Anti-cancer activity of ethanolic extract of Jackfruit (*Artocarpus heterophyllus*) skin on human melanoma and colon cancer cell lines

V. Gayathri¹, A. Ruckmani¹*, R. Arunkumar¹, M. R. Ganesh², MadhaviEerike⁴ and M. Meera³

¹Department of Pharmacology, Chettinad Hospital and Research Institute, Chettinad Academy of Research and Education, Kelambakkam-603103, Tamil Nadu, India

²Department of Medical Biotechnology, Faculty of Allied Health Sciences, Chettinad Hospital and Research Institute, Chettinad Academy of Research and Education, Kelambakkam-603103, Tamil Nadu, India

³Interdisciplinary School of Indian System of Medicine, SRM University, Kattankulathur-603203, Tamil Nadu, India

*Corresponding author:

Dr. A. Ruckmani, Professor and Head, Department of Pharmacology, Chettinad Hospital and Research Institute, Chettinad Academy of Research and Education, Kelambakkam-603103, Tamil Nadu, India.

Phone no: 9500102346
E-mail.id: ruckmani.nirmal@gmail.com

ABSTRACT:

The objective is to find out whether the ethanolic extract of Jackfruit skin has anti-cancer activity against human melanoma and colon cancer cell lines. The ethanolic extract of Jackfruit skin (EEJS) was prepared by maceration and filtration technique. The human melanoma (A-431) and colon cancer (HT-29) cell lines were procured from NCCS (National Centre for Cell Science), Pune, India and grown in DMEM (Dulbecco’s Modified Eagle Medium). Anti-cancer activity of EEJS was evaluated in-vitro by MTT (3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide) and LDH (Lactate dehydrogenase) assays in six different concentrations 0.5, 1, 2, 4, 8 and 12mg/ml against these two cell lines. The yield of the extract was 3.08%. EEJS exhibited cytotoxicity in all the doses and a maximum of 93% and 90% was observed at 12mg/ml against A-431 cell lines in MTT and LDH assays respectively. The anti-colon cancer activity was 39% and 38% in both the assays. The anti-cancer activity was found to be higher for melanoma than colon cancer cell lines. EEJS showed cytotoxicity against human melanoma and colon cancer cell lines and the cytotoxicity was highly significant against melanoma than colon cancer cell lines.

Key words: A-431 cells, anti-cancer activity, HT-29 cells, Jackfruit skin

INTRODUCTION:

Cancer is the second leading cause of death worldwide next to cardiovascular disease. Globally colorectal cancer (CRC) is the third common cancer in men and second in women. Global incidence and mortality of CRC was 13,60,000 and 6,94,000 respectively in 2012 and increased to 18,49,518 and 8,80,792 in 2018.

Melanoma accounts for 1 to 3% of all cancers in the world. Global incidence and mortality of melanoma were 2,32,000 and 55,000 in 2012 and increased to 2,87,723 and 60,712 in 2018. Though the incidence is low compared to CRC, it is increasing rapidly since 50 years.

Many plants including edible plants are used in traditional systems of medicine for treatment of cancer. Among the edible fruits Jackfruit (JF - Artocarpus heterophyllus – (AH)) is the largest fruit.

Its seed, wood, and leaf have been reported to have anti-cancer activity against lung, breast, cervical, melanoma and colorectal cancers. Jackfruit being the biggest of all the fruits, large quantity of skin can be obtained from a single fruit which can be used as a resource for various extract preparation and analyses. The skin has been shown to possess anti-cancer activity against liver, breast, cervix and bone cancers.

This study was undertaken to evaluate the anti-cancer activity of JF skin (JFS) against human melanoma and colon cancer cells which has not been assessed so far.

MATERIALS AND METHODS:

Plant material:

JF was purchased from a farm near the institute (Chettinad Hospital and Research Institute, Chennai) and authenticated by Dr. Narasimhan, Former Professor, Department of Botany, Madras Christian College, Chennai. The fruit weighed 7.2 kg. Skin in this study refers to the skin and spines. The skin was carefully removed from the fruit. It weighed 1,040 grams. After excluding the damaged portions 400 gram was taken for study.

Extract preparation:

The skin was cut into small pieces, macerated using a blender and soaked in 80% ethanol in the ratio of 1:5 (100g of skin in 500 ml of ethanol) for 72 hours with periodic shaking. The supernatant fluid was filtered using Whatman filter paper. The filtrate was kept over a hot water bath for 48 hours for evaporation. Finally a brown sticky extract was obtained. The yield was 12.32 g and the percentage yield was 3.08. The extract thus obtained was stored in the refrigerator (2-8°C).

Cell lines:

Human melanoma (A-431) and colon cancer (HT-29) cell lines were procured from National Centre for Cell Science (NCCS), Pune and cultured in DMEM (Dulbecco’s Modified Eagle Medium).

Chemicals:

DMSO (Dimethyl sulfoxide), DMEM medium, 10% FBS (Fetal bovine serum), 100 U/ml penicillin, 0.1 mg/ml Streptomycin and 0.25 µg/ml Amphotericin B were obtained from In Vitrogen laboratories limited, Chennai.
MTT reagent, Ethanol (80%) and LDH kit were obtained from HI Media laboratories private limited, Chennai.

**Assays for anti-cancer activity:**

The anti-cancer activity was assessed by two in-vitro assays- MTT and LDH assays.

**MTT assay:**

In a preliminary study conducted using murine melanoma and human colon cancer cell lines, cell death was observed with the starting dose of 0.5 milligram. Hence the extract was used from 0.5 milligram and its multiples and the assay was done in triplicate. Ethanol was used as negative control. 10000 cells were seeded in 96 well plate and incubated overnight to adhere. Cells were treated with different concentrations (0.5, 1, 2, 4, 8 & 12mg/ml) of jackfruit skin extract. Followed by 24 hrs incubation 10 µl of MTT solution along with 5% FBS added. Incubated for 3 hrs, finally 100 µl of DMSO is added and quantified at 540 nm.

The results were expressed as percentage inhibition of cancer cells calculated using the formula:

\[
100 - \frac{OD \text{ of treated (test) cells}}{OD \text{ of untreated (control) cells}} \times 100
\]

(OD – Optical density)

**LDH assay:**

LDH assay was done with the cell culture medium from each group using LDH assay kit (Sigma). The supernatant from each sample was used in this assay. 20000 cells were seeded in 96 well plate and incubated overnight to adhere. The cells were treated with EEJS in different concentrations (0.5, 1, 2, 4, 8 &12mg/ml) for 24hr. Ethanol was used as negative control. LDH assay was done in triplicate. The cell culture media from the treatment and control groups were collected and centrifuged. 50µL of the supernatant from each well was transferred to fresh 96 well plate and then mixed with equal volumes of LDH substrate and LDH assay buffer reaction mixture. The plate was incubated at 37 °C for 10 min. Absorbance was read at 450 nm.

The results were expressed as percentage of LDH release and calculated using the formula:

\[
\frac{OD \text{ of test}}{OD \text{ of control}} \times 100
\]

**Statistical analysis:**

The values were expressed as change in percentage and compared using unpaired t-test.

P<0.05 was considered as significant, p<0.01 as highly significant and p<0.001 as extremely statistically significant.

**RESULTS:**

**MTT assay results:**

The percentage of cell death in the extract treated wells at the doses of 0.5, 1, 2, 4, 8 and 12 mg/ml was 9%, 15%,
66%, 75%, 92% and 93% for A-431 cells and 3%, 7%, 11%, 14%, 29% and 39%, for HT-29 cells respectively (Figure:1&2). The maximum cell death was achieved with the maximum dose of extract used (12 mg/ml) for both the cell lines.

The percentage of cell death was significant (p<0.05) with the doses 2, 4, 8 and 12 mg/ml for melanoma cells and 4, 8 and 12mg/ml for HT-29 cells when compared to untreated cells. The percentages of cell death with ethanol for A-431 and HT-29 cells were 5% and 2%.

**LDH assay results:**

The result is expressed as percentage of LDH release. The lysis buffer treated cells were kept as positive control, as it killed 100 % of the cancer cells. The extract at the doses of 0.5, 1, 2, 4, 8 and 12 mg/ml caused LDH release from human melanoma cells up to 40%, 45%, 57%, 78%, 81% and 90% and from colon cancer cells up to 5%, 10%, 16%, 22%, 33% and 38% respectively (Figure:3&4).

The maximum percentage of LDH release was achieved with the extract at the dose of 12 mg/ml for both the cell lines. The LDH release was significant with all the doses for melanoma cells and with 4, 8 and 12mg/ml for colon cancer cells. The percentages of LDH release with ethanol for A-431 and HT-29 cells were 9% and 3%.

**DISCUSSION:**

The ethanolic extract of JFS was found to exhibit higher anticancer activity against melanoma cell lines than colon cancer cell lines in this study.

EEJS was found to have better anti-melanoma activity than many other plants screened in earlier studies\textsuperscript{18,19,20,21&22} and lesser activity than the plants screened for anti-colon cancer activity.\textsuperscript{23,24,25,26&27}

Compared to JFS extract other fruit skin extracts such as pomegranate\textsuperscript{28}, banana, orange, guava, papaya and lemon\textsuperscript{29} have produced cytotoxicity against cancer cells at a much lower dose (in microgram). The efficacy of the JFS extract seen at a higher dose may not be a problem if this dose is found to be safe. The skin extract is reported to be safe up to the dose of 2000mg/kg in \textit{in-vivo} acute toxicity study conducted in mice earlier.\textsuperscript{14}

Gupta P et al 2014 observed that saponin and tannin content was higher in JFS than the fruit residues of C. carandas (Bengal currant), A. comosus (Pineapple), A. lachoocha (Monkey fruit), L. sinensis (Lichi), G. asiatica (Phalsa) and B. vulgaris (Common barberry).\textsuperscript{8}Saponins\textsuperscript{30} and tannins\textsuperscript{31} were observed to possess anti-cancer activity. The high content of saponins and tannins could have contributed to the anti-cancer activity of JFS.

Zhang et al 2017\textsuperscript{32} showed that JFS had high content of phenols and flavonoids than other parts of JF like pulp, flake and seed and isolated 53 phytochemicals from JFS. The major components identified were prenly flavonoid (PF), hydroxycinnamic acid (HCA) and glycosides. Prenylated flavonoids inhibit tyrosinase enzyme which is the rate limiting enzyme in melanin biosynthesis.\textsuperscript{33} Increased melanin production in melanoma worsens the progression of the disease by promoting metastasis and decreases the effectiveness of chemotheraphy and radiotherapy in melanoma.\textsuperscript{34} Hence inhibition of melanin synthesis will reduce the progression and metastasis of
melanoma. Prenyl flavonoids in addition to inhibiting melanin synthesis possess antioxidant property. Moreover cytotoxic activity against malignant melanoma, leukemia, colon, breast, cervical and hepatocellular cancer cells has been observed in *in-vitro* studies. HCA was also found to have anti-cancer and anti-oxidant activity. The main ingredients responsible for the anti-cancer activity of JFS can be attributed to prenylated flavonoids and HCA present in JF skin. Moreover total phenolic content of JFS was found to be higher than the fruit skin of pomegranate, orange and banana which could have contributed to the significant anti-melanoma activity.

Thus it was observed in the present study that EEJS possesses significant anti-melanoma activity than several other plants. The activity against colon cancer cell lines was comparatively lower but comparable to other plants. As the extent of cytotoxicity is more than 90% for melanoma cells, if proven in *in-vivo* studies, JFS extract could be used as a single agent in melanoma in future. The cytotoxicity exhibited by JFS on colon cancer cell lines, though 39%, it can be explored for its probable use as an adjuvant in the treatment of colon cancer.

**CONCLUSION:**

It can be concluded from the results of the present study on the ethanolic extract of JF skin that the extract had *in-vitro* anti-cancer activity against human melanoma cell lines (A-431) and human colon cancer celllines (HT-29). The extract had better anti-cancer activity against melanoma cell line. From the available evidences, it can be inferred that the anti-cancer activity could be due to the presence of prenylated flavonoids and hydroxycinnamic acid and the inhibition of tyrosinase enzyme.

However further studies are needed to isolate the active components in JF skin and evaluate the anti-cancer activity *in-vivo*.

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**Declaration of interest statement:**

The authors declared no conflict of interest.

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Figures: MTT and LDH assays result for A-431 & HT-29 cells

1) MTT assay results for human melanoma (A-431) and colon cancer (HT-29) cell lines. Extract treated cells compared with control. **p<0.01, ***p<0.001

2) LDH assay results for human melanoma (A-431) and colon cancer (HT-29) cell lines. Extract treated cells compared with control. **p<0.01, ***p<0.001

3) LDH release (%), Concentration of extract (mg/ml) 0.5 1 2 4 8 12

4) LDH release (%), Concentration of extract (mg/ml) 0.5 1 2 4 8 12

Extract compared with control. *p<0.05, **p<0.01, ***p<0.001