



Research Article

IN-VITRO STUDY ON THE MECHANISM OF ANTIPROLIFERATIVE AND APOPTOTIC EFFECT OF *TEEKSHNA APAMARGA KSHARA* ON ORAL SQUAMOUS CELL CARCINOMA (KB) CELLS

Keerthana P^{1*}, M.S. Deepa², Aneesh S³

¹MS Scholar, Department of Shalyatantra, Government Ayurveda Medical College Thiruvananthapuram.

²Professor and HOD, Department of Shalyatantra, Government Ayurveda Medical College, Kannur, Kerala.

³Professor and HOD, Department of Shalyatantra, Government Ayurveda Medical College, Tripunithura, Kerala.

Article info

Article History:

Received: 23-08-2026

Accepted: 14-09-2026

Published: 04-10-2026

KEYWORDS:

Apamarga Kshara,
Oral squamous cell
carcinoma,
Antiproliferative
activity, Apoptosis,
KB cells, ERK1.

ABSTRACT

Oral squamous cell carcinoma (OSCC) is the sixth most common malignancy worldwide, and India contributes nearly one-third of the global burden of oral cancer. *Apamarga* (*Achyranthes aspera*), a pan-tropical weed described in classical Ayurveda, is processed into *Kshara*, an alkaline preparation credited with *Ksharana* (excision, incision and scraping) properties capable of destroying morbid tissue while sparing normal cells. **Objectives:** To evaluate the anti-proliferative and apoptotic effect of *Teekshna Apamarga Kshara* on oral squamous cell carcinoma (KB) cells and to explore its underlying molecular mechanism. **Materials and Methods:** *Apamarga Kshara* was prepared as per classical reference and standardized physico-chemically. Its effect on KB cells was evaluated using the MTT assay for cell viability, Acridine Orange/Ethidium Bromide (AO/EtBr) dual fluorescent staining for apoptosis, flow cytometry for cell-cycle distribution, and quantitative real-time PCR (qRT-PCR) for expression of ERK1 and PCNA genes. **Results:** *Apamarga Kshara* produced a concentration-dependent decline in KB cell viability, from 91.44% at 6.25µg/mL to 58.90% at 100µg/mL, with an LC50 of 118.373µg/ml. AO/EtBr staining showed increased early and late apoptotic populations relative to control. Flow cytometry revealed an elevated S-phase fraction in treated cells (16.7%) compared to control (8.1%), indicating S-phase arrest. qRT-PCR showed downregulation of ERK1 (~47%) and PCNA (~15%) gene expression relative to control, confirmed by agarose gel electrophoresis. **Conclusion:** *Teekshna Apamarga Kshara* exerts a significant, concentration-dependent antiproliferative and apoptotic effect on KB oral carcinoma cells, plausibly mediated through suppression of the ERK1 pro-survival pathway and PCNA-dependent proliferation, supporting its potential as a botanical anticancer agent that warrants further mechanistic and in-vivo investigation.

INTRODUCTION

Cancer is recognized as one of the foremost causes of mortality across the globe, responsible for nearly 10 million deaths in 2020, corresponding to almost one in six deaths worldwide^[1]. In 2022, approximately 20 million individuals were newly diagnosed with cancer, while close to 9.7 million deaths were attributed to the disease globally^[2].

Oral cancer represents the sixth most prevalent malignancy worldwide, with India contributing nearly one-third of the global incidence and ranking as the second-highest country in terms of oral cancer burden^[3]. Among oral malignancies, oral squamous cell carcinoma (OSCC) accounts for the majority of cases and is commonly associated with potentially malignant disorders, regarded as clinically identifiable precancerous stages of oral cancer development^[4].

Cancer progression is characterized by a series of distinctive biological capabilities collectively termed the hallmarks of cancer, including sustained proliferative signalling, evasion of growth-inhibitory mechanisms, resistance to apoptotic cell death, limitless replicative capacity, angiogenesis, and tissue invasion and metastasis^[5]. Apoptosis, distinguishable

Access this article online

Quick Response Code



<https://doi.org/10.47070/ijapr.v14i9.4414>

Published by Mahadev Publications (Regd.)
publication licensed under a Creative Commons
Attribution-NonCommercial-ShareAlike 4.0
International (CC BY-NC-SA 4.0)

from necrosis by characteristic morphological and biochemical changes such as chromatin condensation and fragmentation, cytoplasmic shrinkage, and loss of membrane asymmetry, is frequently lost in cancer cells^[6]. Since cancer arises when cells proliferate uncontrollably while evading apoptosis, exploiting the cell's intrinsic death machinery to promote apoptosis represents a rational and effective therapeutic strategy.

Both surgical excision and chemotherapy affect normal cells along with malignant cells, are frequently expensive, and are unsuitable for cancer prevention. Plant-derived agents with selective anticancer activity and minimal toxicity to normal cells therefore represent an attractive alternative. *Apamarga* (*Achyranthes aspera*, family *Amaranthaceae*) is a pan-tropical weed found in abundance, containing *Kshara* as its principal active preparation. *Kshara* are alkaline, hygroscopic substances obtained from the water-soluble ash of plant-derived drugs and are traditionally considered among the most potent of the sharp (*Shastra*) and para-surgical (*Anushastra*) instruments in Ayurveda by virtue of their *Ksharana* quality, which imparts excision, incision and scraping (*Chedana*, *Bhedana*, *Lekhana*) effects capable of destroying or desiccating malignant tissue, while simultaneously eliminating morbid *Doshas* from the site of the lesion. Prior studies have reported anticancer and cytotoxic activity of *Apamarga Kshara* against cervical cancer cells^[7] and of *Paneeeya Kshara* against colon cancer cell lines^[8], along with documented clinical utility of *Apamarga Kshara* in gynecological conditions such as *Karnini Yonivyapada*^[9] and in oral mucocele as *Teekshna Pratisaraniya Kshara*^[10]. *Achyranthes aspera* extracts have also demonstrated specific inhibitory activity against pancreatic cancer cells^[11], collectively providing a rationale for evaluating its effect against oral cancer.

Based on this background, the present study was designed to evaluate the mechanism of the antiproliferative and apoptotic effect of *Teekshna Apamarga Kshara* on oral squamous cell carcinoma (KB) cells using an in-vitro experimental approach.

MATERIALS AND METHODS

The study comprised four major components: (1) collection and identification of the plant drug, (2) preparation of *Apamarga Kshara* as per classical textual reference, (3) physicochemical standardization of the prepared *Kshara*, and (4) experimental evaluation of the effect of *Apamarga Kshara* on KB cells. The pharmacognostic and standardization work was carried out at the Drug Standardization Unit (DSU), Government Ayurveda College, Thiruvananthapuram, and the cell-line based

experimental study was conducted at the Biogenix Research Centre, Thirumala, Thiruvananthapuram.

Collection and authentication of plant material

Fresh, mature *Achyranthes aspera panchanga* (whole plant) belonging to the family *Amaranthaceae* was collected for the study. Authentication of the plant material was carried out by experts in *Dravyaguna* based on its pharmacognostic features and physicochemical parameters, which were found to comply with the standards prescribed in the Ayurvedic Pharmacopoeia of India (API).

Preparation of Teekshna Apamarga Kshara

The collected plant material was shade/sun-dried for three weeks. Approximately 2kg of dried *Apamarga panchanga*, free of visible physical impurities, was subjected to complete combustion in an iron vessel under open-air conditions. Following complete incineration, approximately 119.5gm of white ash was obtained and allowed to cool. The ash was mixed with water to prepare *Ksharajala*; as per classical reference, six parts of water were used for one part of ash^[12]. The mixture was stirred thoroughly and left undisturbed overnight for sedimentation. The clear supernatant was decanted the following day and filtered through double-layered clean cotton cloth; this filtration was repeated approximately twenty times, and the final filtrate was collected in a stainless-steel vessel.

A 100ml aliquot of the filtrate was mixed with *Sudha* (lime water), while the remaining *Ksharajala* was boiled down to two-thirds of its original volume. The red-hot *Sudha-Ksharajala* mixture was then added to the reduced *Ksharajala* and boiled further until a semisolid consistency was attained, following which *Sanka Bhasma*, *Shukti Bhasma* and *Chitraka* powder (1 g each) were incorporated. The mixture was allowed to cool partially, transferred to another container, and left to sediment, from which *Teekshna Apamarga Kshara* was collected for use in the study.

Physicochemical standardization

Physicochemical parameters of the prepared *Kshara*, namely pH, loss on drying, total ash, water-soluble ash, and alcohol- and water-soluble extractive values, were determined as per the standards prescribed in the API.

Cell line and culture conditions

The KB human oral cancer cell line, procured from the National Centre for Cell Science (NCCS), was maintained in Dulbecco's Modified Eagle Medium (DMEM; Sigma-Aldrich) supplemented with 10% fetal bovine serum (FBS), L-glutamine, sodium bicarbonate (Merck), and an antibiotic mixture of penicillin (100 U/mL), streptomycin (100µg/mL) and amphotericin B (2.5µg/mL). Cultures were maintained at 37°C in a

humidified incubator with 5% CO₂ (NBS Eppendorf, Germany). For experiments, a two-day-old confluent monolayer was trypsinized and approximately 5×10^3 cells in 100 μ L of growth medium were seeded per well of a 96-well tissue culture plate.

Preparation of Test Compound

A stock solution of the test compound was prepared by dissolving 1mg of *Apamarga Kshara* in 1ml of 0.1% DMSO using a cyclomixer, followed by sterilization through a 0.22 μ m millipore syringe filter.

In-vitro anticancer assay

After 24 h of incubation, the growth medium was discarded and replaced with freshly prepared test compound at concentrations of 6.25, 12.5, 25, 50 and 100 μ g/mL in DMEM, each tested in triplicate, with untreated cells maintained simultaneously as controls. Cellular morphology was examined at 48 h and 72 h under an inverted phase-contrast microscope (Olympus CKX41 with Optika Pro5 CCD camera); rounding, shrinkage, cytoplasmic granulation and vacuolization were recorded as indicators of cytotoxicity.

Cell viability assessment (MTT assay)

Cell viability was quantified by the MTT assay^[13]. MTT reagent (15mg in 3ml phosphate-buffered saline, sterile-filtered) was added (30 μ L/well) to treated and control wells after the incubation period, followed by 4 h incubation at 37°C in 5% CO₂. The supernatant was discarded, and 100 μ L of DMSO was added to each well to dissolve the formazan crystals. Absorbance was measured at 540 nm using a microplate reader. Percentage viability was calculated as: % Viability = (Mean OD of sample / Mean OD of control) $\times$ 100. The LC₅₀ value was computed using ED50 Plus V1.0 software.

Determination of apoptosis by Acridine Orange/Ethidium Bromide (AO/EtBr) double staining

Morphological identification of apoptotic and necrotic cells was carried out using AO/EtBr (Sigma-Aldrich) dual fluorescent staining as described by Zhang et al.^[14]. These nucleic acid-binding dyes differentiate viable from non-viable cells on the basis of membrane integrity: AO penetrates both viable and non-viable cells, emitting green fluorescence on binding double-stranded DNA, whereas EtBr enters only cells with compromised membranes, producing red/orange fluorescence. KB cells were treated with the LC₅₀ concentration of the test sample (118.373 μ g/mL) for 24 h at 37°C in 5% CO₂, alongside

untreated controls. After treatment, cells were washed with cold PBS and stained with AO (100 μ g/mL) and EtBr (100 μ g/mL) for 10 minutes at room temperature, washed twice with $1 \times$ PBS, and examined under a fluorescence microscope with a blue filter (Olympus CKX41 with Optika Pro5 camera). Cells were categorized as viable (normal green nuclei), early apoptotic (bright green nuclei with chromatin condensation/fragmentation), late apoptotic (orange-stained nuclei with condensed/fragmented chromatin), or necrotic (uniformly orange-stained nuclei).

Cell cycle analysis by flow cytometry

Cell-cycle distribution was analysed using the MUSE Cell Cycle Kit, which is based on ethanol fixation and detergent permeabilization, allowing access of propidium iodide (PI) with RNase to intracellular DNA, thereby distinguishing cells in the G₀/G₁, S and G₂/M phases^[15]. KB cells were treated with the LC₅₀ concentration of the test sample (118.373 μ g/mL) for 24 h at 37°C in 5% CO₂, with untreated controls maintained in parallel. A minimum of 1×10^6 cells was centrifuged (3000 rpm, 5 min), washed with PBS, and fixed dropwise into ice-cold 70% ethanol with vortexing, followed by storage at -20°C. After overnight fixation, cells were centrifuged, washed with PBS, and incubated with 250 μ L of cell-cycle reagent in the dark for 30 minutes. Stained samples were analysed by flow cytometry, with gating referenced to untreated controls^[16].

Gene expression analysis by qRT-PCR

KB cells treated with the LC₅₀ concentration of the test sample (118.373 μ g/mL) for 24 h, along with untreated controls, were used for gene expression analysis. Total RNA was isolated from both groups using the TRIzol method (TRIzol reagent, Invitrogen; Product Code 10296010) as per the manufacturer's protocol^[17,18], involving cell lysis, chloroform-based phase separation, isopropanol precipitation, and washing with 75% ethanol, followed by resuspension in TE buffer. First-strand cDNA was synthesized from the isolated RNA using a cDNA Preparation Kit (G Biosciences; Product Codes 786-5019s and 786-5020)^[19], using an RNase-free reaction mix of RT Easy Mix (5 μ L), oligo-dT primer (0.5 μ L) and RNA template (2 μ L, equivalent to 0.5 μ g total RNA) made up to 10 μ L, run on a thermal cycler (Eppendorf Mastercycler) at 42°C for 20 minutes followed by 85°C for 5 minutes (enzyme inactivation), as summarized in Table 1.

Table 1: Thermal cycling conditions for cDNA synthesis

Step	Temperature (°C)	Time (minutes)	Number of cycles
cDNA synthesis	42	20	1
Enzyme inactivation	85	5	1

Gene expression was quantified by quantitative real-time PCR (qRT-PCR) using SYBR Green Master Mix (G Biosciences; Product Code 786-5062)^[20] on a LightCycler 96 instrument (Roche), with each reaction performed in triplicate. Expression data were analysed by the $\Delta\Delta C_t$ method using LightCycler 96 SW 1.1 software. Primer sequences for ERK1, PCNA, and the housekeeping gene GAPDH are given in Table 2, and the thermal cycling protocol is given in Table 3.

Table 2: Primer sequences used for qRT-PCR

Gene	Forward sequence (5'→3')	Tm (°C)	Reverse sequence (5'→3')	Tm (°C)
ERK1	TGGCAAGCACTACCTGGATCAG	62.4	GCAGAGACTGTAGGTAGTTTCGG	62.4
PCNA	AGACTTTCCTCCTTCCCGC	60.0	GCCTCCAACACCTTCTAGT	57.5
GAPDH	AATGCATCCTGCACCACCAACTGC	64.4	GGAGGCCATGTAGGCCATGAGGTC	67.8

Table 3: qRT-PCR thermal cycling protocol

Step	Time	Temperature	Number of cycles
Initial activation	2 minutes	95°C	1
Denaturation	10 seconds	95°C	40 (3-step cycling)
Annealing	1 minute	58°C	40 (3-step cycling)
Extension	1 min/kb	72°C	40 (3-step cycling)
Final hold	Indefinite	4°C	—

Agarose gel electrophoresis

PCR products were resolved on a 1.5% agarose gel prepared in 1× TE buffer containing ethidium bromide (6μl of 10mg/ml), cast and allowed to solidify. Electrophoresis was carried out at 50 V for 30 minutes, and the gel was visualized using a ChemiDoc Imaging System (Bio-Rad)^[21].

Statistical analysis

Experiments were performed in triplicate and results are expressed as Mean±Standard Error (SE). Statistical comparisons between treated and control groups were performed using one-way analysis of variance (ANOVA) followed by Dunnett's test, with $p < 0.0001$ considered statistically significant.

RESULTS

Yield and physicochemical standardization of Apamarga Kshara

Complete combustion of approximately 2kg of dried *Apamarga panchanga* yielded approximately 119.5gm of white ash, which was processed further to obtain *Teekshna Apamarga Kshara*. On physicochemical evaluation, the prepared *Kshara* showed a pH of 13.01 (API reference range 10–13), loss on drying of 0.5%, total ash value of 85.57%, water-soluble ash of 88.25%, alcohol-soluble extractive of 29.5%, and water-soluble extractive of 81.16% (Table 4). The alkaline pH within the API-specified range confirmed the characteristic alkaline nature of the prepared *Kshara*.

Table 4: Physicochemical parameters of Teekshna Apamarga Kshara

Parameter	Obtained value
pH (API range: 10–13)	13.01
Loss on drying (%)	0.5
Total ash (%)	85.57
Water-soluble ash (%)	88.25
Alcohol-soluble extractive (%)	29.5
Water-soluble extractive (%)	81.16

Antiproliferative effect on KB cells (MTT assay)

Apamarga Kshara produced a progressive, concentration-dependent reduction in KB cell viability relative to the untreated control (set at 100%). Percentage viability declined from $91.46 \pm 1.10\%$ at $6.25\mu\text{g/ml}$ to $58.92 \pm 1.27\%$ at $100\mu\text{g/ml}$, with corresponding cytotoxicity increasing from approximately 8.56% to 41.10% over the same concentration range (Table 5). The LC₅₀ value, computed using ED₅₀ Plus V1.0 software, was $118.373\mu\text{g/ml}$, indicating moderate cytotoxic potential of *Apamarga Kshara* against KB cells. Low standard error values across triplicates confirmed the reproducibility of the observed dose-response relationship (one-way ANOVA followed by Dunnett's test, $p < 0.0001$ vs. control).

Table 5: Percentage viability of KB cells following treatment with *Apamarga Kshara* (MTT assay)

Concentration ($\mu\text{g/mL}$)	Mean % viability $\pm$ SE
Control	100.00 $\pm$ 0.00
6.25	91.46 $\pm$ 1.10
12.5	83.82 $\pm$ 0.88
25	76.14 $\pm$ 0.68
50	66.97 $\pm$ 1.36
100	58.92 $\pm$ 1.27

LC50 of *Apamarga Kshara* against KB cells: 118.373 $\mu\text{g/mL}$ (ED50 Plus V1.0 software).

Apoptosis assessment by AO/EtBr double staining

Control cells showed predominantly bright, round, intact green-fluorescing nuclei with well-preserved nuclear architecture and no evidence of chromatin condensation or fragmentation, confirming high baseline viability and the absence of staining-induced artefact. In contrast, cells treated with *Apamarga Kshara* displayed a heterogeneous population comprising viable cells (reduced in proportion relative to control), early apoptotic cells with bright green nuclei showing chromatin condensation, late apoptotic cells with orange-stained nuclei showing condensed or fragmented chromatin, and a minor population of necrotic cells with diffuse orange-red staining. The predominance of organized early and late apoptotic morphology over necrotic change indicated that *Apamarga Kshara* induced cell death predominantly through an apoptotic rather than necrotic pathway.

Cell cycle distribution (flow cytometry)

Flow cytometric DNA content analysis revealed a marked increase in the S-phase fraction of *Apamarga Kshara*-treated KB cells (16.7%) compared with untreated control cells (8.1%)- more than double that of the control- accompanied by a reduction in the G0/G1 fraction (54.9% in treated cells vs. 65.2% in control) and a modest decrease in the G2/M fraction (19.2% in treated cells vs. 21.6% in control) (Table 6). This pattern is consistent with S-phase arrest secondary to disruption of the G1/S checkpoint.

Table 6: Cell cycle distribution of control and *Apamarga Kshara*-treated KB cells

Cell cycle phase	Control (% gated)	<i>Apamarga Kshara</i> -treated (% gated)
G0/G1	65.2	54.9
S	8.1	16.7
G2/M	21.6	19.2

Gene expression analysis (qRT-PCR and agarose gel electrophoresis)

Relative to untreated control (fold change set at 1.0), *Apamarga Kshara*-treated KB cells showed approximately 47% downregulation of ERK1 (Extracellular Signal-Regulated Kinase 1) gene expression. PCNA (Proliferating Cell Nuclear Antigen) expression was also downregulated in the treated group, with a fold-change value of approximately 0.85 relative to control, corresponding to an approximately 15% reduction. These findings were corroborated by 1.5% agarose gel electrophoresis of the RT-PCR products, which showed visibly reduced ERK1 band intensity and a markedly diminished PCNA band in the treated sample compared with the bright, intense bands seen in the control, with PCNA showing more pronounced suppression than ERK1; GAPDH served as the housekeeping control.

Summary of findings

Collectively, these results demonstrate that *Apamarga Kshara* exerts a significant, concentration-dependent antiproliferative effect against KB oral carcinoma cells. Convergent evidence from MTT cytotoxicity profiling (LC50=118.373 $\mu\text{g/mL}$), AO/EtBr fluorescence microscopy-confirmed apoptosis, S-phase cell cycle arrest, and transcriptional downregulation of ERK1 and PCNA supports a multi-mechanistic antiproliferative activity of the drug.

DISCUSSION

Classical Ayurvedic literature does not describe cancer as a distinct clinical entity; references comparable to malignancy are scattered across descriptions of other disease conditions. Systematic screening of botanicals for anticancer activity is therefore essential, and nearly 3,000 herbs have been screened for such activity to date^[22]. Although conventional cancer therapy continues to advance, the toxicity of chemotherapeutic agents towards normal tissue remains a major concern, prompting sustained interest in the development of selectively cytotoxic, plant-derived agents. Oral cancer, being a leading cause of cancer-related mortality worldwide, warrants effective and targeted therapeutic interventions, and

Ayurvedic formulations such as *Apamarga Kshara* have attracted growing scientific attention in this context.

Anti-proliferative and apoptotic activities are two of the principal hallmarks of cancer targeted by anticancer agents, and cell-line-based assays of viability, apoptosis and cell-cycle distribution provide a useful preliminary framework for their evaluation. The MTT assay, a gold-standard colorimetric method that measures mitochondrial activity as an indicator of cellular metabolic activity and viability^[13], clearly demonstrated that *Apamarga Kshara* exerts a significant, concentration-dependent antiproliferative effect on KB cells. The inverse relationship between concentration and viability observed in this study is indicative of classical dose-response cytotoxicity consistent with the behaviour of established chemotherapeutic agents, and this dose-response pattern is characteristic of a genuine cytotoxic interaction between the test sample and cancer cells, rather than a non-specific or cytostatic effect^[23], also enabling determination of the LC50 for dose selection.

The LC50 of *Apamarga Kshara* against KB cells (118.373 µg/mL) places it in the category of moderate cytotoxic activity, suggesting that although the formulation does not exhibit potent cytotoxicity at lower concentrations, its biological effect is real, measurable and dose-dependent. Such moderate activity is not uncommon for Ayurvedic herbal or herbo-mineral formulations, which often act through multi-target mechanisms rather than single high-potency cytotoxicity^[24].

The AO/EtBr dual fluorescent staining method exploits the differential membrane permeability of the two dyes to categorize cell death modalities^[25]: AO is cell-permeant and stains the DNA of all cells green, whereas EtBr is membrane-impermeant and enters only cells with compromised membrane integrity, producing orange-red fluorescence that outcompetes AO emission, thereby allowing simultaneous identification of four distinct nuclear morphologies under fluorescence microscopy^[26]. The dose-dependent reduction in viable cell number, the increase in early and late apoptotic populations relative to control, the preservation of organized nuclear morphology in most dying cells (consistent with caspase-mediated apoptosis rather than random necrotic degradation), and the limited necrotic fraction together indicate that *Apamarga Kshara* induces cell death predominantly through apoptosis rather than through membrane-disrupting necrotic mechanisms^[27].

Flow cytometric DNA content analysis provides information on cell-cycle kinetics and proliferative activity with important biological implications^[28]. The marked increase in S-phase fraction in *Apamarga*

Kshara-treated cells relative to control may reflect S-phase arrest, wherein cells accumulate in S-phase because they are unable to complete DNA replication. The concomitant reduction in the G0/G1 compartment is consistent with either recruitment of cells out of G0/G1 into S-phase or loss of G1 checkpoint integrity due to treatment-induced dysregulation, and together with the rise in S-phase fraction, points towards disruption of the G1/S checkpoint. The modest decrease in the G2/M fraction suggests that the treatment does not preferentially induce G2/M arrest.

Both ERK1 signalling and PCNA are known to play key roles in regulating the S-phase of the cell cycle. Inhibition of the ERK/MAPK pathway impairs the G1-to-S-phase transition, leading to S-phase arrest and inhibition of proliferation^[29], while PCNA is essential for proper DNA replication, and disruption of its function can similarly interfere with replication and precipitate S-phase arrest^[30]. These considerations prompted evaluation of ERK1 and PCNA expression to determine whether their modulation contributed to the observed S-phase arrest. ERK1, encoded by the MAPK3 gene, is one of the most frequently dysregulated signalling nodes in human malignancy; under physiological conditions it is transiently activated by growth-factor stimulation and regulates proliferation, differentiation and survival, whereas constitutive or hyperactivated ERK1 signalling in cancer cells drives uncontrolled cell-cycle progression (particularly at the G1/S transition) and suppresses apoptotic machinery^[31]. The approximately 47% downregulation of ERK1 expression observed in *Apamarga Kshara*-treated cells relative to control provides molecular evidence that this traditional preparation exerts its anticancer effect, at least in part, through suppression of the RAS-RAF-MEK-ERK1 pro-survival and pro-proliferative signalling axis, mechanistically bridging the observed reduction in cell viability (MTT assay) and induction of apoptosis (AO/EtBr staining).

PCNA is a critical nuclear protein that functions as a sliding clamp for DNA polymerase delta, playing an indispensable role in DNA replication, repair, and cell-cycle progression, and is widely regarded as a reliable marker of cellular proliferation^[32]. The downregulation of PCNA expression observed in the present study, corroborated by the markedly diminished PCNA band on agarose gel electrophoresis, further supports suppression of proliferative activity in *Apamarga Kshara*-treated KB cells.

CONCLUSION

Teekshna Apamarga Kshara exerted a significant, concentration-dependent antiproliferative effect against KB oral squamous cell carcinoma cells. Convergent evidence from MTT cytotoxicity profiling

(LC50=118.373µg/mL), AO/EtBr fluorescence microscopy-confirmed apoptosis, flow cytometric demonstration of S-phase cell cycle arrest, and transcriptional downregulation of ERK1 and PCNA collectively supports a multi-mechanistic antiproliferative and apoptotic activity, mediated at least in part through suppression of the ERK1 pro-survival signalling axis and PCNA-dependent proliferation. These findings provide preliminary scientific validation for the traditional Ayurvedic use of *Apamarga Kshara*, by virtue of its *Ksharana* property, in destroying malignant tissue, and support its translational potential as a botanical anticancer agent. Further in-vivo studies, protein-level validation, and clinical trials are warranted to establish its therapeutic efficacy, safety profile, and dosage standardization for clinical application.

REFERENCES

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global cancer statistics 2020.
2. Sung H, et al. Global cancer statistics 2022.
3. Rising trend of oral cancers in India: a comprehensive review. Indian J Res Med Sci. 2025 Sep 16.
4. Warnakulasuriya S. Global epidemiology of oral and oropharyngeal cancer. Oral Oncol. 2009; 45(4-5): 309-316.
5. Hanahan D, Weinberg RA. Hallmarks of Cancer: The Next Generation. Cell. 2011; 144(5): 646-674.
6. Elmore S. Apoptosis: a review of programmed cell death. Toxicol Pathol. 2007; 35(4): 495-516.
7. Mrudula KS. Efficacy of Apamarga Kshara in cervical cancer cells. International Ayurvedic Medical Journal. 2017 Jul; 5(7).
8. Gururaja D, et al. Evaluation of cytotoxic and anticancer activity of Paneeya Kshara on colon cancer cell lines. International Journal of Research in Ayurveda and Pharmacy. 2023; 14(2).
9. Dr. Priyanka Sharma & Dr. Sushila Sharma: A Clinical Study to Evaluate Efficacy of Apamarg Kshara & Dhatakyadi Taila Pichu in the Management of Karnini Yonivyapada w.s.r. to Cervical Erosion. International Ayurvedic medical Journal [online] 2016 [cited 2016 July] Available from: http://www.iamj.in/posts/images/upload/2792_2798.pdf
10. Gayatri KB. A clinical study to evaluate the role of Kshara Karma in oral mucocoele with Teekshna Pratisaraniya Apamarga Kshara. Journal of Ayurveda and Integrated Medical Sciences. 2022 Jul; 7(5).
11. Subbarayan PR. Anti-proliferative and anti-cancer properties of *Achyranthes aspera*: specific inhibitory activity against pancreatic cancer cell. Journal of Ethnopharmacology. 2010 Aug 19; 131(1).
12. Suśruta. Suśrutasamhitā of Suśruta: with the Nibandhasaṅgraha commentary of Śrī Dalhaṇāchārya and the Nyāyacandrikā Pañjikā of Śrī Gayadāsāchārya on Nidānasthāna. Ācārya JT, Ācārya NR, editors. Varanasi: Chaukhamba Sanskrit Sansthan (Kashi Sanskrit Series; vol. 316). Sūtrasthāna, Chapter 11/11, p.46.
13. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods. 1983; 65(1-2): 55-63.
14. Zhang JH, Yu J, Li WX, Cheng CP. Evaluation of Mn²⁺ stimulated and Zn²⁺ inhibited apoptosis in rat corpus luteal cells by flow cytometry and fluorochromes staining. Chin J Physiol. 1998; 41(2): 121-6.
15. Jerard C, Michael BP, Chenicheri S, Vijayakumar N, Ramachandran R. Rosmarinic acid-rich fraction from *Mentha arvensis* synchronizes Bcl/Bax expression and induces G0/G1 arrest in hepatocarcinoma cells. Proc Natl Acad Sci India Sect A Phys Sci. 2020; 90(3): 515-22.
16. Mohan Latha Kumari A, Biju Latha C, Padmanabhan RA, Vijayakumar N, Ramachandran R. Antibacterial peptide nisin activates p53 gene expression and triggers apoptosis in human colon cancer cells. Pept Sci. 2024; [Epub ahead of print]: e24379.
17. Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem. 1987; 162(1): 156-159.
18. Invitrogen (Thermo Fisher Scientific). TRIzol™ Reagent User Guide [Publication No. MAN0001271]. Waltham (MA): Thermo Fisher Scientific; 2016.
19. G Biosciences. cDNA Preparation Kit: Instruction Manual [Product Codes: 786-5019s and 786-5020]. St. Louis (MO): G Biosciences.
20. G Biosciences. SYBR Green Master Mix [Product Code: 786-5062]: Instruction Manual. St. Louis (MO): G Biosciences.
21. Sambrook J, Russell DW. Molecular Cloning: A Laboratory Manual. 3rd ed. Cold Spring Harbor (NY): Cold Spring Harbor Laboratory Press; 2001. Chapter 5, Gel electrophoresis of DNA and pulsed-field agarose gel electrophoresis; p. 5.1-5.73.
22. Boyd MR, Paull KD. Some practical considerations and applications of the National Cancer Institute in vitro anticancer drug discovery screen. Drug Development Research. 1995; 34(2): 91-109.
23. Mishra M, et al. The rationale of dose-response curves in selecting cancer drug candidates. Front Pharmacol. 2019; 10: 1-13.
24. Swerdlow JL, et al. Ayurveda and cancer. J Altern Complement Med. 2011; 17(4): 327-33.

25. Aithal BK, Kumar MR, Rao BN, Udupa N, Rao BS. Cytotoxicity and induction of apoptosis in melanoma (MDA-MB-231) cells by acridine orange/ethidium bromide staining. Indian J Pharm Sci. 2009; 71(5): 500-4.
26. Ponnusamy L, et al. Acridine orange/ethidium bromide (AO/EtBr) double staining for apoptosis detection. Bio Protoc. 2022; 12(11): e4434.
27. Kerr JF, Wyllie AH, Currie AR. Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. Br J Cancer. 1972; 26(4): 239-57.
28. Darzynkiewicz Z, Halicka HD, Zhao H. Analysis of cellular DNA content by flow and laser scanning cytometry. In: Robinson JP, editor. Current Protocols in Cytometry. New York: Wiley; 2000. p. 7.7.1-7.7.23.
29. Chambard JC, Lefloch R, Pouyssegur J, Lenormand P. The ERK1/2 mitogen-activated protein kinase pathway as a master regulator of the G1- to S-phase transition. Oncogene. 2007; 26(22): 3227-39.
30. Moldovan GL, Pfander B, Jentsch S. PCNA, the maestro of the replication fork. Cell. 2007; 129(4): 665-79.
31. Traverse S, Gomez N, Paterson H, Marshall C, Cohen P. Regulation of extracellular signal-regulated protein kinase-1 (ERK-1; pp44/mitogen-activated protein kinase) by epidermal growth factor and nerve growth factor in PC12 cells: implication of ERK1 inhibitory activities. Endocrinology. 1993; 132(6): 2578-85.
32. Maga G, Hubscher U. Proliferating cell nuclear antigen (PCNA): a dancer with many partners. Cell Mol Life Sci. 2003; 60(12): 2630-48.

Cite this article as:

Keerthana P, M.S. Deepa, Aneesh S. In-Vitro Study on the Mechanism of Antiproliferative and Apoptotic Effect of Teekshna Apamarga Kshara on Oral Squamous Cell Carcinoma (KB) Cells. International Journal of Ayurveda and Pharma Research. 2026;14(9):126-133.

<https://doi.org/10.47070/ijapr.v14i9.4414>

Source of support: Nil, Conflict of interest: None Declared

***Address for correspondence**

Dr. Keerthana P

MS Scholar,
Department of Shalyatantra,
Government Ayurveda Medical College,
Thiruvananthapuram., Kerala.
Email: dr.keerthanaps@gmail.com

Disclaimer: IJAPR is solely owned by Mahadev Publications - dedicated to publish quality research, while every effort has been taken to verify the accuracy of the content published in our Journal. IJAPR cannot accept any responsibility or liability for the articles content which are published. The views expressed in articles by our contributing authors are not necessarily those of IJAPR editor or editorial board members.