IJBPAS, May, 2022, 11(5): 2172-2178

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

EFFICACY OF APRICOT KERNEL SEEDS AS AN ANTICANCER AGENT – AN INVITRO STUDY

MITHRA S, RAMANI P, RAMASUBRAMANIAN A, SUKUMARAN G AND RESHMA PK

1: Department of Oral and Maxillofacial Pathology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai, India

2: Professor & Head, Department of Oral and Maxillofacial Pathology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai, India

3: Reader, Department of Oral and Maxillofacial Pathology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai, India

4: Reader, Department of Oral and Maxillofacial Pathology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai, India

5: Senior Lecturer, Department of Oral and Maxillofacial Pathology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai, India

***Corresponding Author: Dr. Mithra Swornappan: E Mail: mithraswornappan@gmail.com**

Received 19th April 2021; Revised 18th May 2021; Accepted 3rd June. 2021; Available online 1st May 2022

https://doi.org/10.31032/IJBPAS/2022/11.5.5874

ABSTRACT

Background: The apricot kernel seeds contain amygdalin, which is considered as a naturally occurring anticancer agent. But recently it is debated that this compound does not positively have any anti-cancer effect. **Aim:** The aim of the study is to perform an in vitro study to assess the efficacy of apricot kernel seeds as an anticancer agent. **Materials and methods:** This study was conducted using KB cells procured from NCCS Pune and maintained in Duelbecco's Modified Eagle's medium. The cells were subcultures and were treated to apricot kernel seed extract at different concentrations 10, 50 and 100 µg/ mL; in different duration 24 h, 48 h and 72 hours and the cells were evaluated for the viability, anti-proliferative and cell cycle progression. **Result:** The cells treated at higher concentrations 100 µg/ mL of apricot kernel

seed extract for longer duration revealed a hindrance in the proliferative effect of the cell predominantly in the S-phase of the cell cycle with few cells losing its viability. At low concentrations of the extract at shorter duration, there was a noticeable stimulatory effect for proliferation of the cells. **Conclusion:** There is positive anti-proliferative effect in the cells treated with apricot kernel seed extract but the viability of the cells is still evident with only few cells becoming non-vital. So, this proves that apricot kernel seeds have anti-cancer effect and thus, the protocol for anti-cancer therapies can be standardized using apricot kernel seeds.

Keywords: Apricot kernel seed; Anti-cancer agent; In vitro study

INTRODUCTION

Apricot kernel is the seed of an apricot, which is located within the hard internal structure of the fruit. The apricot kernel seed is known to contain amygdalin compound. Amygdalin is a naturally occurring chemical compound which is also found in seeds of many plants such as bitter almonds, apple, peach, etc. **[1]**. Amygdalin is also a poisonous compound which is considered to have an anti cancerous effect. The apricot kernel also has polyphenols, such as gallic acid **[2]**. Standard therapies against cancer include chemotherapy, surgery and radiotherapy, but its efficacy is limited in many cases. A desirable anticancer drug should have greater efficacy and potency, which is almost non-existent **[3]**. There are lots of modern approaches has been made against cancer in recent decades but yet no constant approach has been standardized or proven to be effective. One such approach is using amygdalin from apricot kernel seed to prevent cancer. The amygdalin produces hydrogen cyanide which, when absorbed into the bloodstream, is transformed into cyanide. It is assumed that hydrogen cyanide kills cancer cells **[4, 5]**. There are not many evidences to prove the anticancer effect of apricot kernel seed, leaving it questionable still.

An in vitro study has been performed earlier to evaluate anti-proliferative effect of apricot and peach kernel extracts in human colon cancer cells. The authors have concluded that the mechanisms of cancer cell growth inhibition by amygdalin containing the apricot and peach kernel extracts is possible and may contribute towards the development of dietary anti-cancer therapies **[6]**. No other studies have been performed to evaluate the efficacy of anti-cancer effect primarily from the extract of apricot kernel seeds.

The aim of the study is to assess the efficacy of the apricot kernel seeds as an anticancer agent by performing an in vitro procedure.

MATERIALS AND METHODS

Subculture preparation:

The clearance for the study was obtained from the Institutional Ethical Committee. Buccal Keratinocyte (KB)cells were procured from NCCS Pune, Maharashtra and were maintained in Dulbecco's Modified Eagle's Medium, supplemented with 10% heat inactivated Fetal Bovine Serum (FBS) (GIBCO), penicillin (100 µg/mL) and streptomycin (100 µg/mL)(GIBCO). The KB cells were received in tissue culture flasks along with the growth medium. The viability of the cells was checked at the bottom of the flask. The maximum of the KB cells were present at the bottom of the tissue culture flask and very minimal cells were floating in the growth medium, which indicated that those cells were non-vital. Also using an inverted microscope, the flask has been checked for microbial contamination and no contamination was detected. Then the KB cells were grown and maintained in sterile petridishes at a 5% CO^2, 95% humidifiedair atmosphere at 37 °C for 24 hours.

Apricot kernel seed extract:

The raw apricot kernel seeds were purchased from online commercial store [Leeve Dry Fruits, India]. All kernels were grounded using a Stone Mortar and Pestle. The extract were obtained and blended with distilled water. The organic extract were achieved by evaporating the distilled water using a LABQUEST, HME500 at 55 °C to obtain a viscous oily liquid and stored at − 20°C. The kernel extract of weight 1 gram was made using 10 ml of distilled water; making the concentration as 10, 50 and 100 µg/mL solution was made using Deubelco's Modified Eagle's media. Then the organic extract was mixed gently and kept undisturbed for 2 hours.

KB cells and the apricot kernel seed extract:

KB cells were seeded in 96 well plates at 1000 cells/ well and incubated for 24 hours. The cells were exposed to the organic extracts at concentrations of 10, 50 and 100 µg/mL respectively for 24 h, 48 h and 72 hours. After incubation, the cells were observed under an inverted microscope for any morphological differences like clumping of cells, change in cell size, shape and number. Also the viability the KB cells werechecked using the fluorescent microscopy.

Fluorescent microscope:

On sterilised cover slips set in 6-well culture plates, cells were seeded. The cells were treated with specific organic extraction for 24h, 48h and 72h after incubation for 24h to allow attachment were stained with 1 µg/ mL Hoechst 33342 (Sigma) in medium for 30

minutes at 37^0c. Further to this, the cells were washed with PBS three times and placed in 20mM Tris (pH 8.8) and 90 percent glycerol using a solution of 0.5 percent p-phenylenediamine. Finally the cells were assessed using fluorescent microscope using a 450nm emission filter.

Flowcytometry analysis: Cell cycle

Cell cycles G1, S, G2 were investigated in KB cells utilizing propidium iodide to stain the core to decide the measure of DNA. Cells were gathered and made into a pellet by centrifugation at 3500 rpm/6 min. The supernatant poured off and the 3 mL of super cold 95% ethanol was added to each cylinder and were put away at − 20 °C short-term. The ethanol was eliminated by centrifugation at 3500 rpm/6 min and the cells washed with 1 mL PBS at 6 min spans. The silt was re-suspended in 1 mL hypotonic DNA staining cradle, Propidium Iodide (PI)/RNase [Roche], and put away at 4 °C for 30 min prior to perusing the specimens.For each example at any rate 10,000 occasions were gathered and amassed cells were gated out. The quantity of gated cells in the G1, G2/M and S-stage is addressed as %. (RNase arrangement: 250 mL refined water, 0.25 g Tri NaCitrate, 750 µL Triton X-100, 0.025 g PI and 0.005 g RNase A).

RESULTS

Cell morphology and viability:

The KB cells were initially assessed for the morphological changes using an inverted microscopy. More than half of the cells lost its original morphology. The cells treated with the apricot kernel seed extract were shrunken leading to decrease in size and shape of the cell and also there was evident irregularly shaped cells along with clumping of cells (Figure 1). The number of the cells was noticed decreased with time and the concentration of the apricot kernel seed extract to which it was exposed proving it to have considerable cytotoxic effect.

Cell proliferation:

The KB cell proliferation was noted to be inhibited at the end of 72 hours and especially with higher concentration of apricot kernel seed extract, 100 µg/ mL the cell proliferation was merely noted. The KB cells showed increase in stimulatory effect for cell proliferation at 10 and 50 µg/mL at the end of 24 and 48 hours; and 100 µg/ mL showed limited cell proliferative effect within 24 and with 48 hours the proliferation was noted in sparse amount.

Cell cycle phase:

The number of cells was noted more in the S-phase. After 24 h exposure, showed the most significant accumulation of cells in the S-phase. Similar increase in the S-phase cell

fraction was observed after the 48 h and 72 h exposure. The percentage values extractexposed cells in the G1 and S-phases vary greatly between the exposure time and concentration but amount of cells showing G1 phase was very minimal compared to the S phase.

DISCUSSION

The inhibition of cell multiplication and the acceptance of apoptosis could be a significant approach in chemotherapeutic treatment. Seeing how dietary segments control multiplication and cell endurance could assume a basic part being developed of new specialists that can forestall and treat malignancy with diminished danger of harmfulness. Ordinary chemotherapy needs fluid dissolvability, selectivity and because of its vague focusing of malignant growth cells, harms quickly multiplying typical cells **[6, 7]**.

This study results showed that inhibition of the cell proliferation and cell death was noted more in higher concentrations of the apricot kernel seed extract with increased time. Since the apricot kernel seed extract is a natural product with no added chemicals, it could be effective but might take little longer time to act. This is in accordance with the previous literatures. Wagheda Cassiem, *et al*. **[6]** have estimated the anti proliferative effect in HT-29 cells colon cancer cells when exposed to different types of apricot kernel extract and concluded possible mechanisms of growth inhibition by apricot kernel extract and may contribute towards the development of dietary anti-cancer therapies.

Our study results showed that majority of the viable cells were seen in the S-phase. This can be due to the temporary inhibition of the cell proliferation at S-phase and also it depends upon the time and the concentration of the apricot kernel seed extract to which the cells are exposed. The intra-S phase control point triggered by genotoxic insults causes only a temporary, reversible delay in the progression of the cell cycle, mainly by inhibiting the initiation of new replicas' and thus slowing down the replication of DNA, but not permanently stopping DNA replication **[6, 8]**. This is not in accordance with Wagheda Cassiem, *et al*. experiment **[6]** where they found that cell cycle progression revealed a significant G1 and G2 phase peak in the cells. Another study amygdalin promoted arrest of cell cycle in G0/G1 phase followed by decreasing number of S and G2/M phase cells and positively concluded saying amygdalin causes deceleration of cell cycle by blocking cell proliferation and growth **[9]**. This discrepancy between the studies could be because of the type of the

apricot kernel seeds used and also the differences in the concentration of the extract used.

Owing to a lack of clinical evidence about its efficacy, apricot kernel seeds are not approved as a cancer treatment by the FDA. There are more clinical trials needed to prove the efficacy, determination and quantification of apricot kernel seeds against the cancer cells.

CONCLUSION

Our study revealed that there were noticeable alterations in the morphology of the cell with minimal decrease in the amount of the cells treated with apricot kernel seed extract. The cells showed preponderance of inhibition of cell proliferation at 100 µL/ ml at 72 hour period with cells temporarily inhibited in S – phase. Many more studies should be done in order to explore the true genotoxicity, cytotoxicity, anti-proliferative, apoptotic and mitotic effect of apricot kernel seeds.

REFERENCES

[1] Considine, Douglas M. (2012). Foods and Food Production Encyclopedia. Springer Science & Business Media. p. 776. ISBN 978-1-4684-8511-0.

[2] Korekar G, Stobdan T, Arora R, Yadav A, Singh SB. (2011). Antioxidant capacity and phenolics content of apricot (*Prunus armeniaca* L.) kernel as a function of genotype. Plant Foods Hum Nutr.; 66(4): 376–83.

[3] Das AK. (2015). Anticancer Effect of Anti Malarial Artemisinin Compounds. Ann Med Health Sci Res.; 5(2): 93-102. doi:10.4103/2141-9248.153609

[4] Milazzo S, Horneber M (2015). "Laetrile treatment for cancer". Cochrane Database Syst Rev (Systematic review) (4): CD005476. doi:10.1002/14651858.CD005476.pub4.

[5] Laetrile/Amygdalin (PDQ) –Health Professional Version. https://www.cancer.gov/about-cancer/treatment/cam/patient/laetrile-pdq

[6] Wagheda Cassiem, Maryna de Kock. (2019). The anti-proliferative effect of apricot and peach kernel extracts on human colon cancer cells in vitro. Cassiem and Kock BMC Complementary and Alternative Medicine 19: 32. https://doi.org/10.1186/s12906-019-2437-4.

[7] Moorthi C, Manavalan R, Kathiresan K. (2011). Nanotherapeutics to overcome conventional cancer

chemotherapy limitations. J Pharm Pharm Sci.; 14(1): 67–77.

[8] Sjögren C, Nasmyth K (2001). Sister chromatid cohesion is required forpostreplicative double-strand break repair in *Saccharomyces cerevisiae*. Curr Biol.; 11(12): 991–5.

[9] Makarević, J. (2016). Amygdalin delays cell cycle progression and blocks growth of prostate cancer cells *in vitro. Life Sci.*, 147, 137-142.