ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 10, Issue 12, 2017

Online - 2455-3891
Print - 0974-2441

Research Article

ANTICANCER ACTIVITY OF *GARCINIA MORELLA* CHLOROFORM FRACTION AND ITS ACTIVE COMPOUND GARCINOL ON NEUROBLASTOMA

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Received: 03 July 2017, Revised and Accepted: 25 August 2017

ABSTRACT

Objective: The aim of the present study is to evaluate the anticancer activity of *G. morella* fruit chloroform fraction and its isolated bioactive molecule garcinol on neuroblastoma cell line (SH-SY5Y).

Method: Methanol extraction was performed for the *G. morella* fruits through cold maceration and further fractionated with chloroform. The presence of Garcinol was confirmed by measuring the melting point. Further, the bioactive chloroform fraction and pure Garcinol was tested for anticancer activity against SH-SY5Y cells through MTT assay.

Result: The present study reveals the anticancer ability of bioactive chloroform fraction of *G. morella* fruit and its active molecule garcinol.

Conclusion: *G. morella* fruit and its bioactive compound Garcinol have significant activity against neuroblastoma. This study opens an avenue to further elucidate the mechanism of action and development of alternative treatment of this dreaded disease.

Keywords: *Garcinia morella*, Neuroblastoma, Garcinol, Anticancer activity.

 DOI: http://dx.doi.org/10.22159/ajpcr.2017.v10i12.21066

INTRODUCTION

Plants are integral part of the medicinal system from ancient time. Many local and tribal groups all over the world still depend completely on natural remedies based mainly on plants. According to an official report by the World Health Organization approximately 80% of the world populations still rely on phytotherapy [1]. However, owing to the lack of scientific validation and proper documentation of the processing of such plants for medicinal purposes, they are yet to attain their status. Hence, researchers from all over the world are now interested in plant-based drug discovery research. 25% of the small drug molecule approved in 2014 are natural product based. The biological properties of plants can be attributed to their chemical constituents. Privileged chemical structures from nature are chosen as backbone for many new chemical entities developed for targeting various diseases [2].

Plants of the genus *Garcinia* are reported to possess antibacterial [3,4], antifungal [5], antioxidant [6], anti-inflammatory [7], and anticancer [8,9] activity. Many important chemical constituents are reported to be isolated from plants of these genus such as garcinol, isogarcinol, alpha mangosteen, beta mangosteen, gambogenic acid [10-12]. *Garcinia morella* is a lesser explored plant of this genus and mainly found in northeastern region of India, Southern region of India, China, and Sri Lanka. It is commonly called as Kuji Thekera in Assam. The fruit of this plant is used in traditional medicine system for treatment of inflammatory disorders, bowel syndromes, wound healing, and tumors.

Neuroblastoma is childhood tumor associated with the sympathetic nervous system. It is aggressive tumor with severe clinical complexities often leading to high rate of mortality. Neuroblastoma is characterized to have cellular heterogeneity and human neuroblastoma derived cell lines retain the heterogeneous character [13]. Even with the recent advancements of medical field, still, neuroblastoma accounts for approximately 15% of cancer deaths in younger children [14]. Hence, novel chemotherapeutic agents from natural sources are much sought for treatment of such form of deadly cancer.

In our previous report, we demonstrated the anticancer activity of *G. morella* fruit on T-cell murine lymphoma in in vitro and in vivo condition [9]. Since there are no earlier reports of anticancer activity of *G. morella* fruit on neuroblastoma, we have taken up this study to support our claim of *G. morella* fruit to a be a rich source of anticancer compounds. Moreover, Garcinol isolated from plants of Garcinia family are reported to have remarkable anticancer efficacy, but there are no reports highlighting its effect on neuroblastoma. Thereby, we embarked on this preliminary study to establish the anticancer potential of Garcinol on neuroblastoma cell line SH-SY5Y.

METHODS

Collection and identification of plant samples

G. morella fruits were collected from Sorbhog, Patchala district, Assam, India (N26.33′37E091.00′99) during the month of February, 2012. The plant sample was identified and authenticated by taxonomist at Northeast Indian Ayurvedic Research Institute (Government of India) Guwahati, Assam, India. The fruit sample was preserved and herbarium was prepared and deposited in drug Discovery Laboratory, Institute of Advanced study in Science and Technology, Guwahati with voucher no IASST/BCCS/HNO112/2012.

Preparation of extract

The fruit samples were finely cut into small pieces and air dried. The dried pericarps were grounded to coarse powder and extracted at room temperature by continuous stirring in methanol for 3 consecutive days. The extract was filtered and the solvent was evaporated by rotar evaporator at 45°C. The dried extract obtained was further aseptically air dried and stored in closed container at 4°C.

Phytochemical analysis

Methanol extract of *G. morella* was phytochemically investigated by standard protocols [15].

Fractionation of *G. morella* crude extract

1 kg of *G. morella* crude methanol extract was loaded to a silica gel column and eluted with solvents in increasing polarity such as hexane: Ethyl acetate, ethyl acetate, chloroform, and methanol: Chloroform. The fractions were collected separately and rotor evaporated. The dried samples were stored in tight containers at 4°C for biological assays.

Identification of garcinol in chloroform fraction

The chloroform fraction was further fractionated in flash chromatography. 1.2 g chloroform fraction was loaded in 12 g silica gel column and eluted with hexane: Ethyl acetate gradient. The eluent of each peak was separately collected with regular monitoring by thin-layer chromatography. A yellow powder obtained in subfraction 23 was identified by determination of melting point of the compound.

Anticancer activity

Chemicals

Dulbecco's modified eagle's medium (DMEM), fetal bovine serum (FBS), trypsin ethylenediaminetetraacetic acid (EDTA), phosphate buffered saline, pentsrep, dimethyl sulfoxide (DMSO), and 3-(4,5–dimethyl thiazol–2–yl)–2,5–diphenyl tetrazolium bromide (MTT) were procured from Sigma Aldrich Co., St. Louis USA. Solvents Methanol, Chloroform, Hexane, and Ethylacetate were purchased from Merck Ltd., Mumbai, India. Garcinol was procured from Santa Cruz Biotechnology Inc.

Cell culture

Neuroblastoma cell line (SH-SY5Y) was obtained from National centre for cell science, Pune. SH-SY5Y cells were maintained in DMEM with 10% FBS and 1% Pen strep in humidified atmosphere of 5% CO_2 incubator at 37°C. The cells were grown in T-25 flask till confluent then they were dissociated from the flask by trypsin EDTA. The dissociated cells were counted using cell counter. All the cytotoxic assays were carried out using 96 well culture plates (Tarsons, India Pvt. Ltd., Kolkata, India).

Drug preparation

Stock solution of *Garcinia morella* fruit chloroform fraction (GFCH) (100 mg/ml) was prepared in 100% DMSO. Different dilutions of GFCH were made in incomplete sterile DMEM. Garcinol was dissolved in 100% DMSO at a concentration of 10 mm. The stock solution was further diluted in incomplete DMEM for experiments.

Evaluation of cytoxicity of the test samples by MTT assay

Anticancer activity of chloroform fraction GFCH and garcinol on neuroblastoma cell line SHSY5Y was determined by MTT assay [16-18]. Briefly, SHSY5Y cells were seeded in 96 well plates at a density of 5000 cells per well in DMEM media and 10% FBS and 1% Pen strep and plates were incubated overnight in a 5% CO_2 incubator. After 24 hrs, GFCH at doses of 1.56, 3.12, 6.25, 12.5, and 25 µg/ml and garcinol at concentrations of 2.5, 5, 10, and 20 µm were added to the respective wells. The plates were incubated for 24, 48, and 72 hrs. At the commencement of drug treatment period of each plate, they were removed from the incubator, and the media containing drug was disposed. Fresh media was filled in each well preceded by addition of 4 mg/ml of MTT solution. The plates were then kept at 37°C incubator in dark for 4 hrs. After the reaction period, the media containing MTT was disposed from the wells and the Formazan formed at the bottom of the well was suspended in DMSO. The plates were then read at 570 nm in a multimode reader. The optical density of each well was recorded and the percentage inhibition of proliferation of the neuroblastoma cells by treatment with different doses of the drugs were calculated by the formula:

$$\text{Percentage of live cells } (\%) = \frac{\text{Mean OD of test sample} - \text{Mean OD of blank}}{\text{Mean OD of control} - \text{Mean OD of blank}} \times 100$$

Percentage inhibition of the drug = 100-percentage of live cells (%).

RESULTS

Phytochemical analysis

From Table 1, *G. morella* fruit was found to be composed of phytochemicals such as flavonoid, phenol, saponin, tannin, steroid, and terpenoid.

Compound 23 was found to have a melting point 132°C. With literature survey of other plants of *Garcinia* family, compound 23 was hinted to be garcinol.

Anticancer assays

The results of the MTT assay clearly show the dose and time-dependent activity of GFCH against neuroblastoma cell line SHSY5Y. IC_{50} of GFCH on SH-SY5Y cells after an exposure time of 24 h, 48 h and 72 h was found to be 5.3, 4.32, and 3.96 µg/ml, respectively. At all durations of exposure to GFCH, the activity of GFCH on SHSY5Y was found to be dose dependent, i.e., the percentage inhibition of proliferation of SHSY5Y was increasing with increasing concentrations of GFCH (Fig. 1) Lower value of IC_{50} at longer duration of treatment period indicated that GFCH-induced highest activity at 72 hrs (Table 2). In a similar study through MTT assay, IC_{50} of *Datura metel* leaf and stem extract was evaluated against Vero and MCF7 cell line [19]. In another study, IC_{50} of *Stephania elegans* on MCF7 was reported to be 158.7 ± 0.13 µg/ml [20].

Similarly, anticancer activity of different concentrations of garcinol on neuroblastoma cell line SHSY5Y for different doses and period revealed that garcinol-induced significant dose and time activity against SHSY5Y cells (Fig. 2). IC_{50} of garcinol on SHSY5Y at 24, 48, and 72 hrs was found to be 7.78, 6.80, and 6.30 µm, respectively (Table 2). Interestingly, IC50 doses of both GFCH and Garcinol were found to decrease upon increasing the exposure time. Thus, at the longest duration of the treatment period (72 h), GFCH and garcinol induced the highest activity.

DISCUSSION

According to American cancer society 2009-2013, Neuroblastoma is the third most popular type of cancer detected in children and adolescents. Neuroblastoma is reported to be resistant to chemotherapeutic drugs and display traits of recurrence [21]. Thus, research towards the identification of novel anti-neuroblastoma agents and their subsequent development into drugs is still recommended to improve the patient condition.

Recent studies have revealed the capability of various plant based products in inhibiting the proliferation of several cancer cell lines including neuroblastoma [22-24]. Plants of Garcinia family are cited to have anticancer activity against different types of cancer cell lines. But to our knowledge, the activity of *G. morella* has never been investigated against neuroblastoma. Thereby, in the present study, for the first time, we reported the remarkable efficacy of *G. morella* fruit against neuroblastoma. In similar studies, the n-Hexane fraction of *Nardostachys jatamansi* was reported to have exhibited 54 % and 91% inhibition against neuroblastoma cell line at 30 and 100 µg/ml respectively[24]. Similarly, *Saussurea lappa* Clarke root extract was found to induce dose dependent activity against neuroblastoma cell line via apoptotic pathway [25]. From our results, we can interpret that GFCH has a higher cytotoxic potential (IC50 5.3 µg/ml at 24 h) on neuroblastoma than the other previously reported plant crude extracts. In our previous study, we have demonstrated the apoptosis inducing the effect of *G. morella* fruit extract on Dalton's ascites lymphoma cells by activation of caspases [9]. In the present study, we can hypothesise the involvement of apoptotic markers in bestowing cytotoxic potential to *G. morella* fruit against neuroblastoma.

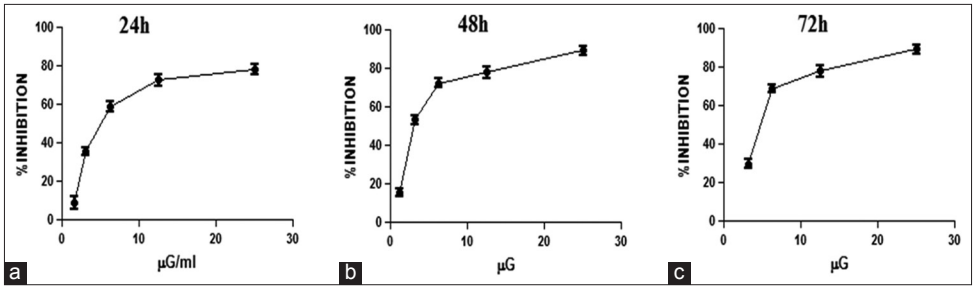

Fig. 1: Effect of GFCH (µg/ml) on the proliferation of SH-SY5Y cells after treatment for (A) 24 h, (B) 48h and (C) 72h. Results are expressed as mean ± S.D. (standard deviations), (N=6). GFCH: chloroform fraction of *Garcinia morella*.

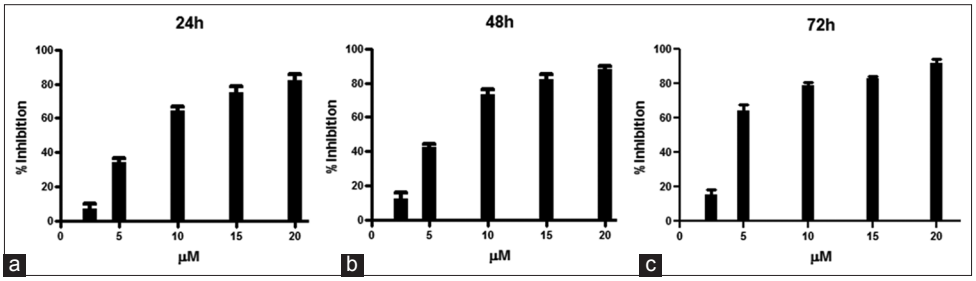

Fig 2: Effect of Garcinol (µM) on the proliferation of SH-SY5Y after treatment for (A) 24 h, (B) 48 h and (C) 72 h. All the results are expressed as mean ± S.D. (standard deviations), (n=6).

Table 1: Phytochemical analysis of *G. morella* fruit chloroform fraction

Extracts	Flavonoid	Tannin	Saponin	Phenol	Steroid	Terpenoid
GFM	+++	+++	+++	+++	++	+++

GFM: *Garcinia morella* fruit methanol extract. *G. Morella: Garcinia morella*

Polyisoprenylated benzophenone, Garcinol is isolated from different species of Garcinia family especially from *G. indica* and *G. mangostana*. Many studies have reported the significant anticancer activity of Garcinol on a diverse range of cancer cell lines. Garcinol is reported to have dose dependent activity against prostate cancer cell lines PC-3, LNCaP and DU-145 [26]. The types of cancer on which cytotoxic effect of garcinol is tested are breast cancer [27], Burkitt lymphoma [28], Colon cancer [29], esophageal cancer [27], hepatocellular carcinoma [30]. We assume that garcinol exhibits anticancer activity against neuroblastoma through triggering the apoptotic cascade.

CONCLUSION

The results of this study indicate that *G. morella* fruit can be a potential anticancer source against neuroblastoma. More importantly, garcinol the main bioactive molecule of *G. morella* chloroform fraction was found to be highly effective against this cancer. Thereby, our study instigates new research in this direction which may lead to the development of a novel drug for treatment of neuroblastoma.

ACKNOWLEDGMENT

Authors are very much thankful to Department of Science and Technology, the Government of India, New Delhi for providing the financial support to conduct this study and the host institute, Institute of Advanced study in science and technology in providing necessary facilities.

AUTHOR CONTRIBUTION

Ms. Choudhury is the main author of this research work including study design, experimental setup and writing of the manuscript. She performed all the experiments. Mr. Kandimalla contributed towards study design and compilation of data. Dr. Kotoky and Dr. Bharali supervised the design of experiments and assessment of results.

Table 2: IC_{50} of GFCH and garcinol on SH-SY5Y

Drugs	24 hrs	48 hrs	72 hrs
GFCH (µg/ml)	5.3	4.32	3.96
Garcinol (µm)	7.78	6.80	6.30

GFCH: *Garcinia morella* fruit chloroform fraction, IC_{50}: Inhibitory concentration

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