INTERNATIONAL JOURNAL OF BIOPHARMACEUTICAL & TOXICOLOGICAL RESEARCH

ESTIMATION OF THE ANTI- PROLIFERATIVE POTENTIAL OF COSTUS PICTUS ON MOLT- 4 HUMAN CANCER CELL LINE

Ajit Kumar Rajpoot

Jyotirmoy Das Choudhury[1*], Anup Kumar Maurya[2], Smriti Tripathi[2], Reetu Ranga[2], Monica Kahrana[3], Manohar Lal Samyal[4].

Keywords:
Costus pictus, Lymphocytes, MTT assay, Cytotoxicity

Corresponding Author-
Jyotirmoy Das Choudhury
Department of Pharmacology,
Royal College of Pharmacy & Health Sciences, Berhampur (Gm) - 760002, (Odisha), India.
Phone no:- +91
Email id:- jdc_pharma@yahoo.in

ABSTRACT:

Introduction: The present study is aimed at evaluating the anti-proliferative and apoptotic of Costus pictus on MOLT- 4 human cancer cell line, and also evaluating its safety to normal human lymphocytes.

Materials and Methods: Dried leaves of C. pictus plant were used for aqueous and alcohol extraction. Different concentration of these were evaluated for their cytotoxicity by trypan blue dye exclusion method and 3-(4, 5- dimethylthiazol-2yl)-2, 5-diphenyl tetrazolium bromide (MTT) assay on the cancer cell line (Human acute lymphoblastic leukemia) MOLT- 4 and normal cell line (Human peripheral lymphocytes). The apoptic potential was analyzed by DNA fragmentation analysis of the treated cells.

Results: The ethnolic extract of C.pictus was found to be anti-proliferative and cytotoxic at lower concentration and induced cell death in MOLT- 4 cells. The ethanolic extract at the same concentration had no cytotoxicity on normal lymphocytes. Campared to the ethanolic extract, aqueous and methanol extracts were less effective.

Conclusion: the present investigation, for the first time, reveals the anticancer potential of the ethanolic extract of C.pictus on MOLT- 4 cells. It is very likely that the result can be extrapolated to animal or human system. The extracts can be used for further purification of the active component for future application.

Introduction:

Medicinal plants are frequently used by traditional healers to treat various ailments and symptoms, including diabetes and cancer. Cancer is the second leading cause of death after cardiovascular diseases in India. [1] Cancer cells, which are already irreversibly developed, obtain the capability to escape apoptosis by a number of ways.

The role of anticancer agents is to trigger the apoptosis-signaling system in these cancer cells while disturbing their proliferation. [2] Zingiberaceae plants

are well known for their medicinal properties and many of these species are effectively used in the treatment of disease. Curcuma longa L., for example, has proven anticancer properties. [3] The rhizomes of these plants possess several biologic activities such as antioxidant,[4] cytotoxic, and antitumor properties.[5-7] Costus pictus, commonly known as 'Spiral ginger', 'Step ladder', or 'Insulin plant' is a member of Zingiberaceae family and is newly introduced plant in India, originated probably in Mexico. In India, it is grown in gardens, especially in the state of Kerala; here, patients with diabetes consume the fresh raw leaves. In vitro studies on methanol, aqueous ethyl acetate, and ethanol extract of C.pictus have revealed good inhibitory effect on carbohydrate hydrolyzing enzymes like α-glucosidase and α-amylase. [8]Toxicity studies and antidiabetic activity of methanol extract of this plant have been reported previously. [9-10] Since there are no earlier reports about testing the anticancer property of C.pictus, we tried to evaluate potential anticancer effect of C.pictus ethanol, aqueous and methanol extracts on MOLT- 4 cell line and also their effects on human peripheral lymphocytes.

MATERIALS AND METHODS

Selection of medicinal plant

Costus pictus (insulin plant) was collected in the month of August 2010, from IIIM Medicinal garden, Jammu, India, and identified by Dr. S. N. Sharma, a senior scientist in the Botany Division of the Indian Institute of Integrative Medicine (IIIM), Jammu, India. A voucher specimen was retained and deposited at the crude drug repository of the herbarium of IIIM, Jammu. The leaves were shade dried, reduced to coarse powder and stored in airtight container till further use.

Extraction using ethanol

The dried leaves of the plant (50 g) were crushed, soaked in 75 ml of ethanol (80% v/v) for 24 h and then percolated (5 h, 30 drops/min). The extract obtained was concentrated by a rotary evaporator and dried in oven at 40°C. Then 20 mg of solid residue was dissolved to 100 ml with distil water. The resulting solution was filtered through 0.22 micro filters. The dilution was continued so that final concentration of extract was 40, 80 and 120µg/ml. [11]

Extraction using methanol

Dried leaf samples were ground using an electric blender. Two grams of ground material was soaked in 25 ml of methanol for 24 h and was filtered using filter paper. The material was again mixed with 25 ml of fresh methanol and filtered after 24 h. The process was repeated (25 ml x 3 times).The filtrate was then transferred to vials and allowed to dry. Dry extract was resuspended in 2ml of DMSO. The concentration of extract was 1mg/ml. [12] The dilution was continued so that final concentrations of extracts used were 40, 80, and 120µg/ml.

Aqueous extraction

Dried plant (50 g) was ground and mixed with 1 L boiling distilled water for 1 h. The mixture was filtered twice through a funnel by using suction pump. The extract was evaporated under reduced pressure till completely dry in a lyophilizer. The extract (1 mg/ml) was prepared by dilution of the stock with sterile distilled water. [13]

Cell line selected

Human peripheral lymphocytes were isolated in the laboratory and MOLT- 4 cell line was procured from National Center for Cell Sciences (NCCS) Pune, India. MOLT-4 (Human acute lymphoblastic leukemia) cells were grown in complete DMEM medium (2mM L-glutamine, 100g/ml of streptomycin, and 100U/ml of penicillin) supplemented with 10% fetal bovine serum and maintained in a 5% CO2 humidified incubator at 37°C. Cells were seeded at a density of 1X105cells/ml, except where otherwise indicated.

Isolation of lymphocytes

Lymphocytes were obtained from the blood of five healthy male and female individuals, of age about 20 years, apparently free from infection by pathogenic agents, and had not been under any treatment for the last six months. Hisep medium (HIMEDIA, India) was used for the isolation. Cells were suspended in complete RPMI 1640 medium supplement with 10% fetal bovine serum (HIMEDIA, India), 5g/ml phytohemagglutinin (PHA), and maintained at 37°C in a 5% CO2 humidified incubator. Lymphocytes were used as control cells to assess the cytotoxicity of plant extracts.

Determination of cell concentration and viability by Trypan blue dye exclusion

At the end of treatment period, the cells were counted with the aid of hemocytometer and cells viability was determined by trypan blue dye exclusion method. Trypan blue was prepared at a concentration of 0.4% in phosphate buffered saline (PBS). [14]

MTT assay

MTT assay was performed to assess the cytotoxicity of the plant extracts. (MTT is a yellow dye, which is reduced into purple formazan crystals by the activity of mitochondrial succinate dehydrogenase enzyme in viable cells). Cells were cultured in 96- well microtiter plates. Cells were treated with varying concentration of plant extracts for 24 h (9 wells for each concentration) and incubated. At the end of treatment period, to each well, 20 µl of MTT was added. After addition of MTT, the plates were incubated for 3 h in a dark chamber. Then, 100 µl of DMSO was added to dissolve the formazan crystals. The absorbance was read at 540 nm using ELISA reader. [15]

Analysis of DNA fragmentation

In a medium containing 10% FBS, 1.0X105 cells were incubated for 24 h. After 24 h, cells were treated with C.pictus leaf, (aqueous, methanol, and ethanol) at different concentrations. After 24 h, cells were collected by trypsinization; cells from different wells of the same concentration were pooled and rinsed twice in cold PBS (PH 7.4). Genomic DNA was extracts from MOLT- 4 cells as described earlier.[16] Briefly, cells were re-suspended twice in a lysis buffer containing 1% Nonidet-P40, 20mM Ethylenediaminetetraacetic acid (EDTA), and 50 mM Tris-HCl at PH 8. The cells were centrifuge at 1,600g for 10 min, recovered supernatant were combined and incubated with 0.5 mg/ml RNase A (Bangalore Genei, India) at 37°C for 4 h. The DNA was precipitated by the addition of 1/10 volume of 7.5 M ammonium acetate and two volumes of ethanol and analyzed by agarose gel electrophoresis.

Morphology observation under inverted microscope

Morphological changes were observed under the 40X lens of an inverted microscope and photographs were taken.

Statistical analysis

All experiments were carried out in triplicates. The results were calculated as mean±standard error (SE) values. Statistical significance was calculated using one-way analysis of variance (ANOVA) and Student's test. A value of $P<0.05$ was taken as statistically significant.

RESULTS

When different concentrations of C.pictus were added to normal lymphocytes, the aqueous extract induced proliferation in the lymphocytes. The cell concentration and cell survival both increased, as the concentration of the extract increased [Figure 1]. The ethanol extract had no effect on the lymphocytes. Methanol extract showed slight cytotoxicity when compared to the other two extracts.

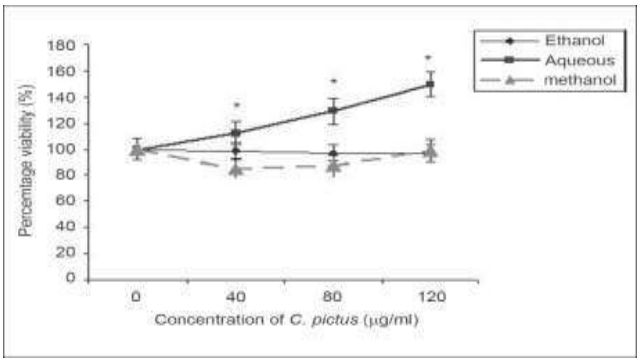

Figure 1: Effect of C. pictus on the percentage viability of normal lymphocytes as measured by MTT assay. Values are expressed as mean±S.E.M. (n=9). *P<0.05 (in comparison with control group)

When cell count was taken by hemocytometry, it was observed that [Figure 2] the ethanol extract had no significant effect on the peripheral blood lymphocytes, whereas the methanol extract was slightly cytotoxic and the aqueous extract had increased the cell count significantly.

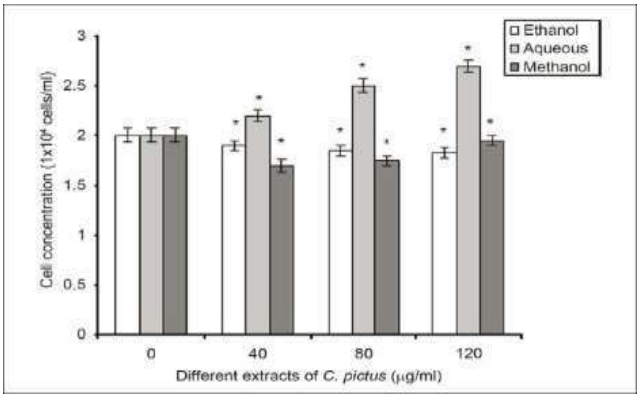

Figure 2: Effect of C. pictus on the cell concentration of normal lymphocytes measured by Trypan blue dye exclusion method. Values are expressed as mean±S.E.M. (n=4). *P<0.05 (in comparison with control group)

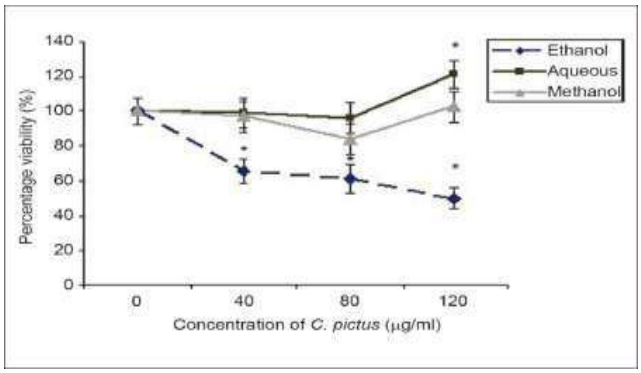

Figure 3: Effect of C. pictus on the percentage viability of MOLT-4 human cancer cells as measured by MTT assay. Values are expressed as mean±S.E.M. (n=9). *P<0.05 (in comparison with control group)

C.pictus aqueous extract administered to MOLT- 4 cells at 40 and 80 µg/ml concentration showed slight cytotoxicity, through it was not significant. The ethanol extract was highly cytotoxic; reducing the cell viability to less than 50% at 120 µg/ml concentrations (IC50) value was found to be 120 µg/ml from the graph.

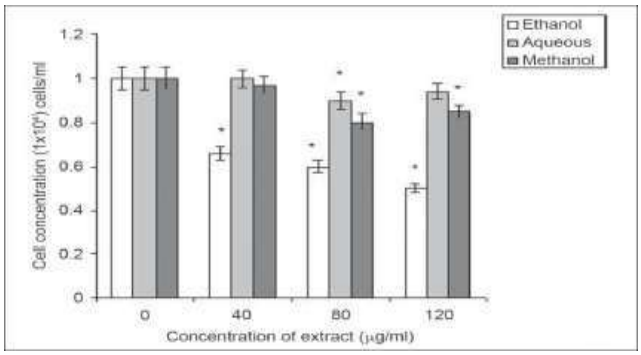

Figure 4: Effect of C. pictus on the cell concentration of MOLT-4 human cancer cells measured by trypan blue dye exclusion method. Values are expressed as mean±S.E.M. (n=4). *P<0.05 (in comparison with control group)

As the concentration of the extract increased, cell viability decreased. The methanol extract was cytotoxic at 80 and 120 µg/ml concentration. By trypan blue dye exclusion method, the cell concentration and cell viability were determined with the help of a hemocytometer [Figure 4].

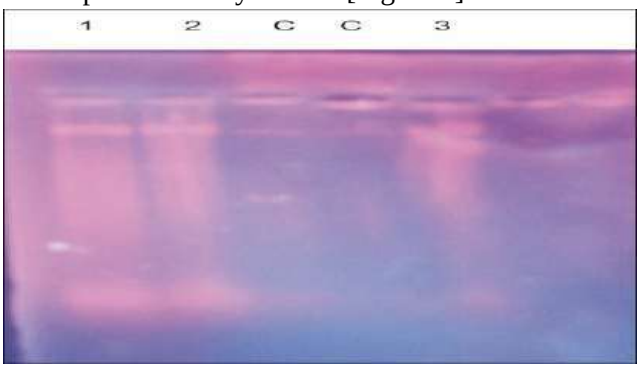

Figure 5: DNA fragmentation assay of HT-1080 cells treated with different concentrations of C. pictus ethanol extract for 24 h. Lane C- control, Lane 3- 40 µg/ml, Lane 2- 80 µg/ml, and Lane 1- 120 µg/ml of C. pictus ethanol extract.

The ethanol extract (120 µg/ml) had reduced the cell concentration to half of that of the control. The ethanol extract was safe to normal human lymphocytes but has shown cytotoxicity to the cancer cell line.

DNA fragmentation was tested by agarose gel electrophoresis. [Figure 5] indicates a significant increase in inter- nucleosomal DNA fragmentation of MOLT-4 cells. When the DNA isolate from C.pictus-treated cells was subjected to agarose gel electrophoresis, a DNA ladder characteristic of apoptotic DNA was observed in the cells treated with different concentration of the ethanol extract.

The morphology of MOLT-4 cells was observed under the inverted microscope, and it was obvious that the control cells were firmly attached to the substrate [Figure 6] and C.pictus (80 µg/ml) - treated cells started becoming round and were detached [Figure 7] from the substrate. The ethanol extract had induced apoptosis in the fibrosarcoma cells.

Figure 6: Control MOLT- 4 cells observed under the 40X inverted microscope

Figure 7: MOLT- 4 cells treated with C. pictus (80 µg/ml) as observed under the 40X of inverted microscope

DISCUSSION

Accumulating evidence suggests that many dietary factors may be used alone or in combition with traditional chemotherapeutic agents to prevent or treat cancer. The main disadvantage of using natural or dietary compounds as anticancer remedy is that they seem to have low toxicity and show very few adverse side effects. Ginger (Zingiber officinale Roscoe), is widely used all over the world as a spice and condiment in daily cooking. It has been shown to have anticancer and antioxidant effects.[17] The antidiabetic activity of the aqueous, ethanol ethyl acetate and methanol extract of C.pictus has been reported earlier. [8-10] Effect of C.pictus on glucose uptake by L6 myotube cell line ((Skeletal muscle) has also been reported. [18] However, to best of our knowledge, this is the first report on the first report on the cytotoxic activity of C.pictus on any human cancer cell line.

CONCLUSION

In conclusion, the result of the present work show that the ethanol extract of C.pictus activated the apoptotic pathway in (Human acute lymphoblastic leukemia cell line) MOLT- 4 cells. The ability of the extract to trigger and execute apoptosis in cancer cells is unclear but the MTT assay suggests a mitochondrial involvement. Anticancer activity of the ethanol extract of C.pictus has not been reported in literature. In our present in vitro study through MTT assay, Trypan blue dye-exclusion method, and DNA fragmentation analysis, we confirm the pro-apoptotic and anticancer potential of C.pictus ethanol extract. The IC50 value was found to be 120µg/ml. Additionally; it was revealed that the key bioactive compound was in the ethanol fraction, showing its promising anticancer drugs used in cancer therapy are toxic and have adverse side effects. Thus, studies pinpointing confirmed efficacy of a particular fraction or compound among several found in crude extract are important for therpeacutic purpose. Although the efficacy of C.pictus ethanol extract has presently been tested against an in vitro cancer cell line, it is very likely that the result can be extrapolated to animal or human systems. However, to determine this, more experiments should be carried out on in vivo animal models, which will hopefully be taken up in the next phase of our program.

REFERENCES

1. Bingham S, Riboli E. Diet and cancer- the European prospective investigation into cancer and nutrition. Nature Rev Cancer 2004; 4:206-15.
2. Bold RJ, Termuhlen PM, and McConkey Dj. Apoptosis, Cancer and cancer therapy. Surg Oncol 1997; 6:133-42.
3. Cousins M, Adelbergj, Chen F. Antioxidant capacity of fresh and dreied rhizome from four clones of turmeric (Curcuma longa) grown in vitro. Ind crop Prod 2007; 25:129-35.
4. Selvam R, Subramanian L, Gayathri R, Angayarkanni N. The antioxidant activity of turmeric (Curcuma longa). J Ethanopharmacol 1995; 47:59-67.
5. Itokawa H, Morita H, Sumitomo T, Totsuka N, Takeya K, antitumor principles from Alpinia galanga. Planta Med 1987; 53:32-3.
6. Murakami A. jiwajiinda S, Koshimizu K, Ohigashi H. Screening for in-vitro Anti-tumor promoting activities of edible plants from Thailand. Cancer Lett 1995; 95:137-46.
7. Pal S, Choudhuri T, Chattopadhyay S. Bhattacharya A, Datta GK, Das T, et al. Mechanisms of curcumin- induced apoptosis of ehrlichs ascites carcinoma cells. Biochem Biophy Res Comm 2001;288:658-65.
8. Jayasri MA, Radha A, Mathew TL. α – Amylase and α – glucosidase inhibitory activity of Costus pictus D. Don in the management of diabetes. J herb Med Toxicol 2009; 3:91-4.
9. Dicarli MF, janises J, Grunberger j, Ager J. Role of chronic hyperglycemia in the pathogenesis of coronary microvascular dysfunction in diabetes j Am coll cardiol 2003;41:1387-93.
10. Merina B. Toxicity studies of the herb costus pictus D.Don (online journal) Pharmainfo.net. 2004, Available from: http;//www.pharmainfo.net/reviews/toxicity-studies-of-the-herb-costus-pictus-d.don.
11. Rakhi S, Daman S, Bilikere S, Dwarakanth, Madhu C. inhibition of human cervical cancer cell growth by ethanolic of Boerhaavia diffusa Linn. (Punarnava) root. Evidence-based Complementry and Alternative Medicine 2010;doi:10.1093/ecam/nep223:1-13.
12. Hojjat S, Ahmed E, Morteza S, Babak S, Abbas j. Evaluation of in-vitro cytotoxic effect of juniperus foetidissima and juniperus Sabina extracts against

a panel of cancer cells. Iran j Pharm Res 2009; 8:281-6.

13. Jeymesson RC, De Souza IA, Do Nasimento SC, Sonia PL. Indigofera suffruticosa: An Alternative Anticancer Therapy. Oxf J 2007; 4:355-9.
14. Biswas R, Mandal SK, Dutta S, Bhattacharyya SS, Boujedaini N, Khuda-Bukhush AR. Thujone-rich fraction of Thuja occidentals demonstrates major anti-cancer potentials: Evidence-based Complementary and Alternative Medicine 2010;doi:10.1093/ecam/neg042 : 1-15.
15. Ferda O, Engin U, Arzu YO, Mustafa Z, Ozel, Ozkan K. The differential anticancer effect of green tea in estrogen receptor- negative human breast cancer cell lines. Adv Mol med 2008; 3:69-75.
16. Negri C, Bernardi R, Donzelli M, Scovassi Al. Induction of apoptotic cell death by DNA topoisomerase ll inhibitors. Biochimie 1995; 77:893-9.
17. Surh Yj. Cancer chemoprevention with dietary phytochemical. Nat Rev Cancer 2003; 3:768-80.
18. Pareek A, Suthar M, Godarvathi A, Goyal M, Bansal V. Negative regulation of glucose uptake by Costus pictus in L6 myotube cell line. J Pharm Negat Results 2010;1:24-6.