics of the fourteen studies on the anticancer effects of OLE that were included. Thirteen studies presented results from *in vitro* studies (Bao et al., 2016; Garofalo et al., 2017; Ko et al., 2018; Li et al., 2008, 2020; Ma et al., 2015; Newman et al., 2007; Pan et al., 2017; Pathak et al., 2000; Raghavendra et al., 2007; Smith et al., 2001; Yang et al., 2009, 2014b) (Table 3). Table 4 presents the characteristics of the studies with animal experimental results (Garofalo et al., 2017; Li et al., 2021). One experiment presented both *in vivo* and *in vitro* information (Garofalo et al., 2017).

From the fourteen studies included, eight (57%) were conducted in the USA (Li et al., 2008; Newman et al., 2007; Pan et al., 2017; Pathak et al., 2000; Raghavendra et al., 2007; Smith et al., 2001; Yang et al., 2009, 2014b), four (29%) in China (Bao et al., 2016; Li et al., 2020,

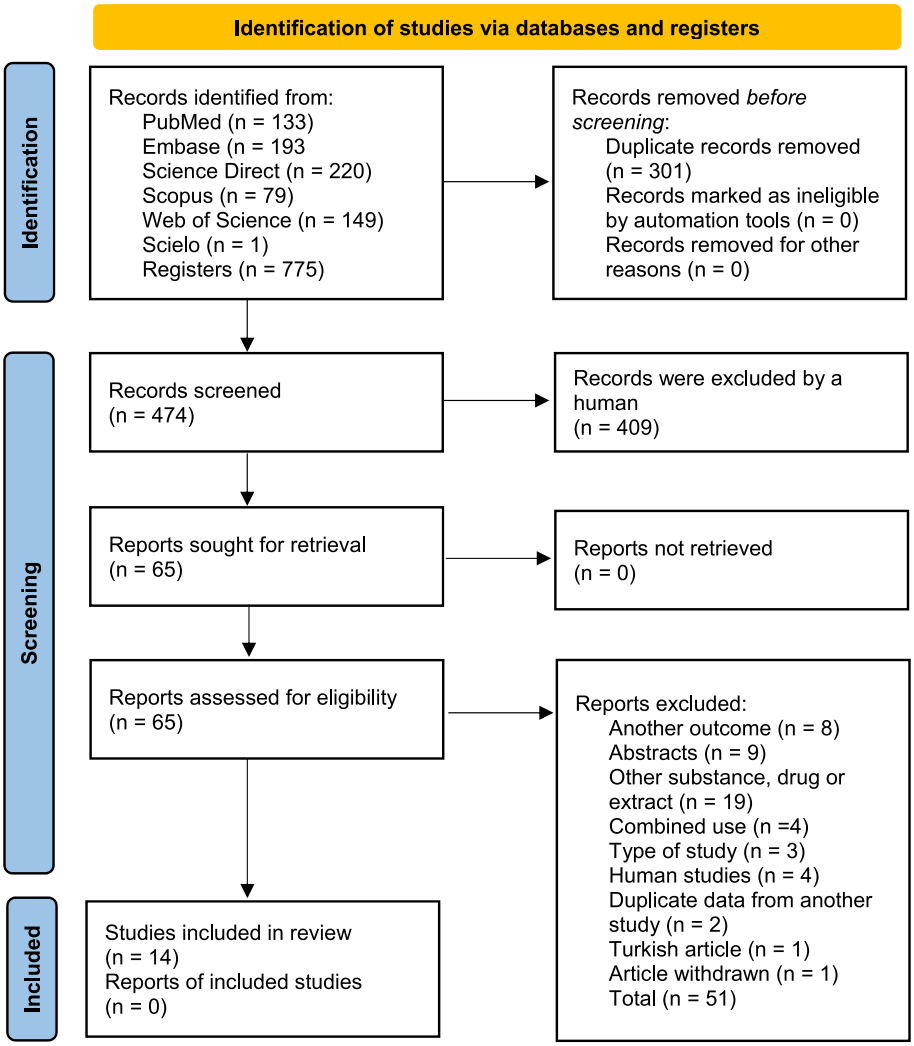

Fig. 1. Flowchart of the selection of studies on the antitumor effects of oleandrin on different types of cancers.

Table 2
Excluded articles and reason for exclusions.

Number	Title	Reason for exclusion
1	Afaq F, Saleem M, Aziz MH, Mukhtar H. Inhibition of 12-O-tetradecanoylphorbol-13-acetate-induced tumor promotion markers in CD-1 mouse skin by oleandrin. Toxicol Appl Pharmacol. 2004 Mar 15; 195(3):361-9. https://doi.org/10.1016/j.taap.2003.09.027.	1
2	Ahmad, Mohammad Kaleem; Mahdi, Abbas Ali; Ansari, Jamal Akhtar; Khan, Abdul Rahman; Rastogi, Namrata; Khan, Homa Jilani; Fatima, Nishat. O38 Evaluation of Anticancer Activity of Indian Medicinal Plants. Journal: Indian Journal of Clinical Biochemistry - Volume 30, Issue 0, pp. S39 - published 2015-01-01. Abstracts of 42nd National Conference of Association of Clinical Biochemists of India (ACBICON 2015) Association of Clinical Biochemists of India 2015	2
3	Al-Hakak, Zahra M., Zinah Ibraheem Khaleel, and Mouhamed Abbas Fadel. "Study the effect of the toxic alcoholic extract of Nerium Oleander on the liver cancer cell line *in vivo* and the effects on the liver histology in Mus Musculus." Journal of Pharmaceutical Sciences and Research 11.1 (2019): 201–205.	3
4	Apostolou, Panagiotis, et al. "Anvirzel™ in combination with cisplatin in breast, colon, lung, prostate, melanoma and pancreatic cancer cell lines." BMC Pharmacology and Toxicology 14.1 (2013): 1–6.	4
5	Ayouaz, Siham, et al. "LC-DAD-ESI-MS/MS analysis and cytotoxic and antiproliferative effects of chlorogenic acid derivative rich extract from Nerium oleander L. pink flowers." Food & Function 12.8 (2021): 3624–3634.	3
6	Bai, Liming, et al. "Polar cardenolide monoglycosides from stems and twigs of Nerium oleander and their biological activities." *Journal of wood science* 57.1 (2011): 47–55.	1
7	Bidyasar, S. et al. "A first-in-human phase I trial of PBI-05204 (oleandrin), an inhibitor of Akt, FGF-2, NF-Kb, and p70S6K in advanced solid tumor patients." Journal of Clinical Oncology 27.15_suppl (2009): 3537-3537.	2
8	Calderón Montaño, José Manuel et al. "A hydroalcoholic extract from the leaves of Nerium oleander inhibits glycolysis and induces selective killing of lung cancer cells." *Planta medica, 79 (12), 1017–1023* (2013).	3
9	Calderón-Montaño, José Manuel et al. Does the Nerium oleander extract PBI-05204 have potential for pancreatic cancer therapy? Investigational new drugs, v. 33, n. 3, p. 787-787, 2015.	3
10	Colapietro, Alessandro, et al. "The Botanical Drug PBI-05204, a Supercritical CO2 Extract of Nerium Oleander, Inhibits Growth of Human Glioblastoma, Reduces Akt/mTOR Activities, and Modulates GSC Cell-Renewal Properties." Frontiers in Pharmacology 11 (2020): 1438.	3
11	Demirel Kars, M. E. L. T. E. M. et al. "Effect of Nerium oleander distillate on MCF-7 breast cancer cell lines." (2011).	2
12	Ding, Kan, et al. "Characterization of a Rhamnogalacturonan and a Xyloglucan from Nerium i ndicum and Their Activities on PC12 Pheochromocytoma Cells." Journal of natural products 66.1 (2003): 7–10.	3
13	Elmaci, İlhan, et al. "Neuroprotective and tumoricidal activities of cardiac glycosides. Could oleandrin be a new weapon against stroke and glioblastoma?" International Journal of Neuroscience 128.9 (2018): 865–877.	5
14	El-Seedi, Hesham R. et al. "The traditional medical uses and cytotoxic activities of sixty-one Egyptian plants: discovery of an active cardiac glycoside from Urginea maritima." *Journal of Ethnopharmacology* 145.3 (2013): 746–757.	3
15	Felth J, Rickardson L, Rosén J, Wickström M, Fryknäs M, Lindskog M, Bohlin L, Gullbo J. Cytotoxic effects of cardiac glycosides in colon cancer cells, alone and in combination with standard chemotherapeutic drugs. J Nat Prod. 2009 Nov; 72(11):1969-74. https://doi.org/10.1021/np900210m.	1
16	Fiebig, Heinz H. et al. Abstract 5572: "Breastin a natural product from Nerium Oleander exhibits high activity in a panel of human tumor cell lines." (2013): 5572-5572.	2
17	Henary, H. et al. Final results of a first-in-human phase I trial of PBI-05204, an inhibitor of Akt, FGF-2, NF-Kb and p70S6K in advanced cancer patients. Breast, v. 5, p. 10–19, 2011. (Duplicado do Bidyasar, S. et al., 2009).	2
18	KARAKOYUN, Çiğdem et al. Five new cardenolides transformed from oleandrin and nerigoside by Alternaria eureka 1E1BL1 and Phaeosphaeriasp. 1E4CS-1 and their cytotoxic activities. Phytochemistry Letters, v. 41, p. 152–157, 2021.	1
19	Hong, D. S. et al. "First-in-human study of pbi-05204, an oleander-derived inhibitor of akt, fgf-2, nf-κB and p70s6k, in patients with advanced solid tumors." *Investigational new drugs* 32.6 (2014): 1204–1212.	6
20	Lin, Y; Felix, E; Ho, DH; Kempen, E; Newman, RA; Lin, Y; Felix, E; Ho, DH; Kempen, E; Newman, RA; Oleandrin-mediated cytotoxicity and its relationship to specific isoform expression of Na+, K + -ATPase in tumor cells. CLINICAL CANCER RESEARCH - Volume 7, Issue 11, pp. 3706S–3706S - published 2001-11-01	2
21	Lin, Yun, Dah H. Ho, and Robert A. Newman. "Human tumor cell sensitivity to oleandrin is dependent on relative expression of Na+, K + -ATPase subunits." *Journal of experimental therapeutics & oncology* 8.4 (2010).	1
22	Ma Y, Zhu B, Yong L et al. Regulation of Intrinsic and Extrinsic Apoptotic Pathways in Osteosarcoma Cells Following Oleandrin Treatment. *Int J Mol Sci*. 2016; 17 (11):1950. Published 2016 Nov 23. https://doi.org/10.3390/ijms17111950	7[a]
23	Manna SK, Sah NK, Newman RA, Cisneros A, Aggarwal BB. Oleandrin suppresses activation of nuclear transcription factor-kappaB, activator protein-1, and c-Jun NH2-terminal kinase. Cancer Res. 2000 Jul 15; 60 (14):3838-47. PMID: 10919658.	1
24	Mekhail, Tarek, et al. "Phase 1 trial of Anvirzel™ in patients with refractory solid tumors." *Investigational new drugs* 24.5 (2006): 423–427.	6
25	MCCONKEY, David J. et al. Cardiac glycosides stimulate Ca2+ increases and apoptosis in androgen-independent, metastatic human prostate adenocarcinoma cells. Cancer Research, v. 60, n. 14, p. 3807–3812, 2000.	1
26	Mijatovic, Tatjana, et al. "Nucleolus and c-Myc: potential targets of cardenolide-mediated antitumor activity." *Molecular cancer therapeutics* 7.5 (2008): 1285–1296.	3
27	Mohapatra, Shubhasmita, et al. "Leaf Extract of Nerium oleander L. Inhibits Cell Proliferation, Migration and Arrest of Cell Cycle at G2/M Phase in HeLa Cervical Cancer Cell." *Anti-Cancer Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Cancer Agents)* 21.5 (2021): 649–657.	3
28	Mouhcine, Messaoudi, et al. "Cytotoxic, antioxidant and antimicrobial activities of Nerium oleander collected in Morocco." *Asian Pacific Journal of Tropical Medicine* 12.1 (2019): 32.	3
29	Newman RA, Yang P, Hittelman WN, Lu T, Ho DH, Ni D, Chan D, Vijjeswarapu M, Cartwright C, Dixon S, Felix E, Addington C. Oleandrin-mediated oxidative stress in human melanoma cells. J Exp Ther Oncol. 2006; 5(3):167-81.	4
30	Newman, Robert A., and Peiying Yang. "Response to: Does the Nerium oleander extract PBI-05204 have	3

(*continued on next page*)

Table 2 (*continued*)

Number	Title	Reason for exclusion
	potential for pancreatic cancer?" *Investigational new drugs* 33.3 (2015): 788.	
31	Owens, M., A. Hill, and A. Hopkins. "Cardiac glycosides as potential anti breast cancer agents: O20." British Journal of Surgery 99 (2012).	2
32	Owens, M., A. D. K. Hill, and A. M. Hopkins. "Cardiac glycosides inhibit breast cancer cell proliferation and migration." Irish Journal Of Medical Science. Vol. 180. 236 Grays Inn Rd, 6th Floor, London Wc1x 8 hl, England: Springer London Ltd, 2011.	2
33	Parsonidis, P. et al. "Effects of natural substances from Boswellia sacra and Nerium oleander (breastin) in colon and pancreatic cancer." *Annals of Oncology* 30 (2019): iv19.	2
34	Pathak, Sen, et al. "Oleander extract induces cell death in human but not murine cancer cells." *Anti-Cancer Drugs* 12.7 (2001): 637–638.	3
35	Raghavendra PB, Sreenivasan Y, Ramesh GT, Manna SK. Cardiac glycoside induces cell death via FasL by activating calcineurin and NF-AT, but apoptosis initially proceeds through activation of caspases [retracted in: Apoptosis. 2013 Jul; 18(7):910]. Apoptosis. 2007; 12(2):307–318. https://doi.org/10.1007/s10495-006-0626-3	7[b]
36	Roth, Marc T. et al. "A Phase II, Single-Arm, Open-Label, Bayesian Adaptive Efficacy and Safety Study of PBI-05204 in Patients with Stage IV Metastatic Pancreatic Adenocarcinoma." The Oncologist 25.10 (2020): e1446-e1450.	6
37	Roth, Marc Thomas et al. "Phase II clinical trial of novel agent PBI-05204 in patients with metastatic pancreatic adenocarcinoma (mPDA)." (2020): 698-698.	6
38	Ruacan, S.A.; Firat, D. Screening of the extracts of Nerium oleander for antineoplastic activity against murine leukemia systems. Journal: Kanser - Volume 6, Issue 1, pp. 49–57, 1976.	8
39	Saha, A., and V. V. Yakovlev. "Towards a rational drug design: Raman micro-spectroscopy analysis of prostate cancer cells treated with an aqueous extract of Nerium Oleander." *Journal of Raman Spectroscopy: An International Journal for Original Work in all Aspects of Raman Spectroscopy, Including Higher Order Processes, and also Brillouin and Rayleigh Scattering* 40.11 (2009): 1459–1460.	3
40	Siddiqui BS, Khatoon N, Begum S, Farooq AD, Qamar K, Bhatti HA, Ali SK. Flavonoid and cardenolide glycosides and a pentacyclic triterpene from the leaves of Nerium oleander and evaluation of cytotoxicity. Phytochemistry. 2012 May; 77:238–44. https://doi.org/10.1016/j.phytochem.2012.01.001.	1
41	Sreenivasan, Y., Raghavendra, P.B. & Manna, S.K. RETRACTED Article: Oleandrin-Mediated Expression of Fas Potentiates Apoptosis in Tumor Cells. J Clin Immunol 26, 308–322 (2006). https://doi.org/10.1007/s10875-006-9028-0	9
42	Zhu, Jing-jing et al. "Delivery of acetylthevetin B, an antitumor cardiac glycoside, using polymeric micelles for enhanced therapeutic efficacy against lung cancer cells." *Acta pharmacologica Sinica* 38.2 (2017): 290–300.	3
43	Turan, Nevruz, et al. "Cytotoxic effects of leaf, stem and root extracts of Nerium oleander on leukemia cell lines and role of the p-glycoprotein in this effect." *Journal of experimental therapeutics & oncology* 6.1 (2006).	3
44	Wahyuningsih, Mae Sri Hartati et al. "5α-Oleandrin reduce Bcl-2 protein and increase Bax protein expression on Hela cervical cancer cell." *Universa Medicina* 36.2 (2017): 102–109.	3
45	Wang, Xiaomin, et al. "LC/MS/MS analyses of an oleander extract for cancer treatment." Analytical Chemistry 72.15 (2000): 3547–3552.	3
46		5
	Winnicka K, Bielawski K, Bielawska A. "Cardiac glycosides in cancer research and cancer therapy." Acta Pol Pharm 63.2 (2006): 109–115.	
47	Yong, Lei, et al. "Oleandrin sensitizes human osteosarcoma cells to cisplatin by preventing degradation of the copper transporter 1." *Phytotherapy Research* 33.7 (2019): 1837–1850.	4
48	Zhao, Ming, et al. "Bioactive cardenolides from the stems and twigs of Nerium oleander." Journal of natural products 70.7 (2007): 1098–1103.	3
49	Sreenivasan, Yashin, Pongali B. Raghavendra, and Sunil K. Manna. "Retraction Note: Oleandrin-Mediated Expression of Fas Potentiates Apoptosis in Tumor Cells." *Journal of Clinical Immunology* 33.5 (2013): 1031-1031.	5
50	Pan Y, Rhea P, Tan L, Cartwright C, Lee HJ, Ravoori MK, Addington C, Gagea M, Kundra V, Kim SJ, Newman RA, Yang P. PBI-05204, a supercritical CO_2 extract of Nerium oleander, inhibits growth of human pancreatic cancer via targeting the PI3K/mTOR pathway. Invest New Drugs. 2015 Apr; 33(2):271-9. https://doi.org/10.1007/s10637-014-0190-6. Epub 2014 Dec 6. PMID: 25476893; PMCID: PMC4387257.	3
51	Terzioglu-Usak S, Nalli A, Elibol B, Ozek E, Hatiboglu MA. AnvirzelTMregulates cell death through inhibiting GSK-3 activity in human U87 glioma cells. Neurol Res. 2020 Jan; 42(1):68–75. https://doi.org/10.1080/01616412.2019.1709744.	3

[1] Another outcome; [2] Abstract; [3] Another substance, drug or extract; [4] Combined use; [5] Type of study; [6] Human studies; [7] Duplicate data from another study; [8] Turkish article; [9] Removed article; [10] Does not mention cancer in the methodology.

[a] Ma Y, Zhu B, Liu X, Yu H, Yong L, Liu X, Shao J, Liu Z. Inhibition of oleandrin on the proliferation show and invasion of osteosarcoma cells *in vitro* by suppressing Wnt/β-catenin signaling pathway. J Exp Clin Cancer Res. 2015 Oct 6; 34:115. https://doi.org/10.1186/s13046-015-0232-8.

[b] RAGHAVENDRA, Pongali B.; SREENIVASAN, Yashin; MANNA, Sunil K. Oleandrin induces apoptosis in human, but not in murine cells: dephosphorylation of Akt, expression of FasL, and alteration of membrane fluidity. Molecular immunology, v. 44, n. 9, p. 2292–2302, 2007.

2021; Ma et al., 2015), one (7%) in Italy (Garofalo et al., 2017) and other one (7%) in Korea (Ko et al., 2018).

In *in vitro* studies, the effects of oleandrin on the following cells were investigated: lung carcinoma (Bao et al., 2016; Yang et al., 2009), glioma and glioblastoma multiform (Garofalo et al., 2017), breast cancer (Ko et al., 2018; Li et al., 2020; Yang et al., 2009), osteosarcoma (Ma et al., 2015), pancreatic cancer (Newman et al., 2007; Yang et al., 2009), colon cancer (Pan et al., 2015; Yang et al., 2009, 2014b), prostate cancer (Pathak et al., 2000; Smith et al., 2001), melanoma (Li et al., 2008; Pathak et al., 2000; Yang et al., 2009), histiocytic lymphoma (Raghavendra et al., 2007), oral cancer (Yang et al., 2009) and ovarian cancer (Yang et al., 2009).

In experimental animal studies, the effects of OLE were investigated on glioma tumor size (Garofalo et al., 2017) and breast cancer (Li et al., 2021).

3.3. In vitro *study*

3.3.1. *Cell proliferation*

Six studies presented results on the effects of OLE on cell proliferation in *in vitro* models. After treatment with OLE, cell proliferation was monitored using the xCELLigence RTCA system and MTT assay (Newman et al., 2007; Li et al., 2008; Yang et al., 2009, 2014a; Pan et al., 2015, 2017; Li et al., 2020).

OLE at different dosages significantly inhibited the growth of breast cancer cells (MCF-7, SK-BR-3 and MDA-MB-231) (Li et al., 2020), pancreatic cancer cells PANC-1 (Newman et al., 2007), human colon

Table 3
Characteristics of the studies included in the systematic review involving *in vitro* studies.

Author/Year/ Country	Type of cancer	Type of cell/ (model)	Intervention	Exposure time	Antitumoral activity			Quality of evidence
					Cell proliferation *in vitro*	Cell viability	Apoptosis and/or cell cycle arrest	
Bao et al. (2016) China	Lung carcinoma	A549 cell (human)	Oleandrin 0,01 μg/ml, 0,02 μg/ml, 0,04 μg/ml	24 h	-		**Flow cytometry** Apoptosis was induced by oleandrin (0.02 μg/ml) $p < 0.001$	⊕⊕⊕◯ MODERATE
Garofalo et al. (2017) Italy	Glioma Glioblastoma multiforme	Primary glioma cells GL261, U87MG (human) Human glioblastoma multiforme (GBM) cells (human)	Oleandrin 0,3, 3, or 30 μm	3, 8, 20, and 40 h	-	**MTT Assay** The oleandrin reduced the viability in all the GBM human cells in a time-dependent way, even in the lowest dose ($n = 4$, $^{**}p < 0,01$), while no effect on viability was observed in GL261 cells.	**Flow cytometry** Apoptosis was induced by oleandrin (3 μ m for 10 h) $p < 0.001$.	⊕⊕⊕◯ MODERATE
Ko et al. (2018) Korea	Breast cancer	MDA-MB-231 cells and RT-R-MDA-MB-231 (human)	Oleandrin 1, 10, 30, 50, 100 and 500 nM to 37 °C	24 h 48 h (50 nM)	–	**MTT Assay** The oleandrin reduced the cell viability of breast cancer cells at concentrations in a dose dependent manner (1, 10, 30, 50, 100 e 500 nM).	-	⊕⊕⊕◯ MODERATE
Li Xiao-xi et al., 2020 China	Breast cancer	MCF7 (luminal A subtype), SK-BR-3 (HER-2 + subtype) and MDA-MB-231 (triple negative breast cancer, TNBC) cells. (human)	Oleandrin MCF7 e SK-BR-3 cells were treated with concentrations varying from 50 nM a 0,78 nM. MDA-MB-231 was treated with concentrations varying from 100 nM a 1,56 nM.	24 h 72 h	**Monitored by the system RTCA-MP** Oleandrin significantly inhibited the growth of breast cancer cells (MCF-7, SK-BR-3 and MDA-MB-231) in a dose- and time-dependent manner.	**MTT Assay** Oleandrin decreases the viability of primary breast cancer cells in Lumina A subtype, Lumina B subtype, HER-2 + subtype and TNBC.	**Flow cytometry** Oleandrin induced apoptosis of breast cancer cells.	⊕⊕⊕⊕ HIGH
Li et al., 2008 USA	Melanoma	BRO Cells (human) B16 Cells (murine)	Oleandrin 2 nM–1000 nM	48 h	**MTT assay** . Growth inhibition >80% in BRO even at low doses (10 nM), No growth inhibition in B16 cells even at concentrations higher than 1000 nM.	–	**Flow cytometry** Apoptosis was induced in BRO cells with 50 nM oleandrin.	⊕⊕⊕◯ MODERATE
Ma et al. (2015) China	Osteosarcoma	U2OS and SaOS-2 cells (human)	Oleandrin 25, 50, 75 and 100 nM	24, 48 and 72 h	–	**MTT Assay** For U2OS, cell viability was significantly reduced after treatment with 50 nM oleandrin for 24 h ($p < 0.01$) and 48 h ($p < 0.01$). For SaOS-2, however, both 25 nM and 50 nM oleandrin significantly decreased cell viability after treatment for 24 h ($p < 0.01$) and 48 h ($p < 0.01$).	**Flow cytometry** After treatment with 50 nM oleandrin, the total number of apoptotic cells in both U2OS and SaOS-2 cell lines increased significantly. The apoptosis rates of U2OS cells at 0, 24 and 48 h were 15.8%, 29.0% and 46.0%, respectively (24 or 48 vs. 0 h:$p = 0.005$ or $p = 0.000$; 24 vs. 48 h: $p = 0.001$). The apoptosis rates of SaOS-2 cells were 10.6%, 22.2% and 31.8%, respectively	⊕⊕⊕⊕ HIGH

(*continued on next page*)

Table 3 (*continued*)

Author/Year/ Country	Type of cancer	Type of cell/ (model)	Intervention	Exposure time	Antitumoral activity			Quality of evidence
					Cell proliferation *in vitro*	Cell viability	Apoptosis and/or cell cycle arrest	
							(24 or 48 vs. 0 h: p = 0.007 or p = 0.000; 24 vs. 48 h: p = 0.015).	
Pan et al. (2017) USA	Colon cancer	Colon Cancer Cells SW480 (human)	Oleandrin 0.4 nM–3 μM	24, 48, and 72 h	**MTT Assay** Oleandrin has a significant inhibitory effect on SW480 cell proliferation without significantly reducing the viability of normal human colon epithelial cells	**MTT Assay** Oleandrin significantly decreased cell viability in SW480 cells.	**Flow cytometry** The doses of oleandrin (0.01, 0.02, 0.05 μM) that induced SW480 cell death reached 10.12, 16.48 and 24.57% compared to 3.87% for the control group.	⊕⊕⊕⊕ HIGH
Newman et al. (2007) USA	Pancreatic tumor cells	PANC-1 pancreatic cancer cells (human)	Oleandrin 5, 10, 25 and 50 nM	24 h	**MTT Assay** Oleandrin markedly inhibited the growth of PANC-1 cells with an IC50 value of 0.005 μM apoptosis.	-	**DNA Flowmetric Analysis** Cell cycle inhibition occurs without any apparent induction of apoptosis as evidenced by the lack of cells in sub-G1. The cytotoxic effect of oleandrin is not attributable to induction of apoptosis.	⊕⊕⊕◯ MODERATE
Pathak et al. (2000) USA	Prostate Cancer Melanoma	PC-3M prostate cancer cell lines (human) Melanoma cell line K1735 clone X21 (Murine) Oral melanoma cell line CML-1 (Canine) U-937, HL-60, HeLa, and MCF-7 cells (Human) SP-2, J774, C2C12, P338D1 and NIH3T3 cells (Murine)	Oleandrin 1,0 ng/ml, 10,0 ng/ml, 0,1 mg/ml e 1,0 mg/ml K1735-X21 cells were treated 5 and 50 mg/ml CML-1 were treated with Oleandrin 0,1, 1 and 5 mg/ml	24 h	-	-	**Flow cytometry** Oleandrin induce cell death in PC-3M cells . Oleandrin do not induce metastatic cell death in murine melanoma K1735-X21. Oleandrin induces cell death in a canine melanoma cell line (CML-1).	⊕⊕⊕◯ MODERATE
Raghavendra et al. (2007) USA	Histiocytic lymphoma		Oleandrin 100 ng/ml	24 h	–	**MTT Assay** . Oleandrin decreased cell viability in a dose-dependent manner in human cells, but not in murine cells.	**Flow cytometry** Oleandrin-induced cell death by 33%, 27% and 36% in U-937, HeLa and MCF-7 cells. There was no oleandrin-induced cell death in murine cells, NIH3T3 cells, C2C12 cells, and P338D1 cells,	⊕⊕⊕◯ MODERATE
Smith et al. (2001) USA	Prostate tumor	DU145 and PC3 Cells (human)	Oleandrin 0,05 or 0,1 ng/mL	72 h	–	**MTT Assay** Oleandrin (0.1 ng/mL) produced a 49.9% inhibition of DU145 cells.	–	⊕⊕⊕◯ MODERATE
Yang et al., 2014a, 2014b USA	Colorectal cancer	CaCO-2 Cellls (human)	Oleandrin 0,2–25 nM	48 h – cell ploriferation 24 h – Cell viability	**MTT Assay** 20% inhibition of cell growth $p < 0,01$ Oleandrin exerted a three times stronger antiproliferative activity in undifferentiated CaCO-2 cells (IC50,	**MITT Assay** There was inhibition of CaCO2 proliferation and induction of cell death in a concentration-	**Flow cytometry** Oleandrin (10–20 nM) inhibited cell proliferation by inducing autophagic cell death in undifferentiated CaCO-2 cells.	⊕⊕⊕⊕ HIGH

(*continued on next page*)

Table 3 (*continued*)

Author/Year/ Country	Type of cancer	Type of cell/ (model)	Intervention	Exposure time	Antitumoral activity			Quality of evidence
					Cell proliferation *in vitro*	Cell viability	Apoptosis and/or cell cycle arrest	
					8.25 nM) than in differentiated CaCO-2 cells (IC50,> 25 nM)	dependent manner.		
Yang et al. (2009) USA	-pancreatic cancer -colon cancer - melanoma - human breast cancer - human oral cancer cells - ovary cancer - human non-small cell lung cancer	- Panc-1, BxPC3 and MiaPaca cells (human) - CaCO-2, DOD-1, HCT-116, HT29, RKO and LST174 cells (human) - B16 melanoma (murine) - SUM149, MCF-7 and MDA231 cells (human) - SCC9 and CAL-27 cells (human) - ES3, TOV1120 and SKOV cells (human) - A549 and H1299 cells (human)	Oleandrin 0, 5, 20 and 50 nmol/L	6, 24 or 72 h.	**MTT Assay** Oleandrin differentially inhibited the proliferation of rodent (mouse Panc-02) and human pancreatic cancer (Panc-1, MiaPaca and BxPC3) cell lines. Human melanoma and leukemia cells were 100-fold more sensitive to oleandrin than murine tumor cells, normal human epithelial cells, peripheral blood mononuclear cells or neutrophils	–	–	⊕⊕⊕◯ MODERATE

Non-triple negative breast cancer: TNBC

cancer cells SW480 (Pan et al., 2017), human colorectal cancer cells CaCO-2 (Yang et al., 2014a), mouse Panc-02 and human pancreatic cancer cells Panc-1, MiaPaca and BxPC3 (Yang et al., 2009). In addition, human melanoma and leukemia cells were 100 times more sensitive to OLE in inhibiting cell growth than murine tumor cells, normal human epithelial cells, peripheral blood mononuclear cells or neutrophils (Yang et al., 2009). Another study also reported that there was greater than 80% inhibition of cell growth in human BRO melanoma cells and mouse B16 cells even at low doses (10 nM) (Li et al., 2008).

3.3.2. *Cell viability*

Eight studies investigated the effects of OLE on cell viability. To assess *in vitro* cell viability after treatment, the MTT assay was performed (Smith et al., 2001; Yang et al., 2009; Ma et al., 2015; Garofalo et al., 2017; Pan et al., 2017; Ko et al., 2018; Lin et al., 2020).

OLE reduced viability in all GBM human glioma cells (Garofalo et al., 2017), MDA-MB-231 and RT-R-MDA-MB-231 human breast cancer cells (Ko et al., 2018), MCF7 (luminal A subtype), SK-BR-3 (HER-2 + subtype) and MDA-MB-231 (triple negative breast cancer, TNBC) (Lin et al., 2020), in human U2OS and SaOS-2 osteosarcoma cells (Ma et al., 2015), human SW480 colon cancer cells (Pan et al., 2017), in human DU145 and PC3 prostate tumor cells (Smith et al., 2001) and human CaCO-2 colorectal cancer cells (Yang et al., 2014a, 2014b). In human lymphoma tumor cells U-937, HL-60, HeLa and MCF-7, OLE decreased cell viability in a dose-dependent manner, but not in murine SP-2, J774, C2C12, P338D1 and NIH3T3 cells (Raghavendra et al., 2007).

The reduction of cell viability occurred at different concentrations of OLE (Smith et al., 2001; Yang et al., 2014a, 2014b; Ma et al., 2015; Pan et al., 2017; Ko et al., 2018; Li et al., 2020) and even at lower doses (Garofalo et al., 2017) as shown in Table 1.

3.3.3. *Apoptosis and/or cell cycle arrest*

Ten studies presented results on apoptosis and/or cell cycle arrest in cancer cells (Bao et al., 2016; Garofalo et al., 2017; Li et al., 2008, 2020; Ma et al., 2015; Newman et al., 2007; Pan et al., 2017; Pathak et al., 2000; Raghavendra et al., 2007; Yang et al., 2014b). The analysis method to investigate apoptosis and cell cycle arrest was flow cytometry (Bao et al., 2016; Garofalo et al., 2017; Li et al., 2008, 2020; Ma et al., 2015; Pan et al., 2017; Pathak et al., 2000; Raghavendra et al., 2007; Yang et al., 2014b) and DNA flowmetry analysis (Newman et al., 2007).

Apoptosis was induced in A549 human lung carcinoma cells (Bao et al., 2016), U87MG glioma cells (Garofalo et al., 2017), non-triple negative MCF7 and triple negative MDA-MB-231 breast cancer cells (Li et al., 2020), U2OS and SaOS-2 human osteosarcoma cells (Ma et al., 2015), PC-3M human prostate cancer (Pathak et al., 2000) and canine melanoma cell line (CML-1) (Pathak et al., 2000), at different concentrations of OLE as shown in Table 3.

Only no OLE-induced cell death occurred for human pancreatic cancer cells PANC-1, metastatic cells in murine melanoma K1735-X21 and murine lymphoma cells NIH3T3, C2C12 and P338D1 (Newman et al., 2007; Pathak et al., 2000; Raghavendra et al., 2007).

3.4. In vivo *studies*

The potential antitumor activity of OLE was tested in two animal studies involving U251, GBM19 human and murine GL261 glioma cells (Garofalo et al., 2017) and in murine mammary carcinoma EMT6 (Li et al., 2021). One study has found that OLE reduced tumor growth in mice (Garofalo et al., 2017). The authors reported that the effects of OLE may be mediated by induction of apoptosis and reduction of tumor cell proliferation in human glioma, whereas in murine cells, the reduction of tumor growth does not depend on activation of apoptotic pathways (Garofalo et al., 2017).

According to these authors, the indirect effect of OLE is mediated in the brain parenchyma, where OLE stimulated the production and release of neurotrophin (brain-derived neurotrophic factor-BDNF) by neuronal cells, that plays a key role in glioma growth. OLE is dependent on this factor for modulation of its anticancer activity since there is reduction of neurotrophin (BNDF factor in mice) or silencing of TrKB receptors that abolished antitumor action of this cardiac glycoside. In addition, OLE reduced microglial infiltration, decreased astrogliosis, and counteracted tumor cell infiltration into the healthy parenchyma (Garofalo et al.,

Table 4
Characteristics of the studies included in the systematic review involving studies with experimental animal models.

Author/ Year/ Country	Type of cancer	Experimental model	Histopathological evaluation of tumor volume	Intervention			Outcome/Antitumor activity		Adverse Effects	Conflicts of interest	Ethical Approval	Quality of evidence
				Treatment	Dose (mg/kg)/ administration pathway	*Follow Up*	Tumor size	Cell proliferation and death				
Garofalo et al. (2017) [11] Italy	Glioma Human U251, GBM19 (5 × 10 5) or murine (syngeneic) GL261 (7.5 × 10 4) cells.	2-month-old male C57BL/6 (WT) murine (n = 6).	Intracranial glioma injection. Brains of murine with glioma were isolated and fixed in 4% buffered formaldehyde.	Oleandrin	0,03, 0,3 or 3 mg/kg/day Intravenous and oral pathway .	Every 2 days for a total of 4 times with a 2 week break.	U251, control: 0.34 ± 00.8 mm3, oleandrin: 0.07 ± 0.01 mm3, **p < 0.01 n = 5 GBM19, control: 22.6 ± 4.7 mm3, oleandrin: 4.9 ± 2.3 mm3, *p < 0.05 n = 5 Oleandrin doses below the lethal dose (0.3 mg/kg) significantly increased survival time from 32.6 ± 1.4 d to 53.8 ± 9.6 d in mice injected with U87MG cells (n = 5–11; **p < 0.01, log-rank test) and from 23.37 ± 1.2 d to 34.38 ± 3.3 d (n = 5–11; **p < 0.01.	Oleandrin significantly reduced the extent of BrdU positive cells and increased the percentage of cells positive for cleaved caspase 3 in the U87MG tumor mass (**p < 0.01, *p < 0.05; n = 4–8 mice). In mice with GL261 tumors, oleandrin treatment caused a reduction in BrdU-positive cells, with no changes in the level of cleaved caspase 3.	High concentrations of oleandrin (3 mg/ kg) were fatal in both models.	None	Yes	⊕⊕⊕◯ MODERATE
Li et al., 2021 [4] China	Breast cancer	Female BALB/ C murine (5 weeks old). 3 groups with 5 murine in each group.	EMT6 murine mammary carcinoma cells were implanted into mammary fat pads. After 7 days of administration, the mice were sacrificed and the tumors were weighed. Mouse tumor samples were fixed in 4% paraformaldehyde and embedded in paraffin.	Oleandrin	Groups of 0.3 mg/kg of oleandrin Groups of 0.6 mg/kg of oleandrin Control group Intraperitoneal pathway	7 days	The mean tumor size of 0.3 mg/kg of the treatment group remained unchanged compared to day 0, while the mean tumor size of 0.6 mg/kg of the treatment group was even smaller than on day 0 (p < 0.05, p < 0.01). The average tumor weight of the 0.6 mg/ kg treatment group was 1.58 times lower than that of the 0.3 mg/kg treatment group and was 2.66 times lower than that of the control group (p < 0.01).	–	–	None	Yes	⊕⊕⊕◯ MODERATE

2017).

When investigated, EMT6 murine mammary carcinoma in mice, OLE triggered inhibition the tumor growth with intraperitoneal administration at 0.3 and 0.6 mg/kg. The treatment increased the number of $CD45^{+}$ cells infiltrating the tumor, including DCsCD11c+, TCD4+ and TCD8+ cells. The increases in T-cell numbers were dose-dependent, especially in $CD8^{+}$ T-cells. The results suggested that OLE treatment induced immunogenic cell death and stimulated dendritic cell-mediated immune responses *in vivo* (Li et al., 2021).

3.5. Adverse events

High doses of intraperitoneal OLE (3 mg/kg) were fatal to mice with U251 glioma cells, human GBM19 and murine GL261 cells (Garofalo et al., 2017).

3.6. Quality assessment and risk of bias

The quality of the *in vitro* studies investigated by the GRADE tool indicated that in general they presented moderate and high quality, as indicated in Table 1. As for the risk of bias of the animal experiments (Fig. 2), both showed low risk of bias. They reported complete outcome data, all expected results were reported, and other biases were not stated. In addition, the animal studies were approved by the Animal Research Ethics Committee and did not declare conflicts of interest (Garofalo et al., 2017; Li et al., 2021) (Table 2).

4. Discussion

The treatment of diseases with synthetic drugs is ancient and this has increased the quality of life and longevity of the population, moreover, the advent of biotechnological drugs in recent decades has further enhanced the treatment of various diseases (Cirmi et al., 2017). However, the investigation of the therapeutic potential derived from nature, as in the case of plants, comes to meet the use of practices understood in alternative medicine in various parts of the world (WHO, 2013).

N. oleander is an ornamental plant that is distributed in tropical and subtropical regions of the planet. It is known for presenting cardiotoxicity derived from different cardioactive glycosides (GCs) such as oleandrin, present in all parts of the plant (Botelho et al., 2018). Recently many studies have reported OLE applicability to prevent progression against various types of cancer cells in experimental models of cell cultures (Bao et al., 2016; Garofalo et al., 2017; Ko et al., 2018; Li et al., 2008, 2020; Ma et al., 2015; Newman et al., 2007; Pan et al., 2017; Pathak et al., 2000; Raghavendra et al., 2007; Smith et al., 2001; Yang et al., 2009, 2014b) and *in vivo* models (Garofalo et al., 2017; Li et al., 2021).

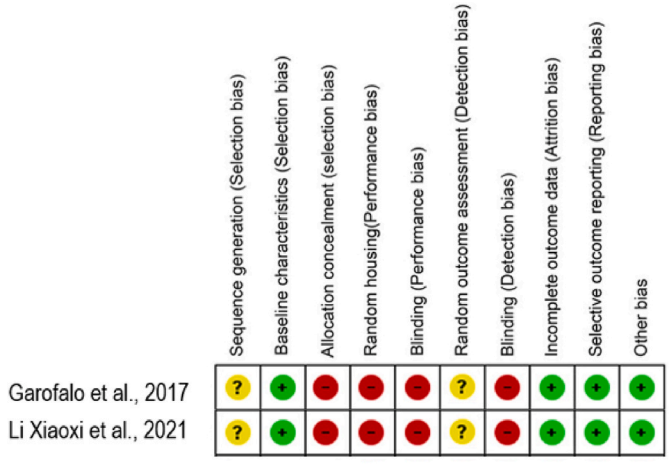

Fig. 2. Summary of the assessment of risk of bias for *in vivo* studies (animal).

To the best of our knowledge, this systematic review is the first to gather the data available in the literature on the antitumor effects of oleandrin on cell proliferation, cell viability and apoptosis *in vitro* and its effects on tumor size and volume in animal experiments. Data from 14 studies were included, of which 13 presented results from *in vitro* experiments, one *in vitro* and *in vivo* (in animals) (Garofalo et al., 2017) and one with only results from *in vivo* experiment (Li et al., 2021).

We found evidence that purified OLE at different concentrations was able to inhibit cell proliferation, decrease cell viability, and induce apoptosis and/or cell cycle arrest in different types of cancer such as lung cancer, glioma, glioblastoma, breast cancer, melanoma, osteosarcoma, pancreatic and colon tumor cells, prostate cancer, histiocytic lymphoma, colorectal cancer, human oral cancer, ovarian cancer, and human non-small lung cell cancer (Bao et al., 2016; Garofalo et al., 2017; Ko et al., 2018; Li et al., 2008, 2020Ma et al., 2015; Newman et al., 2007; Pan et al., 2017; Pathak et al., 2000; Raghavendra et al., 2007; Smith et al., 2001; Yang et al., 2009, 2014b). Furthermore, two *in vivo* experiments performed in mice indicated that OLE reduced tumor growth of human and murine glioma cells (Garofalo et al., 2017), as well as in murine breast cancer cell line EMT6 after grafting into BALB/c mice (Li et al., 2021).

In *in vivo* experiments, oleandrin significantly increased mice survival and reduced tumor growth, both directly on tumor cells and indirectly by promoting an antitumor brain microenvironment with a protective role played by brain-derived neurotrophic factor (Garofalo et al., 2017). In murine breast cancer cell line, oleandrin inhibited tumor growth and induced immune-mediated destruction by immunogenic cell death of breast cancer cells. The authors of this study indicated that oleandrin combined with immune checkpoint inhibitors may improve the efficacy of immunotherapy (Li et al., 2021).

In the *in vitro* experiments, OLE was investigated on different tumor cell types at different concentrations, given as microgram per milliliter and nanomolar concentrations. Different mechanisms of action were involved in inhibiting cell proliferation and apoptosis, and in decreasing cell viability. In human osteosarcoma cells, OLE exerted a strong antitumor effect by suppressing the Wnt/β-catenin signaling pathway, which interfered with cell proliferation and invasion, as well as induced apoptosis. In addition, the expression and activities of MMP-2 and MMP-9 were negatively regulated by OLE, which contributed to the decreased invasion of the cells (Ma et al., 2015).

In breast cancer, the effects of OLE can be attributed to inhibition of invasion through phospho-STAT-3-mediated pathways that are involved in the regulation of this process (Ko et al., 2018). Furthermore, oleandrin induced apoptosis of MDA-MB-231 and MCF7 cells after 24 h, mediated by mitochondria by activating endoplasmic reticulum stress, in breast cancer (Li et al., 2020).

In pancreatic tumor cells, transfection of active Akt into PANC-1 cells blocked oleandrin-mediated inhibition of cell growth. The authors suggested that this cell survival pathway is important in explaining, at least in part, which might lead to oleandrin-mediated tumor cell death (Newman et al., 2007). Furthermore, pancreatic cells exhibit differential sensitivities to oleandrin treatment. Rodent tumor cells express the α3 subunits of Na+/K + -ATPase that may serve as a novel target for cancer therapy (Yang et al., 2009). These findings confirm the importance of this subunit in the antiproliferative action of this cardioactive glycoside in pancreatic tumor cells.

In lung cancer, OLE at low concentration (0.02 μg/ml) can significantly induce A549 cell death related to DNA damage repair. Oleandrin may be a novel homologous recombination inhibitor by suppressing Rad51 expression thus preventing this repair and confirming its antitumor potential (Bao et al., 2016). It is worth noting that the studies, included in the present review, found dose dependent results except for histiocytic lymphoma tumor cells (Raghavendra et al., 2007).

In human lymphoma cells, but not mice cells, OLE interacted with the plasma membrane, altered its fluidity, inhibited Na+/K + -ATPase activity and increased the level of intracellular free Ca^{2+} followed by

calcineurin activity in human cells, but not in murine cells (Raghavendra et al., 2007). In prostate cancer, OLE inhibited FGF-2 export *in vitro* in a concentration-dependent manner and may therefore contribute to antitumor activity (Smith et al., 2001).

In human colorectal cancer cells, OLE induces apoptosis via the mitochondrial pathway (Pan et al., 2017). Furthermore, the antiproliferative activity of OLE is three times stronger in undifferentiated cells than in differentiated CaCO-2 cells (Yang et al., 2014b). These data demonstrate that the intracellular localization of the Na+/K + -ATPase α3 isoform is altered in human colorectal cancer compared to normal cells. The changes in cellular α3 localization and abundance may indicate a potential target for cancer therapy (Yang et al., 2014b).

Importantly, although it was not the aim of this study to investigate OLE *in vivo* experiments in humans, four clinical trials have tested the drugs Anvirzel and PBI-05204, an extract of *N. oleander* containing the cardiac glycoside OLE (Bidyasar et al., 2009; Hong et al., 2014; Mekhail et al., 2006; Roth et al., 2020). Both the results of the present review and the phase I and II clinical trials conducted so far for solid tumors suggest promising effects of OLE in antitumor activity.

Overall evaluation of the studies provides insight into OLE's possible pharmacological targets. OLE anticancer activity is multitargeted and ultimately associated with apoptosis, reduced cell growth, decrease of proliferation and cell viability (Kanwal et al., 2020). The most known effect is the inhibition of the NKA pump, that directly leads to increase intracellular Na+ and secondary increase in both H^+ and Ca^{2+}. Cell acidification stimulates apoptosis. (McConkey et al., 2000). Other apoptosis pathways are activated by oleandrin, including caspases and oxidative stress (McConkey et al., 2000**;** Newman et al., 2007**).** Studies also suggest that oleandrin provokes cells cycle arrest at G2/M, however the full mechanism is not yet confirmed (Newman et al., 2007; Pan et al., 2015).

All *in vitro* studies have important limitations, especially regarding the toxic potential of OLE towards non-targeted cells, a concern often associated with chemotherapy agents. OLE poisoning is often described, mostly due to ingestion of the plant parts by animals and children (Bandara et al., 2010). The main clinical signs are associated with it cardiotoxicity. The known cardiotoxic action of OLE is detrimental to its anti-tumor therapeutic potential, which has motivated not only research on cancer cells, but also on microorganisms in an attempt to clarify the still undistinguished mechanisms of toxicity of this substance (Ruta et al., 2020).

Known inhibition of NKA pump represents both a pharmacological target in cancer cells and a potential toxic site at the cardiomyocyte level. Disruption of Na^+ and K^+, leads to a severe electrochemical imbalance of the heart, ultimately causing disruptions of the action potential and mitochondrial activity (Botelho et al., 2019).

Arrhythmias and conduction disruption are mainly associated with faulty function of cardiomyocytes. Such altered cardiac cells with changes in mitochondrial morphology associated electrical deficiency and in the mechanical functions that together provide an adequate cellular support for the development of major cardiac arrhythmias, which is a limiting factor in the therapeutic use of OLE due to this cardiovascular toxicity (Botelho et al., 2017).

An *in vivo* investigation involving three GCs, including OLE at low doses (50 μg/kg) for 21 days, caused cardiovascular alterations that represent a restraining factor not only in the treatment of heart failure, but also in anti-cancer therapy (Botelho et al., 2020). The study characterized the arrhythmogenic potential of OLE in an experimental animal model and confirmed its major clinical limitation.

In order to determine whether human and veterinary cancer patients can benefit from the use of OLE as an anticancer agent, future studies should focus on OLE dosages and extract compositions that can confer effects on tumor biology without causing cardiotoxicity, which is the major limiting factor of this active ingredient (The Digitalis Investigation Group, 1997; Kanwal et al., 2020). Future studies should also, besides investigate the effects of OLE or extracts in combination with chemotherapies and targeted therapies, look for ways to mitigate the toxicity of this very promising cardiotonic glycoside for the treatment and cure of cancer.

5. Strengths and limitations

As a strength of this review, we highlight the realization of a comprehensive search, on the antimoral effects of purified OLE, in different types of cancer. In addition, our search was conducted without restriction on year of publication and there analyzed the quality of the studies. However, we consider that this systematic review has some limitations, such as not considering human clinical trials as well as studies with *N. oleander* extract. There was considerable heterogeneity in the included studies, noted by the different dosages and time of intervention with the application of oleandrin, so it was not possible to perform meta-analysis with the data presented.

6. Conclusions

Oleandrin, as a cytotoxic agent, has shown antitumor effects in different types of cancers, which encourages its development as a possible adjuvant agent in human clinical trials despite its dose-dependent cardiotoxicity. These results encourage further studies to investigate its therapeutic potential in increasing patient survival, its antimetastatic properties, as well as the decrease in tumor markers.

Credit author statement

Study concept:Cristiane Raquel Dias Francischini, Ana Flávia Machado Botelho; **Study design**: Cristiane Raquel Dias Francischini, Ana Flávia Machado Botelho, Carolina Rodrigues Mendonça.; **Literature Search**: Cristiane Raquel Dias Francischini, Carolina Rodrigues Mendonça; **Data extraction**: Cristiane Raquel Dias Francischini, Ana Flávia Machado Botelho; Carolina Rodrigues Mendonça; Kênia Alves Barcelos; **Data Analysis**: Cristiane Raquel Dias Francischini, Ana Flávia Machado Botelho; Carolina Rodrigues Mendonça, Kênia Alves Barcelos; Marco Augusto Machado Silva.

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This article contains no studies with human or animal participants conducted by any of the authors.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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