Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 7, Supple 1, 2015

Original Article

IN-VITRO CYTOTOXIC EFFECT OF *CANTHIUM DICOCCUM* ON DIFFERENT CANCER CELL LINES

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Received: 07 Aug 2015 Revised and Accepted: 14 Sep 2015

ABSTRACT

Objective: Cancer remains one of the most dreaded diseases causing an astonishingly high death rate. Despite the use of surgical resection and aggressive chemotherapy, nearly 50% of patients with carcinoma develop recurrent disease, highlighting the need for improved therapies. Henceforth, complementary and alternative medicine is slowly emerging as an option. A variety of ingredients of traditional medicines and herbs are being widely investigated in several parts of the world to analyze their potential as therapeutic agents against cancer. In the present study we investigated the efficacy of methanol extracts of *Canthium dicoccum,* for its clonogenic inhibition on Human Breast cancer (MD-MB-231), Prostate cancer (PC-3) and Lung cancer (Calu-6) cell lines.

Methods: The cytotoxic effect of methanolic extract of *Canthium dicoccum* was evaluated by MTT assay on MD-MB-231, Calu-6, and PC-3 cells.

Results: The methanol extract of *C. dicoccum* showed significant cytotoxicity against MD-MB-231and Calu-6, when compared to PC-3 cells.

Conclusion: The methanol extracts of *C. dicoccum* showed effective cytotoxic activities in a dose and time dependent manner. Future work will be interesting to know the chemical composition and also better understanding the mechanism of action will help in developing it as drug for therapeutic application.

Keywords: Anti-cancer, MTT assay, Alternative medicine, Inhibition, Drugs, Therapeutics.

INTRODUCTION

Nature stands as an inexhaustible source of novel chemotypes and pharmacophores, and has been a source of medicinal agents for thousands of years, and an impressive number of modern drugs find their origin in natural products. Natural product chemistry has experienced explosive and diversified growth, making natural products the subject of much interest and promise in the present day research directed towards drug design and discovery [1, 2]. The therapeutic areas of infectious diseases and oncology have benefited much from numerous drug classes derived from the natural form and also as templates for synthetic modification. About 40 new drugs launched on the market between 2000 and 2010, originating from natural sources [3]. In fact, about 60% of anticancer drugs (like vincristine, vinblastin etc.,) are derived from herbal source [4, 5]. Despite major scientific and technological progress in combinatorial chemistry, drugs derived from natural product still make an enormous contribution to drug discovery today. In this respect although, most of the plants have been identified for its anti-cancerous properties, yet their efficacy is needed to be verified.

Canthium dicoccum belonging to the family Rubiaceae is one of the medicinally important plants which is commonly known as "nallamandharam" in Tamil and in Malayalam as Nanyul [6]. It is commonly found in Western Ghats of India and is a medium sized tree. In India, traditionally the bark is used as febrifuge and also applied as plasters [7]. The decoction of roots is used internally for treating diarrhea [7]. Furthermore, the bark powder boiled with sesame oil is used for external application for rheumatic pains [7]. Plant is also known to possess antipyretic activities, anti-diabetic, anti-inflammatory [8] and nephro protective activity [9]. The plant is reported to contain ursolic acid, quercetin, rutin, 7-O-(6-O-benzoyl-β-glucopyranosyl)-rutin, spathulenol, caryophyllene oxide, cedren-13-ol and ledene oxide [6] and the presence of these constituents was shown to provide the scientific evidences for the antimicrobial, Immuno modulatory and antioxidant properties of the plant [6]. Till date, as there was no report on its anticancer potential henceforth, in this study we have attempted to investigate the *in vitro* anti-cancer potential of methanolic extract of *Canthium dicoccum* (*C. dicoccum*) of its cytotoxic efficacy on Human Breast cancer (MD-MB-231), Prostate cancer (PC-3) and Lung cancer (Calu-6) cell lines.

MATERIALS AND METHODS

Materials

All the chemical reagents and solvents of analytical grade were purchased from SRL Chemicals, India. 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT, No M5655) purchased from Sigma (St Louis, MO, USA).

Plant material and extracts preparation

Selection of medicinal plants

Canthium dicoccum (*C. dicoccum*) were collected from the jungles of Western Ghats in and around Karnataka under the guidance of Forest Officer. Plant were analyzed for botanical identity, scientific name including genus, species, subspecies or variety and the family of the plant were recorded and verified for taxonomical status, as recommended by the national pharmacopoeia. Further, the plants were identified by a Taxonomist and the voucher specimens (SY-P-52) were kept at G7 Synergon Private Limited.

Preparation of extracts

C. dicoccum plant parts were washed with distilled water, dried and crushed into fine powder by using electric grinder. The finely powdered material was extracted with methanol in a Soxhlet apparatus for 24 h. The extract was dehydrated/concentrated under reduced pressure using a rotary flash evaporator (Buchi Flawil, Switzerland) and preserved aseptically at 5 °C in airtight bottle until further use [10].

In-vitro cytotoxic assays

Cell culture assay

The human carcinoma cell lines, MDA-MB-231, PC 3 and Calu 6 were obtained from American Type culture Collection (ATCC). The cells were grown in DMEM containing 2 mM L-glutamine supplemented with 10% fetal bovine serum and 100 U/ml of penicillin-streptomycin. The cells were incubated at 37 °C in a humidified 5% CO_2 incubator. All the cell lines used in this study were of early passage number.

MTT assay

Cytotoxicity assay

Cytotoxicity test were carried out using MTT assay [11, 12]. The Trypsinize 70-80% confluent cell lines (MDA-MB-231, PC 3 and Calu 6) of 1×10^5 cells/well are seeded in a 96 well plate and incubate for 24 hr at 37°C, and varying concentrations (0-500 µg/ml) of *C. dicoccum* were added. After incubation of different time points, 20 µl of MTT reagent were added to each well and incubated for 4 hr at 37 °C. The incubated cells were washed twice with PBS and DMSO (100µL/well) reagent which dissolved the insoluble crystalline formazan product. The efficacy of the sample was determined based on the reduced dye at 570 nm by UV spectrophotometer. Doxorubicin at 50 um/ml was used as a standard and appropriate controls were taken the effect of the samples on the proliferation of cell lines were expressed as the % cell viability, using the following formula: % cell viability = A570 of treated cells/A570 of control cells × 100%.

Statistical analysis

The experiments were carried out in triplicate and results are given as the mean±standard deviation. The data in all the experiments were analyzed (Microsoft Excel 2007) for statistical significance using Students *t*-test and differences were considered significant at $p<0.05$.

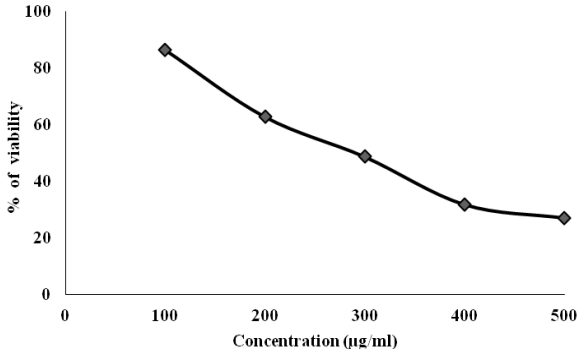

Fig. 1: Cytotoxic activity of *C. dicoccum* methanolic extracts in Human Breast cancer (MD-MB-231) cell lines. Extract were incubated with 10^5 viable cells at concentrations ranging from 0 to 500 µg/ml for 48 h. Cell viability was determined by the MTT method. Results represent means±standard deviation (n = 3)

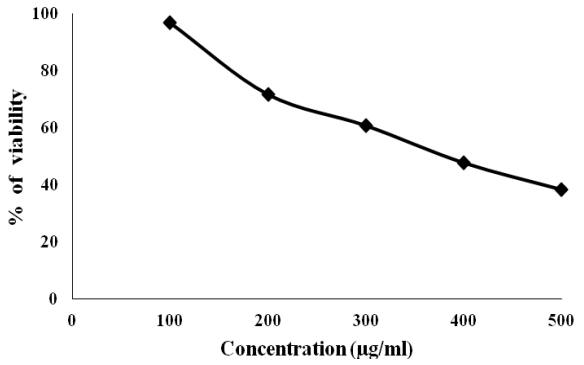

Fig. 2: Cytotoxic activity of *C. dicoccum* methanolic extracts in Prostate cancer (PC-3) cell lines. Extract were incubated with 10^5 viable cells at concentrations ranging from 0 to 500 µg/ml for 48 h. Cell viability was determined by the MTT method. Results represent means±standard deviation (n = 3)

Table 1: IC_{50} values of methanolic extract of *C. dicoccum*

Cell Lines	IC_{50} Value (µg/ml)
MD-MB-231	347.13±1.2
PC-3	462.42±3.2
Calu-6	387.36±2.8

Results represent means±standard deviation (n = 3).

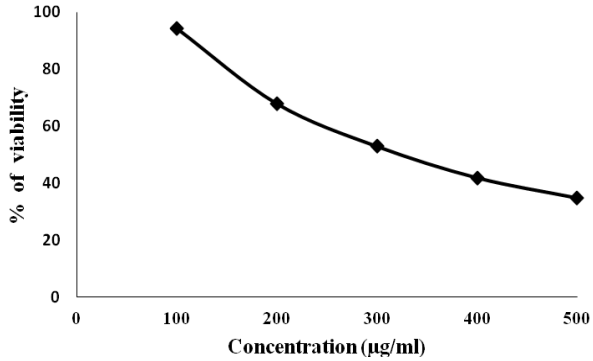

Fig. 3: Cytotoxic activity of *C. dicoccum* methanolic extracts in Lung cancer (Calu-6) cell lines. Extract were incubated with 10^5 viable cells at concentrations ranging from 0 to 500 µg/ml for 48 h. Cell viability was determined by the MTT method. Results represent means±standard deviation (n = 3)

RESULTS AND DISCUSSION

Plants have always been a potential source of new drug molecule and research work in this domain has resulted in discovery of more efficient drugs for cancer treatment [3, 5]. MTT is a simple, reliable technique, which measures cell viability and can be used for screening anti-proliferative agents [11, 12]. MTT assay is a a spectrophotometric analysis, which uses (3-[4,5-dimethyl-thiazol-2-yl]-2,5-diphenyl tetrazolium bromide), known as MTT, a yellow collor and water soluble compound. The MTT enters the cells through the plasma membrane and, in contact with superoxide produced by the mitochondrial activity, is oxidized to MTT-formazan, a slat purplish color, which is insoluble in water. Then the oxidation of MTT is proportional to the mitochondrial activity and therefore to cell viability [11, 12].

In the present study, we investigated the efficacy of methanol extracts of *C. dicoccum* for its clonogenic inhibition on Human Breast cancer (MD-MB-231), Prostate cancer (PC-3) and Lung cancer (Calu-6). When evaluated at different concentration (0-400µg/ml) it was observed that in a dose dependent manner the extract *C. dicoccum* was effective in inducing cytotoxic effect upon MD-MB-231 (fig. 1), PC-3 (fig. 2) and Calu-6 (fig. 3) cell lines. It was observed that *C. dicoccum* exhibited up to ~52% of inhibition against the tested cell lines. However, doxorubicin which acted as positive control exhibited up to ~86%. Based on the IC_{50} values (table. 1), it is observed that the methanol extract *C. dicoccum* showed significant cytotoxicity against MD-MB-231and Calu-6, when compared to and PC-3 cells. Furthermore, it was observed that the control assays that were carried out for samples containing only the appropriate volumes of blank solutions showed no effect on cell growth.

The plant is reported to contain ursolic acid, quercetin, rutin, 7-O-(6-O-benzoyl-β--glucopyranosyl)-rutin, spathulenol (20.76 %), caryophyllene oxide (19.25 %), cedren-13-ol (10.62 %) and ledene oxide (5.24 %) [8]. Further it was reported to contain sesquiterpenoids (55.87%), nitrogenous compounds (12.93 %), aldehydes (8.7 %), terpinolene (6.41 %) and phenols (4.26 %) [6]. Polyphenols are known for inducing cytotoxic effects and thus act as anticancer agents [13, 14]. Similarly in this extract the polyphenols and other constituents that are present in the methanolic extract of *C. dicoccum* might be bringing out the anti-proliferative or cytotoxic effect. Therefore, it will be interesting to understand the chemical composition and better understand the mechanism of action of the bioactive constituents of the extract for developing it as drug for therapeutic application.

CONCLUSION

The results of this study indicate anticancer activities for *C. dicoccum* extract. *C. dicoccum* showed significant cytotoxicity against MD-MB-231and Calu-6, when compared to and PC-3 cells. The potential anticancer activities of *C. dicoccum* extracts may be due to the presence of polyphemols and other constituents. The experimental

evidence could be useful for validate the traditional use of plant as source of easily available effective anticancer agents to the people, particularly in developing countries. Future investigations will be interesting to find medicinal compound and also better understanding its mechanism of action against cancer.

ACKNOWLEDGEMENT

The authors are grateful to Department of Phytochemistry, G7 Synergon Private Limited, and the ICMR for providing financial support. DBL thank Jain University for the constant support and encouragement for carrying out research aspects.

CONFLICTS OF INTERESTS

Declared None

REFERENCES

1. Aggarwal BB, Prasad S, Reuter S, Kannappan R, Vivek R Yadav, Byoungduck. Identification of novel anti-inflammatory agents from ayurvedic medicine for prevention of chronic diseases: “Reverse Pharmacology” and “Bedside to Bench” Approach. Curr Drug Targets 2011;12:1595-653.
2. Patwardhan B, Vaidya ADB. Natural product drug discovery: Accelerating the clinical candidate development using reverse pharmacology approaches. Indian J Exp Biol 2010;48:220–7.
3. Dev S. Impact of natural products in modern drug development. Indian J Exp Biol 2010;48:191–8.
4. Cragg GM, Newman DJ. Plants as a source of anti-cancer agents. J Ethnopharmacol 2005;100:72–9.
5. Tan G, Gyllenhaal C, Soejarto DD. Biodiversity as a source of anticancer drugs. Curr Drug Targets 2006;7:265–77.
6. Raja Rajeshwari, Ramalakshmi S, Muthuchelian K. GC-MS Analysis of bioactive components from the ethanolic leaf extract of *Canthium dicoccum* (Gaertn.) Teijsm and Binn. J Chem Pharm Res 2011;3:792-8.
7. Neelima M, Prasad GP, Sudarsanam G, Penchala PG, Jothi B. Ethnobotanical studies in rapur forest division of nellore district in Andhra Pradesh. Life Sci Leaflets 2011;11:335-45.
8. Vuyyuri B, Tripurana R, Gangeyula J, Ali F. Anti-inflammatory activity of ethanolic extract of *Canthium Dicoccum*. Int J Pharm Phytopharm Res 2013;3:226-30.
9. Santhan S, Janartan S, Zuber Ali M. Evaluation of Anti-diabetic and nephro protective activity of 95% ethanolic extract of *Canthium dicoccum* whole plant using albino rats. J Chem Pharm Sci 2013;6:218-22.
10. Harborne JB. Phytochemical methods: A guide to modern techniques of plant analysis. 3rd edition. Chapman and Hall pub. London. UK; 1998.
11. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 1983;65:55-63.
12. Edmondson JM, Armstrong LS, Martinez AO. A rapid and simple MTT-based spectrophotometric assay for determining drug sensitivity in monolayer cultures. J Tissue Cult Methods 1988;11:15-7.
13. Pandey KB, Rizvi SI. Plant polypenols as dietary antioxidants in human health and disease. Oxid Med Cell Longevity 2009;2:270-8.
14. León-González AJ, Auger C, Schini-Kerth VB. Pro-oxidant activity of polyphenols and its implications on cancer chemoprevention and chemotherapy. Biochem Pharmacol 2015. doi:10.1016/j.bcp.2015.07.017. [Article in Press]