Research Article

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ANTICANCER PROPERTY OF AN AYURVEDIC POLYHERBAL PREPARATION CHIEFLY CONTAINING ARECA NUT AND BETEL LEAF

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ABSTRACT

Background: Areca nut, the nut of the areca palm (*Areca catechu* L.) and the leaf of betel vine (*Piper betle* L.) are the two inevitable ingredients of the common chewing mixture popularly known as betel quid. Both traditional and modern research findings proved that these two plant products have many medicinal values, including anticancer properties. With this information, we formulated a polyherbal preparation chiefly containing these two plant products and evaluated its anticancer efficacy on the specific cancer cell line. Methods: The polyherbal preparation called 'Pugasaram' was prepared using 12 different plant products, including areca nut and betel leaf, in different proportions. It was screened for its anticancer properties against A549 cancer cell lines. Results: The study showed a dose-dependent cytotoxicity of 'Pugasaram' on cultured A549 cancer cells with the IC_{50} value of 109.32 µg/ml. Conclusion: The present study reveals great potential for 'Pugasaram' to develop as an effective anticancer drug. This information could be the basis for further studies in this field.

Keywords: Pugasaram, areca nut, betel leaf, betel quid, anticancer, A549, cytotoxicity.

INTRODUCTION

Areca nut (the endosperm of the fruit of areca palm, *Areca catechu* L. of Palmae family) is called 'Puga' in Sanskrit, and betel leaf (the glossy heart-shaped leaf of betel vine, *Piper betle* L. of Piperaceae family) are the two ubiquitous ingredients of betel quid or 'tambula', the most common traditional type of chewing mixture used for mastication by nearly 600 million people worldwide[1]. Chewing such conventional forms of betel quid is reported beneficial to human health[2-5]. In India, the antiquity of chewing betel quid goes back to 650BC[6]. In certain other countries, such as Vietnam, the archaeological observations revealed the presence of betel quid stains on the human dentition of the Bronze Age[7].

Medicinal plants and their derivative phytochemicals are increasingly recognized and used as preferred complementary treatments against several human diseases. Such medicinal uses of both areca nut[8-10] and betel leaf[11-13] are systematically documented in scientific literature. Further, the anticancer properties of areca nut[14-17] and betel leaf[18-21] are well studied and reported. Taking this scientific information into consideration, an Ayurvedic preparation called 'Pugasaram' was formulated by using these two plant products as main ingredients, along with certain other plants and plant products having medicinal properties, and tested against the A549 cancer cell line *in vitro.* The results are presented and discussed in this report.

MATERIALS AND METHODS

'Pugasaram', a polyherbal preparation, was prepared using 12 different plant products, including areca nut (endosperm of unripe fresh fruit) and betel leaf (fresh green leaf) as the main ingredients, as shown in Table 1. The whole unripe endosperm of areca fruit and fresh betel leaf are macerated together using distilled water, strained, and the juice was obtained. Other herbs are powdered individually and mixed with the above juice, ground well and dried in the shade. After proper drying, the mixture is minced and macerated well with honey into a thick emulsion called 'Pugasaram'. The authentication of the medicinal plants was done at Jeddu Ayurveda Adhyayana evam Anusandhana Samstha, Alike - 574235, Dakshina Kannada, Karnataka, India.

The anticancer effect of 'Pugasaram' was assessed using Methyl Thiazolyl Tetrazolium (MTT) assay[22]. Human cancer cells (A549) were used in this study. The cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% Foetal Bovine Serum (FBS) and 1% antibiotic-antimycotic solution. Cells were maintained at 37 ^{0}C and 5% CO_2 in a humidified atmosphere throughout the experiment.

The cancer cells were seeded onto 96-well microtiter plates at a seeding density of 5000 cells / well. After adherence, they were treated with different concentrations of 'Pugasaram' (solubilised in DMEM) viz., 100, 200, 300, 400 and 500 µg/ml. Forty-eight hours post–incubation with 'Pugasaram', MTT reagent was added to each well and incubated at 37^{o}C for 4 hours. Formazan

crystals formed were solubilised using DMSO, and absorbance was recorded at 570 nm using a multimode microplate reader (FluoSTAR Omega, BMG Labtech). The anticancer effect of 'Pugasaram' was assessed based on the percentage of its toxicity on cancer cells.

Table 1: The constituents of 'Pugasaram'

Name in English/ Sanskrit	Scientific name	Part used	Quantity (%)
Areca nut/Puga	*Areca catechu* L.	Dried endosperm of unripe fruit	20
Betel leaf/Nagavalli	*Piper betle* L.	Dried leaf	20
Malabar plum/Bakula	*Mimusops elengi* L.	Dried bark	4
Turmeric/Haridra	*Curcuma longa* L.	Dried rhizome	2
Catechu/Khadira	*Acacia catechu* (L. f) Willd.	Dried bark	2
Long pepper/Pippali	*Piper longum* L.	Dried fruit	2
Snap ginger/Rasna	*Alpinia calcarata* Ros.	Dried rhizome	2
Clove/Lavanga	*Syzygium aromaticum* L.	Dried flower buds	2
Neem/Nimba	*Azadirachta indica* A. Juss.	Dried bark	2
Banyan tree/Vata	*Ficus bengalensis* L.	Dried bark	2
Pongam tree/Karanja	*Pongamia pinnata* (L.) Pierre	Dried bark	2
Honey/Madhu	From Apis *mellifera* L.	Honey	40
Distilled water	-	-	As required

Table 2: Cytotoxicity of 'Pugasaram' on A549 cancer cells after 48 hours of treatment

Concentration of 'Pugasaram' (µg/ml)	Cytotoxicity % (Mean ±SD)
100	37.29 ± 7.47
200	72.41 ± 2.18
300	74.58 ± 0.73
400	80.63 ± 3.00
500	85.64 ± 4.54

RESULTS

The study showed dose-dependent cytotoxicity of 'Pugasaram' on cultured A549 cancer cells (Table 2). At the lowest dose tested (100 µg/ml), the cytotoxicity was 37.29 ± 7.47%, whereas it was 72.41 ± 2.18% at 200 µg/ml, 74.58 ± 0.73% at 300 µg/ml, 80.63 ± 3.00% at 400 µg/ml and 85.64 ± 4.54 % at 500 µg/ml with the IC_{50} value of 109.32 µg/ml.

DISCUSSION

In the present study, the Ayurvedic formulation 'Pugasaram', prepared by incorporating areca nut and betel leaf as its main ingredients, showed effective cytotoxicity on cancer cells. This conforms with several other observations wherein it was reported that both areca nut and betel leaf exhibited anticancer properties.

In a study conducted *in vitro* on HSC-2 and HSC-3 human oral squamous carcinoma cell lines using the ethanol extract of areca nut, there was a significant increase in apoptosis on both these cancer cell lines after their exposure to areca nut extract for 24 to 48 hours[17]. The apoptosis assay in HSC-2 cells in 24 hours of exposure showed a significant increase of 83.82% in treated cultures compared to 15.54% in the untreated control. In HSC-3 cells, areca nut extract induced early apoptosis after 24 hours, but early and late apoptosis was markedly enhanced after 48 hours. The authors postulated that areca nut could be used as a chemotherapeutic agent against human oral squamous cell carcinoma. Areca nut extract was also reported to suppress the growth of human colon cancer WiDr cells[15]; MCF-7 breast cancer cells[23-24], gastric and liver cancer cells[25] etc.

Even in cancer-induced mice, the application of areca nut extract was reported to inhibit the growth of cancer[14]. In the experiments conducted by them on mice by topical application of 5 µg of a chemical carcinogen, benzpyrene (BP), three times a week showed tumour growth in all the treated animals (10/10) at the end of the 39th week, but when areca nut extract (0.1 ml of 2.0 % solution) was applied along with BP no such tumour (0/10) was observed until that period.

Arecoline, one of the active chemical components of areca nut, also exhibited anticancer properties. Exposure of this compound for 24 hours at concentrations ranging from 10 to 100 µg/ml inhibited the growth of basal cell carcinoma BCC-1/KMC cells in a dose-dependent manner but did not affect HaCaT cells[26]. It was reported that the arecoline hydrobromide (AH) inhibited the activity of the enzyme ACAT1(acetyl-COA acetyltransferase 1), leading to the attenuation of cancer cell proliferation and tumour growth in xenograft mice[27]. In the drug treatment experiments conducted by them through intraperitoneal administration of AH, there was a dose-dependent reduction in the size of tumours developed in H1299 xenograft mice and complete inhibition of tumour growth at the maximum tolerated dose of 50 mg/kg/day of AH treatment.

Apart from areca nut, betel leaf also exhibits anticancer activity against several cancers, including oral, fore-stomach, skin and breast cancers[28]. The LD_{50} value of betel leaf extract against colon cancer HT-29 cells was reported to be 98.44 µl/ml[29]. The hydroxychavicol (HC), the most abundant phenol compound (26.59%) in betel leaf, was mainly responsible for such activity[18]. HC was also reported to arrest the growth of oral KB carcinoma cells[30]. There was a remarkable inhibition of prostate tumour volume by 72% when HC was orally fed at a dose of 150 mg/kg BW daily for six weeks in xenograft mice[19]. In another study, administration of the methanol extract of betel leaf at 100 mg/kg BW for nine consecutive days to Ehrlich Ascites Carcinoma (EAC) bearing mice, there was a significant reduction in tumour burden, its volume, weight, viable tumour cell count and increase in the life span of such mice[20].

Reports say chewing the traditional betel quid or 'tambula' is a healthy practice[4, 31]. It was again confirmed in a population study conducted on chewers and non-chewers of the conventional form of betel quid (BQ), wherein it was noticed that among 292 BQ chewers, not a single instance of cancer was reported, whereas,

among 232 non-chewers of BQ, there were two such cases[5]. The present study conforms with these observations. Thus, the 'Poogasaram', which contains areca nut and betel leaf, could be effectively exploited as a chemo-preventive Ayurvedic preparation.

CONCLUSION

The present study revealed that the polyherbal preparation called 'Pugasaram', chiefly containing areca nut and betel leaf is an effective drug against the A549 cancer cell line with the IC_{50} value of 109.32 µg/ml. This shows ample scope for this Ayurvedic preparation to develop as an effective anticancer drug against other cancer cells. However, research on animal models is warranted to confirm this further.

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