Original Article

Cytotoxic activity of finger millet against the lung cancer cell line, A549

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Abstract:

Introduction: Millets are an important staple food in India. Studies showed Consumption of millets reduces the risk of developing cancer. Among the various millets, finger millet in one of the important ingredient in south Indian food. Therefore, this study aims to elucidate the cytotoxic activity of finger millet.

Methods: Methanolic extraction was carried out by cold percolation method and the secondary metabolite was quantified. Antioxidant activity was studied using DPPH assay. Cytotoxicity activity was studied using MTT assay and Ao/EtBr was performed to study the mechanism of cell death.

Results: Total polyphenols and flavonoids content was 156.68 µg/ml, and 50.24 µg/ml respectively. The DPPH radicle scavenging activity shows the Ic50 value of 35.70±0.45 µg/ml. MTT assay reveals, the Ic50 value to induce cell death in A549 cells is 65.66%. Early apoptotic and necrotic population was observed in treated cells when compared to the control.

Conclusion: This study showed the cytotoxic activity of the millet extract. Further study is warranted to explore the anticancer activity of the finger millet extract.

Key words: *Finger Millet, Lung cancer, Apoptosis, antioxidant activity.*

Introduction

Millets is an important food and mostly cultivated in the semi-arid and tropical regions. Cultivation of millets possess several advantages including pest resistance, short duration for cultivation and give best yield even in the unfavourable conditions like drought season (Devi et al., 2014). Among the various types of millets, Finger millet is the common food and it is also called as ragi. Most of the lower income people use this millet as the staple food (Singh et al., 2015). Finger millet (*Eleusine coracana L*) is widely cultivated in various parts of India and Africa. In India this millet ranks in the sixth position for cultivation and production. This millet has a red brick colour seed coat with naked caryopisis. This red brick seed coat contains various nutrients and also has polyphenols which possess anti-oxidant activity. High content of calcium in this millet than other commercially available calcium including milk and milk based products. Apart from calcium, this millet is also rich in other micro nutrients including Zinc and Magnesium (Anitha et al., 2021). Many studies were carried out using finger millet to check the nutritional status of the grain and very few bioactivity studies were carried out. Antioxidant activity, antimicrobial screening was carried out in this

finger millet extract (Xiang et al., 2019). Cytotoxicity was less studied using this millet and one study was carried out in liver cancer cell line (Singh et al., 2015). Therefore, the present study was carried out to descrie the cytotoxic property of the finger millet extract against the lung cancer cell line.

Lung cancer is widely observed cancer among the various types and it is diagnosed in both genders. The main risk factor for lung cancer is tobacco consumption and the minor risk factor includes intake of alcohol, pollution in air, occupational hazard etc (Poofery et al., 2020). Surgical procedure is the primary treatment to meet the initial stage of cancer but in the later stages, other treatment methods including chemotherapy, targeted therapy will be carried out (Matsuda et al., 2015). Cancer cells are able to proliferate rapidly by escaping the apoptosis process. These cells able to metastasize in to other organs and controlling the proliferation of cancer cells is the major problem in treatment (Sajid et al., 2019). Many studies showed phytoconstituents can able to hinder the cell proliferation by inhibiting the angiogenesis, metastasis and cell cycle progression (Swaffer et al., 2016). Therefore, screening of various plant may help to find the novel compound with potent cytotoxic activity against cancer cells. The present study showed the cytotoxic activity of finger millet methanolic extract in A549, lung adenocarcinoma cell line.

Methodology

Extraction:

Finger millet was procured from local shop, dried and fine powder was prepared. To 100 gm of the finger millet powder 500 ml of methanol was added and kept it in the shaker for 72 hours. After the filtration process, the sample was dried and stored at -20°c until further use.

Quantification of secondary metabolites in various fractions

Estimation of Total Polyphenol

Polyphenols content in the methanolic extract was estimated using Folin–Ciocalteau reagent. To 100 µl of the sample, Folin-Ciocalteau reagent (20 µl), 25% sodium carbonate (50µl) were added. To this reaction mixture, distilled water was added to make up to 1 ml. After incubating the samples at room temperature for one hour in dark conditions the absorbance was measured at 765 nm. Gallic acid was used as the standard. The total phenol content was expressed as mg of gallic acid equivalents (Jain AK *et al*., 2010).

Estimation of Total Flavonoids

The total flavonoid was estimated by standard protocol. To 50 µl of the sample 750 µl of ethanol (95 %), aluminium chloride (50 µl) and potassium acetate (50 µl) was added. To this reaction mixture, distilled water was added to make up to 3 ml. Incubate the reaction setup for 30 minutes at room temperature. Yellow colour was formed and the absorbance was measured at 415 nm. Quercitin was used as the standard. The total flavonoid content was expressed as mg of quercitin equivalents.

In-vitro antioxidant activity

Free radical scavenging activity

This activity was measured based on donating the hydrogen compounds present in the extract to remove the free radical. To 10 µl of the diverse concentration of samples (2-1000 µg/ml), add 190 µl of DPPH and keep it in the dark. After half an hour, absorbance was measured at 517 nm (Koleva II et al., 2002). Ascorbic acid was used as the control. The percent inhibition was calculated from the formula:

% Inhibition= [Absorbance of control-Absorbance of test sample / Absorbance of control] x 100

Cytotoxic activity

MTT assay

A549 cell line was purchased from National Centre for Cell Sciences (NCCS). The cells were maintained in DMEM medium supplemented with 5% FBS and 1% antibiotic mixture. The cells were incubated at 37°C with 5% $C^{o}2$. To perform the cytotoxic activity, $5x10^{3}$ cells were seeded in 96 well plate. The cells were incubated in the incubator to reach confluency. Once the cell reaches 80% confluency, different concentration of the methanolic extract was added and further incubated for 48 hours. To check the viability of the cells the incubated cells were treated with MTT and further incubated for 3 hours. after the incubation period, the content in the 96 well was removed and DMSO was added to dissolve the formazon crystals. Absorbance was measured at 570 nm. percentage of cell viability was calculated by % of cell viability= absorbance of control-absorbance of treated / absorbance of control * 100.

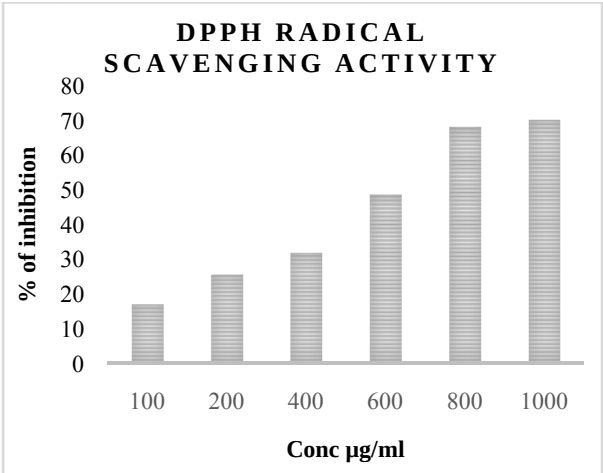
Acridine orange Ethidium Bromide staining (Ao/EtBr):

A549 cells were seeded in 6 well plate at the concentration of $5x10^{4}$ and after reaching confluency the cells were treated with 750 and 1000 µg/ml of the extract. The cells were incubated for 48 hours. after incubation, the media was aspirated and 10 µl of Ao and 10 µl of EtBr was added and observed in the fluorescence microscopy.

Results and discussion

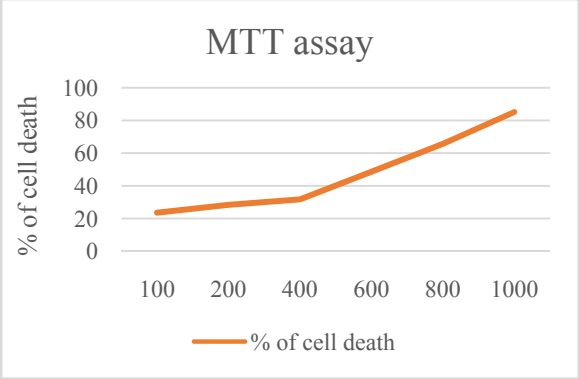
Secondary metabolites will not have any role in the growth but it is produced by the plant mainly for the defence purpose. These secondary metabolite are known to possess anti-oxidant property, anti cancer property. In the present study, the presence of poly phenols and flavonoid was quantified. In the methanolic extract, the total polyphenol content was 156.68 µg/ml, flavonoid content was 50.24 µg/ml. Previous studies also showed that finger millet have more polyphenols when compared to that of flavonoids (Rao and Muralikrishna, 2002).

To screen the anti-oxidant activity of the finger millet extract, DPPH free radical scavenging activity was done. It is the simple and popular method to determine the antioxidant activity of plant extracts. In this assay, a free radical DPPH will get converted to diphenylpicrylhydrazin which is dependent on the hydrogen donating ability of antioxidant compound. In the present study, extract showed the potent scavenging activity against DPPH with the IC_{50} value of 35.70±0.45 µg/ml and it may be due to the presence of high phenolic content (Graph 1). Previous studies also showed that seed coat extract of finger millet showed potent antioxidant activity. They also confirmed that the polyphenol content in the extract is mainly responsible for anti-microbial activity (Viswanath et al., 2009).



Graph 1: Antioxidant activity of the methanolic extract of finger millet.

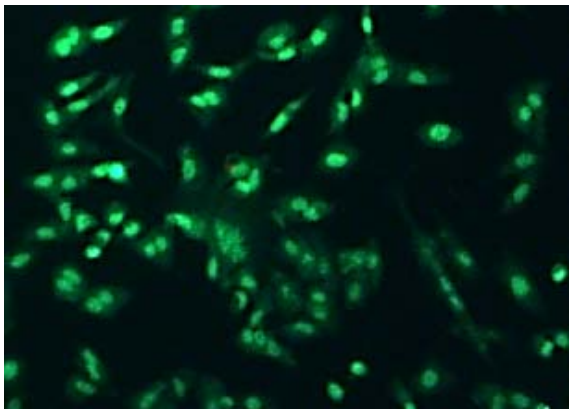
To study cytotoxic activity against lung cancer cell line, A549 was used. The MTT assay revealed that at the concentration of 750 µg/ml 65.66% and at the conc. of 1000 µg/ml, 85.16% of cell death was observed. (graph[2])



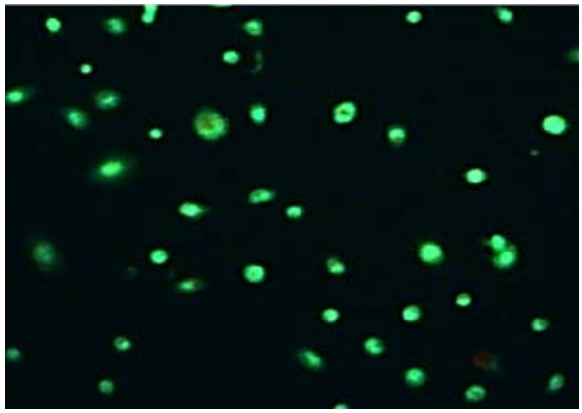
Graph2: Depicts the percentage of cell death in A549 cells treated with finger millet extract.

In the dual staining with acridine orange (A_O) and ethidium bromide (EtBr), no significant apoptotic morphology was observed in the control cells. Early apoptotic cells were observed by crescent shaped nuclei in all the treated group (Fig 1). In the extract treated group reduced cell density with late apoptotic morphology and necrotic cells (Orange colour) was observed.

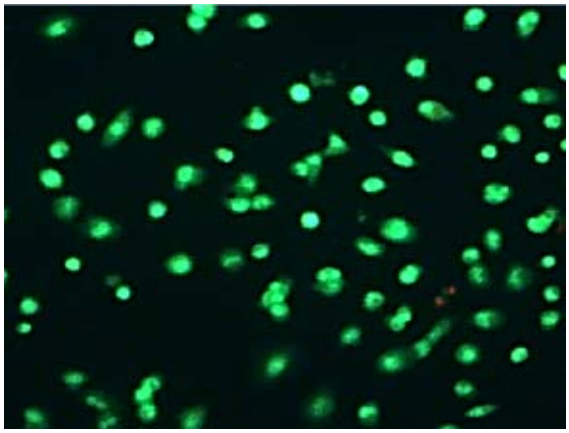
Lung cancer is the most prevalent cancer and the ranks first to increase the mortality in both gender. Though many chemotherapeutic drugs developed, toxicity of the chemotherapy drugs is the main disadvantage. Toxicity is mainly due to the non-specific toxicity to normal cell lines and relatively short survival of compound. Apoptosis is the main pathway to induce death in cancer cells and most of the chemotherapeutic drugs undergo this pathway to invade the tumor cells. In the apoptotic process, the cell membrane get shrinks, membrane blebbing occurs, chromatin gets condensed, fragmentation of DNA occurs. These apoptotic cells will be sensitized by the macrophages and phagocytosis occurs. In the present study, Ao/EtBr staining is used to reveals the presence of early apoptotic, late apoptotic and necrotic cells. A_O stains the cells in green colour and enter only in to the normal and early apoptotic cells. EtBr can stain only the cell with damaged cell membrane as observed in late apoptotic and necrotic cell.



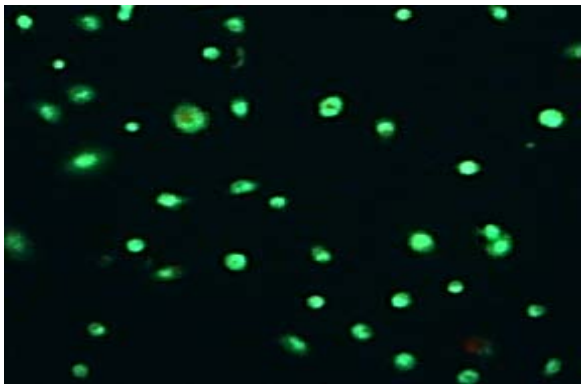
Control



500 µg/ml



750 µg/ml



1000 µg/ml

Fig 1: Early apoptotic and necrosis morphology was observed in treated cells when compared with the control cells.

Conclusion

Thus the present study showed the antioxidant activity against the lung cancer cell line. Continuous research in this millet will explain the possible mechanism against the cancer.

References

1. Devi PB, Vijayabharathi R, Sathyabama S, Malleshi NG, Priyadarisini VB. Health benefits of finger millet (Eleusine coracana L.) polyphenols and dietary fiber: a review. Journal of food science and technology. 2014 Jun; 51(6):1021-40.
2. Singh N, Meenu G, Sekhar A, Jayanthi A. Evaluation of antimicrobial and anticancer properties of finger millet (Eleusine coracana) and pearl millet (Pennisetum glaucum) extracts. The Pharma Innovation. 2015;3(11, Part B):82.
3. Anitha S, Givens DI, Botha R, Kane-Potaka J, Sulaiman NL, Tsusaka TW, Subramaniam K, Rajendran A, Parasannanavar DJ, Bhandari RK. Calcium from Finger Millet—A Systematic Review and Meta-Analysis on Calcium Retention, Bone Resorption, and In Vitro Bioavailability. Sustainability. 2021 Aug 4;13(16):8677.
4. Xiang J, Apea-Bah FB, Ndolo VU, Katundu MC, Beta T. Profile of phenolic compounds and antioxidant activity of finger millet varieties. Food chemistry. 2019 Mar 1;275:361-8.
5. Poofery J, Khaw-On P, Subhawa S, Sripanidkulchai B, Tantraworasin A, Saeteng S, Siwachat S, Lertprasertsuke N, Banjerdpongchai R. Potential of Thai herbal extracts on lung cancer treatment by inducing apoptosis and synergizing chemotherapy. Molecules. 2020 Jan 6;25(1):231.
6. Matsuda T, Machii R. Morphological distribution of lung cancer from Cancer Incidence in Five Continents Vol. X. Japanese journal of clinical oncology. 2015 Apr 1;45(4):404-.
7. Sajid M, Yan C, Li D, Merugu SB, Negi H, Khan MR. Potent anti-cancer activity of Alnus nitida against lung cancer cells; in vitro and in vivo studies. Biomedicine & Pharmacotherapy. 2019 Feb 1;110:254-64.
8. Swaffer MP, Jones AW, Flynn HR, Snijders AP, Nurse P. CDK substrate phosphorylation and ordering the cell cycle. Cell. 2016 Dec 15;167(7):1750-61.
9. Subba Rao MV, Muralikrishna G. Evaluation of the antioxidant properties of free and bound phenolic acids from native and malted finger millet (Ragi, Eleusine coracana Indaf-15). Journal of Agricultural and Food Chemistry. 2002 Feb 13;50(4):889-92.
10. Viswanath V, Urooj A, Malleshi NG. Evaluation of antioxidant and antimicrobial properties of finger millet polyphenols (Eleusine coracana). Food Chemistry. 2009 May 1;114(1):340-6.