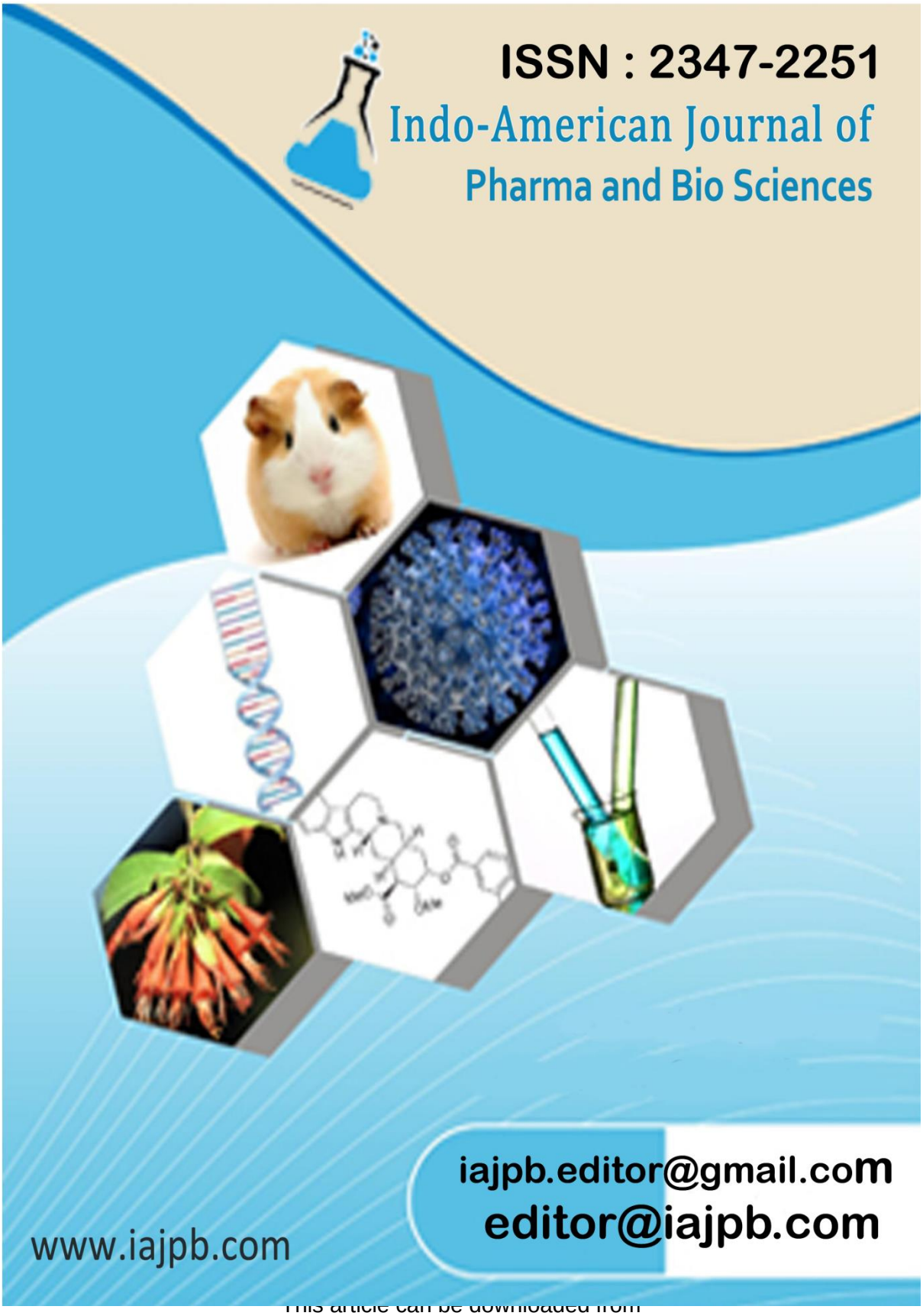


Indo-Am. J. Pharm & Bio. Sc., 2022

ISSN 2347-2251 www.iajpb.com
Vol. 10, Issuse 3, Sep 2022

Indo-American Journal of Pharma and Bio Sciences

Anticancer effect of Andrographolide in oral squamous cell carcinoma cell lines - A Systematic Review

Type of research: Systematic review

Running title: Anticancer effect of Andrographolide in oral squamous cell carcinoma cell lines

Aswani.E, Dr. Gheena.S, Dr.Pratibha Ramani, Dr. Monal B Yuwanti

STRUCTURED ABSTRACT

Background

Andrographolide, a plant extract of Andrographis paniculata has shown promising cytotoxic effects on various cancer in-vitro and animal studies. However, there is scarcity of evidence of its anticancer effect on oral squamous cell carcinoma. Hence, it is important to gather evidence aaavailable in literature.

Methods:

Comprehensive databases were used to retrieve the articles. Studies fulfilling the eligibility criteria were included. Due to heterogeneity in methodology, meta-analysis was not attempted. Quality assessment was carried out using ToxRTool, analyzing the toxic effect of agents.

Results:

Six studies have met the inclusion criteria. These studies demonstrated that andrographolide and its combination with silver lipid nanoparticles and cisplatin is important in inhibiting cell proliferation, cell cycle arrest, apoptosis and regulating proteins involved in carcinogenesis pathways.Hence, combination of andrographolide with nanoparticles shows better potential anticancer effect in OSCC cell lines.

CONCLUSION:

Andrographolide has shown anticancer effects in OSCC and lesser IC50 values which ultimately lead to inhibiting the proliferation of tumor cells. Hence, andrographolide with nanoparticles will exhibit better cytotoxicity in lower concentration.Further studies , combination with standard chemotherapeutic drugs should be explored to increase their cytotoxic potency.

Keywords: andrographolide, apoptosis, protein expression level, cell viability, cell survival, *in vivo*, *in vitro*, OSCC

Department of oral pathology
Saveetha Dental College and Hospitals,
Saveetha Institute of Medical and Technical Sciences,
Saveetha University
Chennai- 600077
Email id: aswaniuma029@gmail.com
Professor,
Department of oral pathology
Saveetha Dental College and Hospitals,

Saveetha Institute of Medical and Technical Sciences,
Saveetha University
Chennai- 600077
Email id:gheena@saveetha.com
Professor and Head,
Department of oral pathology,
Saveetha dental college and hospital,
Saveetha Institute of Medical and Technical Sciences,
Saveetha University,
Chennai-600077.
Email Id: pratibha@saveetha.com
Professor and Head,
Department of General pathology
Saveetha Dental College and Hospitals,
Saveetha Institute of Medical and Technical Sciences,
Saveetha University
Chennai- 600077
Email: monal9817@gmail.com

INTRODUCTION

Oral Squamous cell carcinoma (OSCC) is a major health concern in terms of morbidity and mortality[1]. It is the sixth most common cancer worldwide [1] [2]. These tumors primarily develop in individuals with a prolonged usage history of tobacco and alcohol [3]. Despite the evolving model of multimodality management, which integrates surgical intervention, chemotherapy, and radiation therapy, overall survival remains poor with a five-year relative survival rate below 50%[4],[5]. Currently, there is no effective anticancer drug to improve the survival rate [6]. Those available have various side and adverse effects and have little influence on survivability of oral cancer patients[7].

Over the last few decades, research has been focusing on developing anti-cancer drugs with better efficacy and potent tolerance. Various agents from traditional herbal plants have come to focus due to its antioxidant, anti- inflammatory properties [8]. The development of effective, non-toxic, and affordable novel pharmacological agents is an appealing strategy for preventing the early carcinogenesis and development of OSCC [8]. Notably, chemoprevention by phytochemicals is emerging as one such promising strategy to delay or block the carcinogenic processes of OSCC[9]. Recently, an herbal and non-toxic compound from herbal plants, andrographolide, have been identified as a novel and potential candidate for cancer treatment [10].

Andrographolide is extracted from the stem and leaves of Andrographis paniculata (Burm.f.) Nees, Andrographolide have antioxidant, anti-inflammatory, immunomodulatory, antiseptic,

antimicrobial, antiviral, cytotoxic, hypolipidemic, cardio-protective, hepato-protective, and neuroprotective effects[11] [12] [13].The previous studies have indicated the therapeutic role of andrographolide in human diseases such as neurodegeneration, Parkinson's disease and rheumatoid arthritis, intestinal inflammation and colitis, acute lung injuries, respiratory tract infection, diarrhea, fever and cold, and hand and foot, and mouth disease (HFMD) [14].

Andrographolide has shown cytotoxic effects against a number of cancer cell lines [15]. The andrographolide exerts its anti-cytotoxic effect through different mechanisms. It includes inhibition of JNK-signal transducers and activators of transcription, NF-kB and PI3K signaling pathways, suppression of cyclins and cyclin-dependent kinases (CDKs), metalloproteinase, growth factors and heat shock proteins (hsp-90), and the induction of tumor suppressor proteins p53 and p21[16],[17]. Andrographolide was selectively known to induce apoptosis of human cancer cells via the death-receptor-mediated apoptotic pathway[18].Andrographolide also sensitizes tumor necrosis factor (TNF)-related apoptosis-inducing ligands (TRAIL) to induced apoptosis in TRAIL-resistant human cancer cells [19]. In addition, Andrographolide affects the activity of CDK and induces the expression of cell-cycle inhibitor P27 for restraining tumor cells at G0/G1 [20]. Furthermore, Andrographolide inhibits tumor-induced angiogenesis by decreasing the expression of vascular endothelial growth factor (VEGF), nitric oxide (NO), and various inflammatory cytokines and chemokine while increasing the expression of endogenous anti-angiogenesis factors, such as interleukin (IL)-2 and tissue inhibitors of metalloproteinase [21]. However, its anticancer effect on oral squamous cell carcinoma cells have not been fully explored. This systematic review is intended to gather evidence on the anticancer effect of andrographolide in OSCC cell lines.

STRUCTURED QUESTION

Does andrographolide have an anticancer effect on oral squamous cell carcinoma cell lines?

MATERIALS AND METHODS

The systematic review was reported according to Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA), 2020 guidelines for systematic reviews. (Supplementary S1: PRISMA checklist)

PICO statement

For the present systematic review, following PICO (The population. Intervention, comparison, outcome) was formulated to find answers to questions.

- Population – OSCC Cell lines
- Intervention / Exposure – Andographolide with or without combination
- Comparator – compared with contro group Outcome – Cell viability rate, survival analysis, apoptosis rate

The inclusion criteria for studies were**;** 1. Studies on evaluating the anticancer effect of andrographolide on OSCC cell lines either alone or in combination and as compared with other drugs; 2.Studies in English language were included. Studies were excluded based on the following exclusion criteria; 1. Studies that did not treat oral cancer cell lines with andrographolide; 2. Studies done on cancer cell lines other than oral squamous cell carcinoma;

Search and Sources of Information

PubMed Central, Scopus, Web of Science and Google Scholar were searched to retrieve the relevant published papers. Search strategy was prepared using the keywords from MeSH (Medical Subject Headings) and free text (Supplementary file S1). In addition, an internet search was performed using the free words such as "anticancer activity", "andrographolide" and "Oral Squamous Cell Carcinoma cell lines". The Search results were exported into the reference manager software to remove duplicates.

Screening and Selection of Studies

The title of the papers were screened to identify relevant articles. In case eligibility cannot be ascertained from the title, papers were further evaluated by reading the abstract. Those papers that have fulfilled preliminary criteria were sought for full text retrieval. Two reviewers read the full text for finalization of these papers for inclusion into systematic review. All the papers relevant to research questions and have fulfilled eligibility criteria were included. Bibliography of all the identified studies were screened to find additional papers.

Quality assessment of studies

The studies include the in-vivo and in-vitro studies evaluating the cytotoxicity of andrographolide, a compound from plants. Quality assessment was carried out using the TOXR tool [22]. Studies were scored based on five categories; I: Test substance identification, II: Test system characterization, III: Study design description, IV: Study results documentation, V: Plausibility of study design and data. In total 21 questions for in vivo studies, 18 questions for in vitro studies were assigned. Each question was scored either as "0" or "1". If a criterion is met, 1 point was given. Total points range was 0-21, 0-18 for *in vivo* and *in vitro* study, respectively. Each study was rated based on total number points into one of reliability categorization. The categories were reliable without restrictions (in vivo study, 18-21; in vitro study, 15-18), reliable with restrictions (in vivo study, 13-17; in vitro study, 11-14), not reliable (in vivo study, <13; in vitro study, <11).

Data Collection

Two authors read the full text and extracted predefined data using the Microsoft excel sheet. The following information was extracted from the full text: author names, year of publication, country, study design, cell lines, type of assays, outcome measures (Cell viability/ survival rate, apoptosis rate).

Data were extracted from text, table, figure/ graph (using a digital screen ruler). In addition, authors of articles were contacted to obtain the missing/required data for the experiment.

Synthesis of summary findings

The findings of studies were summarized using descriptive statistics and are presented for each outcome. Quality assessment is presented in tabular format and judged overall as good, moderate and poor based on score. Due to variability and heterogeneity in methodology and material in included studies, meta-analysis was not conducted.

RESULTS

Search findings

The systematic search from the electronic databases of PubMed revealed 222 studies, Google Scholar revealed 3 studies. After removal of duplicates and title scan, 15 studies were identified. After abstract scanning 9 articles were eliminated as they did not meet the inclusion and exclusion criteria. Full text articles for the other 6 studies were obtained for more detailed evaluation (Figure 1: PRISMA Flow chart). The bibliography of these full text articles was scanned manually to include studies apart from the electronic databases. No relevant studies were found from the cross-reference. 6 studies met the inclusion and exclusion criteria of the systematic review.

Study characteristics

The Characteristics of the included studies are presented in Table 1 and 2. Included studies were published between 2011- 2016. 5 studies were from China whereas 1 study was from Taiwan. Three studies evaluated the effect of andrographolide in vitro as well as in vivo. Different cell lines were used in an in-vitro experiment. Only one study extracted the andrographolide from leaves, the rest used the commercially available andrographolide. These included CAL-27, HIOEC, Leuk1, HN6, HN30, SCC-25, HSC-2, HSC-3, HSC-4, Normal human oral cells, HGF, HPLF and HPC, tongue tumor cell (Tb), oral cancer stem cells (ADH+ & CD44+). Cell lines utilized were heterogeneous.Majority studies measured the cell viability and cell apoptosis using different assays (Table 2). Suzuki et al, Wang et al, Huang et al, Suzuki et al compared their efficacy with either saline or other drugs (cisplatin, cannabidiol, 5-FU). Chen et al and Li et al have evaluated andrographolide in combination with cannabidiol and Nano-particles, respectively. The included studies assessed the cell viability or survival using CCK 8, MTT assay, MTS assay, Alamar Blue assay, and Annexin V/7-AAD Binding Assay.

Anti-cancer effect of andrographolide

All studies showed that andropholide has anticancer efficacy mostly at higher concentration in oral squamous cell carcinoma cell lines.Further, andrographolide was found to increase the cytotoxicity effect in combination with DPP at lower concentration of IC50 values. However, andropholide alone at low

concentration does not have significant cytotoxic effect compared to the combination with nanoparticles. Li et al reported increased efficacy after addition of loaded solid lipid nanoparticles with andrographolide at low concentration compared to andrographolide alone which shows cell viability at 38.47 µg/ml and 300 µM concentration (IC50).

Cell Viability and Survival Assay:

Included studies evaluated the Cell viability after 24 hr, 48 hr and 72 hr of exposure to andrographolide. Cell viability in these studies ranged from 4 % to 115 %. Li et al observed that andrographolide with solid lipid nanoparticles in the concentration 11.74 µg/ml shows better cell viability compared to free andrographolide, which is of 19.98 µg/ml. Wang et al states that 200µm at 72 hr shows 40% of the cell viability rate. Yang et al and Suzuki et al observed that 50-µM concentration of andrographolide show about 40% cell survival rate in OCSC and HNSCC cell lines. Chen et al observed a 5% cell survival rate at 800nM concentration of control.

Apoptosis rate

Apoptosis rates reported in these studies ranged between 4.62% (HN6) and 54.40% (SCC-25). Apoptosis rate varied with the concentration of andrographolide (Table 2). When used with combination there increase apoptosis (10.39 % to 39 %). Chen et al reported the rate of apoptosis for combination of ADG-SLN is about 10.39% and 22.62% for HN6 and HN30 and with DPP (cisplatin) of about 47 % in CAL-27. In Wang et al study, the apoptosis rate was 39 %, and 49 % for the 150 µm concentration of andrographolide at 24 hr and 48 hr respectively. Yang et al also observed that cancer stem cell markers like ALDH and CD44 show 70% and 50% of apoptosis rate at 12.5 µm concentration of andrographolide.

Risk of bias across studies

The included studies were considered heterogeneous in terms of source of andrographolide, cell lines, assay, dose and concentration. Out of 6, 3 studies performed in vivo experiment, the quality assessment (ToxRtool) score were from 17 to 21. 5 studies performed in vitro experiment. The quality assessment score for the in vitro experiment was from 17 to 19. All studies were categorized as Reliable without Restrictions (Table 3).

DISCUSSION

The chemotherapeutic drugs have undesirable side effects in oral cancer patients and can develop drug resistance over the period. The development of effective, non-toxic, and affordable novel pharmacological agents is crucial for improving long-term survival and better outcomes. Several natural products and plant extracts were shortlisted as potential chemotherapeutic drugs. Andrographolide, a plant extract, was reported to have a potential cytotoxic effect on cancer cells.

The main advantages for using natural extract compounds were so effective in anticancer effect and even in

chemoprevention has been increased in recent years[23].Unfortunately, studies have shown diverse results.

The present systematic review analyzed the studies evaluating the effect of andrographolide in oral cancer cell lines. There are very few studies in literature that have evaluated the andrographolide inhibitory/anticancer activity in OSCC cell lines. The andrographolide has anticancer efficacy with certain limitations. It inhibits the OSCC in both *in vivo* and *in vitro* conditions. It is consistent with observation with studies conducted in other tumor cell lines.[20] According to Rajagopal S et al, andrographolide has inhibitory effects on different cancer cell lines at different concentrations/doses[20]. However, Chen et al study observed that the andrographolide alone did not have any cytotoxic effect on OSCC cells but increased the cytotoxic effect of DPP drug[24]. Similarly, Li et al observed that there is increased cytotoxic efficacy after it is combined with nano preparation[25]. This shows that there is potential cytotoxicity in andrographolide; it requires additional treatment with an external agent to be effective[25].The minimum inhibitory dose (IC50) of andrographolide for OSCC lines range between 38.47 μg/ml and 300 μM concentration, which can be lowered with help of nano formulation to as low as 0.337l μg/ml[25]. Andrographolide is inhibitory to several different tumor cell lines at 5–15 μM (Rajagopal S et al [20]. Similarly, andrographolide can inhibit several different OSCC cell lines as shown in included studies. This shows that andrographolide is effective in different oral cell lines and can be effective in OSCC irrespective of tumor site.

Most of the plant extract compounds like andrographolide illustrate minimal solubility and even worse bioavailability and so clinical implication gets reduced[23]. Henceforth, Nanotechnology provides surrogate access to conventional therapy that undergoes enhancement in competency and minimal toxicity[26]. According to Li et al ,nano lipids structure will enhance the bioavailability and efficacy of anticancer chemotherapy and prevention of drugs[25].

Andrographolide exerts its cytotoxic effects through several pathways that are observed in in vivo and in vitro studies. It includes decrease proliferation of cancer cells, increase IL-2 and IFN-c, decreasing tumor growth, cell cycle arrest at G2/M phase, decrease in phosphatidylinositol 3-kinase and NF-κB signaling pathways, suppression of hsp 90, cyclins, and cyclin-dependent kinases, MMPs and growth factors, increase tumor suppressor proteins p53 and p21[25],[24],[27],[28].

Andrographolide exposure to OSCC showed variable apoptosis rate, 4.62 % and 54.40 % at low and high concentration, respectively[25]. There is elevated expression p-AKT and p-p53 levels in CAL-27 cells after the andrographolide and/or DDP treatment resulting in apoptosis[24]. In Wang et al study, there was inhibition of aberrant NF-κB activation after andro treats chemically induced oral squamous cell carcinogenesis[27]. In Li et al study, anti-

proliferative effect was due to cell cycle arrest mainly in the G2/M phase[25]. Further, andrographolide can cause inhibition by increasing the radiosensitivity of OSCC cell lines through upregulation of miR-218, thereby reducing Bmi1 expression[28]. Downregulation of Akt and NF-kappaB activity is another mechanism through which andrographolide makes cancer cells susceptible to radiation[27],[24],[29].

The review has certain limitations as included studies have heterogeneous methodology. The included studies have variation in different drug dose/concentration, source of andrographolides, cell lines, and assays. In vivo studies have used different animal species such as mice and hamsters with different monitoring systems and maintained in different set-up[24],[27],[28]. These had reported outcomes using different parameters. Few studies reported cell viability while one study reported cytotoxicity rate[29]. As a result, it was possible to pool the outcome and do the meta-analysis. Cytotoxicity assays were different in included studies. MTT, CCK-8 assay were used, although both assays give the cell viability rate, these have different sensitivity and specificity which could influence outcome[30].

The present review clearly reveals that the field of nanoparticles and its technology has been part of carriers for the andrographolide to enhance its bioavailability and anticancer activity in oral squamous cell carcinoma cell lines.Novel clinical studies and trials will present a done in comprehensively to find the anticancer activity of andrographolide in patients with different types of cancer. Future studies on clinical trials will absolutely yield some light on the consequences of this compound in the enhancement of new chemotherapeutic and complementary strategies in the Oral Squamous Cell Carcinoma.

CONCLUSION:

The andrographolide extracts obtained from commonly used medicinal plants, Andrographis paniculata (Burm.f.) Nees, exhibit anticancer / cytotoxic effect in oral squamous cell carcinoma and can be developed as a potential anticancer agent. However, it is mostly at higher concentration or in combination with drugs or nano-material. Hence, future studies, especially clinical trials, are required to elucidate whether the plant extracts exhibit anticancer activity or cytotoxicity at low concentration.

REFERENCES

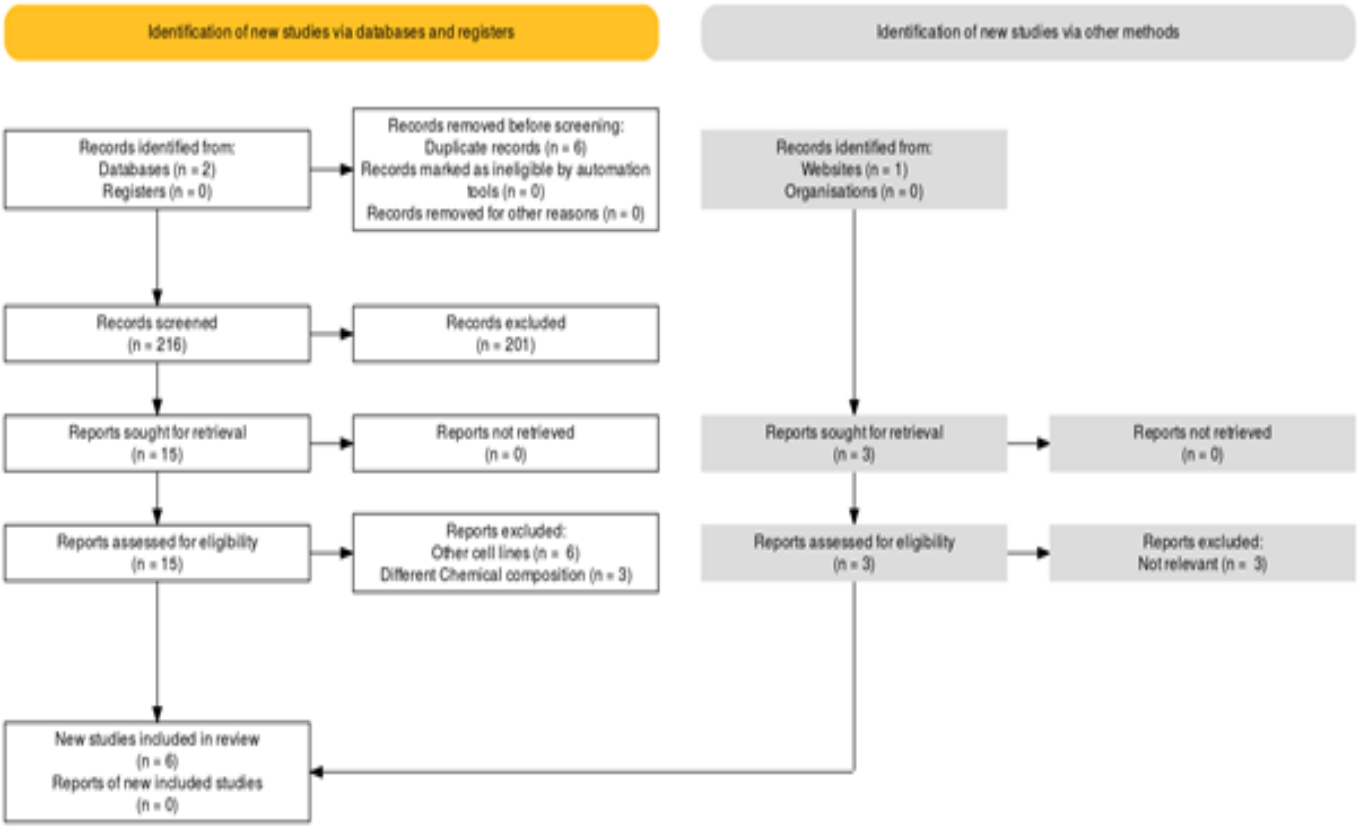
1. Bray F , Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018 Nov;68(6):394–424.
2. Borse V, Konwar AN, Buragohain P. Oral cancer diagnosis and perspectives in India. Sensors International. 2020 Jan 1;1:100046.
3. Coletta RD, Yeudall WA, Salo T. Grand Challenges in Oral Cancers. Front Oral Health 2020 Jun 9;1.https://www.frontiersin.org/article/10.3389/froh.2020.00003/full
4. Chi AC, Day TA, Neville BW. Oral cavity and oropharyngeal squamous

cell carcinoma-an update [Internet]. Vol. 65, CA: A Cancer Journal for Clinicians. 2015. p. 401–21. http://dx.doi.org/10.3322/caac.21293

5. Veluthattil AC, Sudha SP, Kandasamy S, Chakkalakkoombil SV. Effect of Hypofractionated, Palliative Radiotherapy on Quality of Life in Late-Stage Oral Cavity Cancer: A Prospective Clinical Trial. Indian J Palliat Care. 2019 Jul;25(3):383–90.
6. Nör JE, Gutkind JS. Head and Neck Cancer in the New Era of Precision Medicine. J Dent Res. 2018 Jun;97(6):601–2.
7. Dasari S, Bernard Tchounwou P. Cisplatin in cancer therapy: Molecular mechanisms of action. Eur J Pharmacol. 2014 Oct 5;740:364–78.
8. Marur S, Forastiere AA. Head and Neck Squamous Cell Carcinoma: Update on Epidemiology, Diagnosis, and Treatment. Mayo Clin Proc. 2016 Mar;91(3):386–96.
9. Mukhtar E, Mustafa Adhami V, Khan N, Mukhtar H. Apoptosis and Autophagy Induction As Mechanism of Cancer Prevention by Naturally Occurring Dietary Agents. Curr Drug Targets. 2012;13(14):1831–41.
10. Moura CFG de, de Moura CFG, Noguti J, de Jesus GPP, Ribeiro FAP, Garcia FA, et al. Polyphenols as a chemopreventive agent in oral carcinogenesis Vol. 22, European Journal of Cancer Prevention. 2013. p. 467–72. http://dx.doi.org/10.1097/cej.0b013e32835b6a94
11. Yuan H, Sun B, Gao F, Lan M. Synergistic anticancer effects of andrographolide and paclitaxel against A549 NSCLC cells. Pharm Biol. 2016 Nov;54(11):2629–35.
12. Lim JCW, -W. Lim JC, Goh FY, Sagineedu SR, -H. Yong AC, Sidik SM, et al. A semisynthetic diterpenoid lactone inhibits NF-κB signalling to ameliorate inflammation and airway hyperresponsiveness in a mouse asthma model Vol. 302, Toxicology and Applied Pharmacology. 2016. p. 10–22. http://dx.doi.org/10.1016/j.taap.2016.04.004
13. Wang J, Tan XF, Nguyen VS, Yang P, Zhou J, Gao M, et al. A Quantitative Chemical Proteomics Approach to Profile the Specific Cellular Targets of Andrographolide, a Promising Anticancer Agent That Suppresses Tumor Metastasis* . Vol. 13, Molecular & Cellular Proteomics. 2014. p. 876–86. http://dx.doi.org/10.1074/mcp.m113.029793
14. Guo W, Liu W, Chen G, Hong S, Qian C, Xie N, et al. Water-soluble andrographolide sulfonate exerts anti-sepsis action in mice through down-regulating p38 MAPK, STAT3 and NF-κB pathways. Int Immunopharmacol. 2012 Dec;14(4):613–9.
15. Parveen R, Ahmad FJ, Iqbal Z, Samim M, Ahmad S. Solid lipid nanoparticles of anticancer drug andrographolide: formulation,in vitroandin vivostudies . Vol. 40, Drug Development and Industrial Pharmacy. 2014. p. 1206–12. http://dx.doi.org/10.3109/03639045.2013.810636
16. Mishra SK, Tripathi S, Shukla A, Oh SH, Kim HM. Andrographolide and analogues in cancer prevention. Front Biosci . 2015 Jan 1;7:255–66.
17. Islam MT. Andrographolide, an up-coming multi-edged plant-derived sword in cancers. Asian J Ethnopharmacol Med Foods. 2016;2:1–3.
18. Zhou J, Zhang S, Ong CN, Shen HM.

Critical role of pro-apoptotic Bcl-2 family members in andrographolide-induced apoptosis in human cancer cells. Biochem Pharmacol. 2006 Jul 14;72(2):132–44.
19. Zhou J, Lu GD, Ong CS, Ong CN, Shen HM. Andrographolide sensitizes cancer cells to TRAIL-induced apoptosis via p53-mediated death receptor 4 up-regulation. Mol Cancer Ther. 2008 Jul;7(7):2170–80.
20. Rajagopal S, Ajaya Kumar R, Deevi DS, Satyanarayana C, Rajagopalan R. Andrographolide, a potential cancer therapeutic agent isolated from Andrographis paniculata . Vol. 3, Journal of Experimental Therapeutics and Oncology. 2003. p. 147–58. http://dx.doi.org/10.1046/j.1359-4117.2003.01090.x
21. Sheeja K, Guruvayoorappan C, Kuttan G. Antiangiogenic activity of Andrographis paniculata extract and andrographolide. Int Immunopharmacol. 2007 Feb;7(2):211–21.
22. Schneider K, Schwarz M, Burkholder I, Kopp-Schneider A, Edler L, Kinsner-Ovaskainen A, et al. "ToxRTool", a new tool to assess the reliability of toxicological data. Toxicol Lett. 2009 Sep 10;189(2):138–44.
23. Crooker K, Aliani R, Ananth M, Arnold L, Anant S, Thomas SM. A Review of Promising Natural Chemopreventive Agents for Head and Neck Cancer. Vol. 11, Cancer Prevention Research. 2018. p. 441–50. http://dx.doi.org/10.1158/1940-6207.capr-17-0419
24. Chen S, Hu H, Miao S, Zheng J, Xie Z, Zhao H. Anti-tumor effect of cisplatin in human oral squamous cell carcinoma was enhanced by andrographolide via upregulation of phospho-p53 in vitro and in vivo. Tumour Biol. 2017 May;39(5):1010428317705330.
25. Li H, Qu X, Qian W, Song Y, Wang C, Liu W. Andrographolide-loaded solid lipid nanoparticles enhance anti-cancer activity against head and neck cancer and precancerous cells. Oral Dis . 2020 Dec 9;(odi.13751). https://onlinelibrary.wiley.com/doi/10.1111/odi.13751
26. Pentenero M. Nanotechnology: a novel adjunctive aid to fight cancer [Internet]. Vol. 23, Oral Diseases. 2017. p. 273–5. http://dx.doi.org/10.1111/odi.12524
27. Wang LJ, Zhou X, Wang W, Tang F, Qi CL, Yang X, et al. Andrographolide inhibits oral squamous cell carcinogenesis through NF-κB inactivation. J Dent Res. 2011 Oct;90(10):1246–52.
28. Yang PY, Hsieh PL, Wang TH, Yu CC, Lu MY, Liao YW, et al. Andrographolide impedes cancer stemness and enhances radio-sensitivity in oral carcinomas via miR-218 activation. Oncotarget. 2017 Jan 17;8(3):4196–207.
29. Suzuki R, Matsushima Y, Okudaira N, Sakagami H, Shirataki Y. Cytotoxic Components Against Human Oral Squamous Cell Carcinoma Isolated from Andrographis paniculata. Anticancer Res. 2016 Nov;36(11):5931–5.
30. Jiao G, He X, Li X, Qiu J, Xu H, Zhang N, et al. Limitations of MTT and CCK-8 assay for evaluation of graphene cytotoxicity. RSC Adv. 2015 Jun 15;5(66):53240–4.

Figure 1-PRISMA Flow chart



Search strings (Supplementary file S2)

Search ((((((((((oral squamous cell carcinoma cell line) OR (oral squamous cell carcinoma cell lines)) OR (oral cancer cell lines)) OR (oral cancer cell line)) OR (metastatic oral squamous cell carcinoma cell line)) OR (metastatic oral squamous cell carcinoma cell lines)) OR (metastatic oral cancer cell lines)) OR (metastatic oral cancer cell line)) AND ((andrographolide) OR (andrographis paniculata))) AND ((((((((((((((anticancer activity) OR (anticancer activities)) OR (anticancer effect)) OR (anticancer effects)) OR (anti tumour activity)) OR (antitumor activities)) OR (anti tumour effects)) OR (antitumor effect)) OR (anti tumor activity)) OR (anti tumor activities)) OR (anti tumor effect)) OR (anti tumor effects)) OR (anti proliferative activity)) OR (anti proliferative activities)) OR (anti proliferative effect)) OR (anti proliferative effects)

Table 1: Characteristics of Included Studies

S. No	Author, Country	Study type	Comparator	Control	Study methodology	Parameter(S) studied	Anticancer effect direction

1	**Chen et al ,(2017), China**	**In-vitro study**	**Andrographolide**	**Andrographolide + DDP (cisplatin)**	**1.Cell lines and culture. 2.Cell survival assay (CCK 8 kit) 3.Assessment of cell morphology 4.Apoptosis detection assay 5.Flow cytometric analysis 6.Western blotting**	**1. Cell proliferation and apoptosis 2.p-AKT and p-p53 levels in CAL -27 cell**	**No significant cytotoxic effect when used alone**
2	**Yang et al (2017), China**	**In-vitro study**	**Andrographolide**	**Andrographolide + IR**	**1.MTT assay 2.Tumorsphere – forming assay 3.ALDH1 activity assay 4.RT-PCR Western blotting 6.Soft agar colony forming assay miR-218 Sponge.**	**1.Cell proliferation and self-renewal 2. Oncogenicity and radio sensitivity 3. Expression of miR-218 targeting Bmil**	**Significant cytotoxic effect**
3	**Suzuki et al (2016), Japan**	**In-vitro study**	**Andrographolide**	**Doxorubicin (DXR)**	**1.Cell culture 2.MTT assay 3.Western blotting analysis.**	**1.Cytotoxity effect of different compounds of andrographolide 2. Tumor specificity**	**High cytotoxicity and tumor specificity against oral squamous cell carcinoma.**
4	**Wang et al (2011), China**	**In-vitro study**	**4H-Andrographolide**	**Saline**	**1.Cell culture 2.Flow cytometric Analysis. 3.Immunoblotting**	**1.Cell viability 2.Cell proliferation 3.Cell apoptosis 4. Protein expression (p65, phosphorylated p65 (Ser536), and IκBα (Ser32/36), c-myc, cyclin D1)**	**Significant cytotoxic effect**
5	**Huang et al (2020), Taiwan**	**In-vitro study**	**CBD and Andrographolide**	**Cisplatin 5FU**	**1.Cell culture 2.Impedance Measurement by ECIS. 3.Alamar Blue assay. 4.Annexin V/AAD Binding Assay 5.Flow cytometric Analysis**	**1.Cell viability 2.Cell apoptosis**	**Significant cytotoxic effect at higher concentration**

6	**Li et al (2020), China**	**In-vitro study**	**Andrographolide**	**ALG-SLN**	**1.Nano-formulation 2.MTS assay 3.Flow cytometric Analysis 4. HPLC.**	**1.Cell viability 2.Cell cycle arrest 3.Cell apoptosis 4. Cellular uptake**	**No significant cytotoxic effect when used alone**

Table 2: Summation table for included studies

→Author, year	**Sources / Extraction Method**	**Cell line**	**Cell viability**	**Andro (IC50)**	**Andro in combination (IC50) µg/m**	**Apoptosis rate (%)**	**Cytotoxicity (CC50)**	**Tumor growth (gram)**	**Dose in-vivo (Animal model)**
Chen et al 2017	Andrographolide (Sigma-Aldrich) stock solution	CAL-27	48 Hr;	-	-	Andro Only = 18 % +DPP = 39 % Control(DDDP) = 4 %	-	Andro only (1.58 ± 0.18) + DDP (0.62 ± 0.10) Control (1.79 ± 0.20)	Andro (50 mg/ kg, once daily); DDP (5 mg/kg, once per week) combinatorial treatment

Li H et al	Free Andrographolide extracted from high performance liquid chromatography (HPLC)	HIOEC Leuk1 HN6 HN30	48 hr	38.47 µg/ml 26.14 µg/ml 13.75 µg/ml 19.98 µg/ml	0.337l µg/ml 0.717 µg/ml 6.087 µg/ml 11.74 µg/ml	Andro only 9.56% (HIOEC) 15.27% (Leuk1) 4.62% (HN6) 8.01% (HN30) ADG-SLN 12.20% (HIOEC) 23.91% (Leuk1) 10.39% (HN6) 22.62% (HN30)	-	-	-
Huang CC et al 2020	Andrographolide (Sigma-Aldrich) stock solution	SCC-25	24 hr	100 µM	-	Andrographolide (36.40%) Cannabidiol (100%) 5-FU (62.55%) Cisplatin (17.80%)	-	-	-
				300 µM	-	Andrographolide (54.40%) Cannabidiol (100%) 5-FU (70.30%) Cisplatin (64.95%)			
				-	-	Control (DMSO)-(4.40%)			

Suzuki et al 2016	Methanolic extract of leaves of A. paniculata	HSC-2 HSC-3, HSC-4 Normal human oral cells, HGF, HPLF and HPC	48 hr	-	-	-	8±1.0 (HSC-2) 7±1.5 (HSC-3) 10±4.0(HSC-4) 8 (Mean)	-	-
Wang et al (2011)	Andrographolide (Sigma-Aldrich) stock solution	Tb	48 hr	200 μM	-	49 % (Andro 100)* 18 % (Andro 150)*	NA	NA	NA
Yang et al (2016)	Andrographolide (Sigma-Aldrich) stock solution	oral cancer stem cells (ADH^+ & $CD44^+$)	24 hr	50 μM	-	-	-	-	-

* Data extrapolated from graphs/charts

Table: 3 – Quality assessment of selected studies

S.NO	Study	In vivo	In vitro	Reliability Categorization
1	Chen et al (2017)	20	17	Reliable Without Restrictions
2	Yang et al (2016)	17	19	Reliable Without Restrictions
3	Suzuki et al (2016)	-	17	Reliable Without Restrictions

4	Wang et al (2011)	21	17	Reliable Without Restrictions
5	Huang et al (2020)		17	Reliable Without Restrictions
6	Li et al (2020)		17	Reliable Without Restrictions