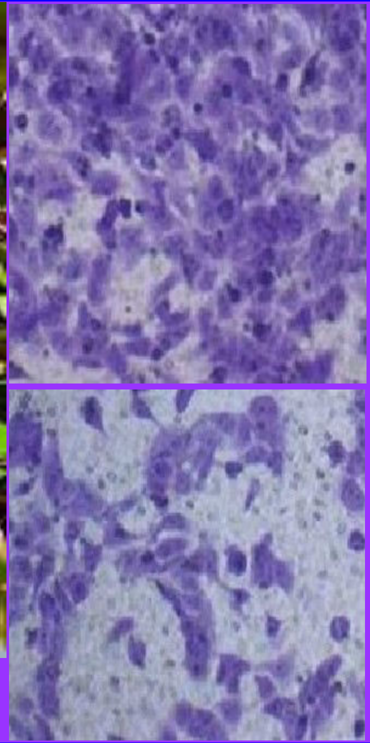

BJP

Bangladesh Journal of Pharmacology

Research Article

Poly-phenolic fraction of *Chryso-phyllum cainito* extract induces cell death in osteosarcoma cells

A Journal of the Bangladesh Pharmacological Society (BDPS) **Bangladesh J Pharmacol 2015; 10: 972-979**
Journal homepage: www.banglajol.info
Abstracted/indexed in Academic Search Complete, Asia Journals Online, Bangladesh Journals Online, Biological Abstracts, BIOSIS Previews, CAB Abstracts, Current Abstracts, Directory of Open Access Journals, EMBASE/Excerpta Medica, Global Health, Google Scholar, HINARI (WHO), International Pharmaceutical Abstracts, Open J-gate, Science Citation Information Expanded (SCIE), SCOPUS and Social Sciences Citation Index;
ISSN: 1991-0088

Poly-phenolic fraction of *Chrysophyllum cainito* extract induces cell death in osteosarcoma cells

Liu-Bing Li, Subin Lin, Jun Yan, Qian-Liang Wang, Zhi-Ying Fan, Qi-Rong Dong, Jian-Zhong Qin and Zong-Gang Xie

Department of Orthopedics, The Second Affiliated Hospital of Soochow University, Suzhou, Jiangsu 215004, China.

Article Info

Received: 18 August 2015
Accepted: 24 September 2015
Available Online: 24 November 2015

DOI: 10.3329/bjp.v10i4.24576
Cite this article:
Li LB, Lin S, Yan J, Wang QL, Fan ZY, Dong QR, Qin JZ, Xie ZG. Poly-phenolic fraction of *Chrysophyllum cainito extract* induces cell death in osteosarcoma cells. Bangladesh J Pharmacol. 2015; 10: 972-79.

Abstract

Osteosarcoma shows resistance to chemotherapy and many side effects. The ethyl acetate fraction of ethanolic extract of *Chrysophyllum cainito* was taken in the present work. The extract was characterized for its phenolic content, antioxidant capacity (ferric reducing antioxidant power and 2,2-diphenyl-1-picrylhydrazyl assays), ability to form reactive oxygen species and annexin V assay. The extract contained phenolic content of 30.1 ± 0.2 mg GAE/g extract. Ferric reducing antioxidant power assay and DPPH assay showed 213.2 ± 12.4 mM Fe^{2+} equivalent per gram extract and 85.6 ± 4.9 TE/g respectively. For production of reactive oxygen species in the osteosarcoma cells, extract at 50 µg GAE/mL concentration was statistically equivalent to 100 µM H_2O_2. EC_{50} of extract was calculated to be 133 µg GAE/mL in cell viability studies.

Introduction

One of most common primary bone sarcoma is osteosarcoma which is a leading cause of cancer death among adolescents, young adults and elder population. It leads to 20% mortality among primary bone cancer and 2.4% among all malignancies in pediatric patients (Huang et al., 2012). Molecular etiology of osteosarcoma is complex and not established (Jones et al., 2012). Chemoresistance is the main cause of treatment failures and is found very common with unclear molecular mechanisms (Huang et al., 2012).

The herbs and their extracts, which are polyphenol rich, have been shown to have effect against hypertension (Ichimura et al., 2006; Oboh et al., 2012), cancer (Ayoub et al., 2010; Hafeez et al., 2006; Martin et al., 2013), Alzheimer's disease (Wang et al., 2008) and cardiovascular diseases.

One of the plant in the category of polyphenolic enriched class is *Chrysophyllum cainito* L., belonging to family *Sapotaceae* (Lawal et al., 2010), commonly known as star apple (Parker et al., 2010). Its various parts have great amount of polyphenolic compounds e.g. 387.1 ± 223.2 mg/100 g in fruit pulp, 73.5 ± 52.0 mg/100 g seed (Parker et al., 2010). *C. cainito* plant as such or its extracts has been studied to cure many diseased conditions (Bautista-Banos et al., 2002; Einbond et al., 2004; Lawal et al., 2010; Luo et al., 2002; Meira et al., 2014; Nguessan et al., 2009).

In the present research work, the polyphenolic extract fraction of *C. cainito* fruit was evaluated for anti-cancer activity against osteosarcoma both *in vitro* and *in vivo.*

Materials and Methods

Materials

Gallic acid, vincristine, propidium iodide, formalin, crystal violet, crystal violet, fetal bovine serum (FBS), chloroquine, penicillin, streptomycin, 2′, 7′-dichlorofluorescein diacetate, Folin-Ciocalteu's phenol reagent and annexin V-FITC apoptosis detection kit were purchased from Sigma-Aldrich. Remaining chemicals and reagents utilized in this research work were of analytical grade and were used as such.

Preparation of polyphenolic fraction of extract

Fruits of *C. cainito* were collected from Hunan Province, China and were supplied by Wu Shi Pharmacy Ltd. Co., China. The identification of the plant was done by Prof. Ding-Xian Han, College of Life Science and Technology, Huazhong University of Science and Technology, China. Fruit pulp was separated from the seeds and cut into small pieces for drying in shades for 120 hours and the dehydrated by lyophilization (freeze drying). Such dried pieces were ground in an electrical grinder and passed through sieve number 5 (4 mm diameter). The polyphenolic fraction of extract was prepared by the method found in literature which was followed with little modifications (Antonio and Brito, 1998; Dhamija et al., 2013). First the alcoholic extract was prepared using coarse, dried powder of fruit of the plant *C. cainito* (250 g) for the hot extraction process (soxhlet) with ethanol (1000 mL) for 20 hours. The concentrated extract was dried by lyophilization. 20 g of ethanolic extract was suspended in water and was fractionated successively and exhaustively with ethyl acetate using separating funnel resulted in extract-fraction of *C. cainito*. The total phenolic content in the extract was estimated by Folin-Ciocalteu method. Gallic acid was taken as standard (standard, 10-150 µg/mL). Total phenolic content was estimated as mg Gallic acid equivalents (GAE)/g of extract (Saeed et al., 2012).

Determination of total phenol content

The total phenolic content of the extract was estimated by Folin-Ciocalteu method as given in literature (Saeed et al., 2012; Tedesco et al., 2013). In brief, 0.5 mg/mL of extract was taken and heated at 90°C for 10 min. 50 µL of extract was mixed with 50 µL distilled water and 500 µL of complex forming reagent (50:50:1:1 of 2% Na_2CO_3, 2% NaOH, 1.5% $CuSO_4$, 2.5% sodium potassium tartrate) and incubated at 37°C for 10 min. After incubation 100 µL Folin-Ciocalteu reagent was added and incubated at 37°C for next 30 min. Absorbance was taken at 750 nm. Gallic acid was taken as standard (10-150 µg mL^{-1}). Total phenolic content was estimated as mg gallic acid equivalents (GAE)/g of extract.

Ferric reducing antioxidant power (FRAP) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay

Reducing or antioxidant capacity of extract was determined by FRAP assay which is based on reduction of ferric tripyridyl triazine (FeIII TPTZ) complex to ferrous form (deep blue color) due to reduction by antioxidants. In particular 100 µL of extract (5 mg/mL) were added to 1 mL of FRAP reagent (TPTZ 1 mM, Fe^{3+} 2 mM in acetate buffer of 30 mM pH 3.6) and incubated for 6 min at room temperature. The change in color and intensity can be monitored by taking absorbance at 593 nm in UV spectrophotometer. The results were expressed in millimolar of ferrous sulfate (standard) (Tedesco et al., 2013).

The scavenging/antioxidant capacity of extract for the stable free radical DPPH was monitored. 100 µM DPPH in methanol was mixed with extract solution (0.5 mg extract/mL) by vortexing for 15 sec and incubated for 30 min in the dark at room temperature. Absorbance was taken at 517 nm. The results were expressed in terms of equivalents of trolox (TE) µmol/g of extract (Alomar, 2015).

U-2 osteosarcoma cell culture

The U-2 osteosarcoma (ATCC HTB-96™) cell line of human osteosarcoma was obtained from ATCC, USA and maintained in Dulbecco's modified Eagle's medium (DMEM) along with fetal bovine serum (10% v/v), L-glutamine (2 mM), penicillin (100 U mL^{-1}), streptomycin (100 µg/mL) at 37°C, in a 5% CO_2 humidified atmosphere and harvested at approximately 90% confluence.

Determination of reactive oxygen species (ROS)

ROS production in the cells was assayed with 2′, 7′-dichlorofluorescein diacetate, a non-fluorescent reagent with free permeation to cells (Abdullah et al., 2015). ROS stimulation by treatment with 0, 10, 50, 100 and 200 µg GAE/g extract and H_2O_2 10 µM (positive control) for 24 hours was determined in the U-2 osteosarcoma cells. Treated cells were washed with 2',7'-dichlorofluorescein diacetate (10 µM) in phosphate buffer and incubated for 30 min to induce oxidation of 2',7'-dichlorofluorescein diacetate to dichlorofluorescein (DCF) by ROS. The cell lysis was carried out using lysis buffer (50 mM Tris-HCl, 1% Triton X-100, 100 mM NaCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 300 mM sucrose, pH 7.4). At an excitation wavelength of 485 nm the fluorescence (Trilogy, Turner Designs Inc) of suspensions was taken at 530 nm.

Cell viability assay

Viability experiment protocol was taken from literature (Hsu et al., 2013; Tedesco et al., 2013). In brief, 0.5 mL of cell suspension of 2 x 10^5/mL was prepared and incubated for 24 hours. Cells were again incubated for 24 hours with lyophilized extract fraction of *C. cainito* of different GAE concentrations (200, 150, 100, 50, 25, 10 and 5 µg GAE/mL in phosphate buffer pH 7.0, 5 mM), taking appropriate control. The cells were separated from medium and washing was done phosphate buffer pH 7.0, 5 mM. Fixing was done by treating the cells with 10% formalin and incubating them for 15 min at room temperature. Formalin treated cells were then treated with crystal violet (0.1% w/v) and was incubated for 30 min at room temperature. Images of cells were taken with inverted microscope in bright field at 400x magnification (Olympus, Japan). After washing, 10% acetic acid was used to solubilize with and absorbance was taken at 590 nm in UV-Vis spectrophotometer.

Apoptotic assays

Expression of phosphatidylserine on outer layer of plasma membrane has been a marker for apoptosis and was measured using annexin-V (assay protein, having strong affinity for phosphatidylserine) labeled with fluoresceinisothiocyanate (FITC, a reporter molecule) (Szliszka et al., 2011; Tedesco et al., 2013). The experiment was carried out as per the manufacturer's protocol. In brief, U-2 osteosarcoma cells (1 x 10^6 /mL) were allowed to grow in 24-well plates for 48 hours then incubated with extract at different concentrations (10, 50, 100 and 200 µg GAE/mL) and standard for 48 hours. The U-2 osteosarcoma cells were washed in saline phosphate buffer and re-suspended in 200 µL of binding buffer (10 mM HEPES, pH 7.4; 140 mM NaCl; 2.5 mM $CaCl_2$). Cells were allowed to incubate with annexin-V FITC (2 µL) and propidium iodide (10 µL) for 10 min in the dark at room temperature. Then, 400 µL of binding buffer was poured to each reaction mixture and mixed properly. Flow cytometer (BD Biosciences, USA) was used for analysis of annexin V-FITC at 530 nm. Low fluorescence debris and necrotic cells, which were permeable to propidium iodide, were omitted out from analysis.

Colorimetric assay kit (Beyotime) for caspase-3 activity determination was utilized. Measurement of p-nitro-anilide (pNA), produced after the caspase-3 activity on the labeled substrate Ac-Asp-Glu-Val-Asp-pNA (DEVD -pNA), was the basis of this assay. U-2 osteosarcoma cells (1 x 10^6 /mL) were incubated with extract (50, 100, 200 and 300 µg GAE/mL) and standard (vincristine 10 µg/mL) for 16 hours. Cells were pelleted and lysed with cell lyses buffer (10 mM HEPES, pH 7.4; 2 mM ethylenediamine tetraacetic acid; 0.1% 3-[(3-cholamido-propyl) dimethylammonio]-1-propanesulfonate; 5 mM dithiothreitol; 1 mM phenylmethyl sulfonylfluoride; 10 µg/mL pepstatin-A; 10 µg/mL aprotinin; 20 µg/mL leupeptin) and incubated on ice for 20 min. After 20 min incubation, centrifugation was carried out (10,000x g for 30 min). The supernatant was taken for caspase activity determination and protein estimation. 300 µL reaction mixture was containing 10 mM DTT, 5 mM DEVD-pNA (substrate) in phosphate buffer (pH 7, 5 mM) and cellular lysate of 10 µg protein. Cell lysate was added in last, mixed and incubated immediately at 37° C for 30 min. Absorbance was taken with an ELISA micro-plate reader (Emax, Molecular Devices) at 405 nm. The activity of caspase-3 was determined by subtraction of appropriate control (untreated) and compared with positive control (vincristine) (Ramasamy et al., 2013).

Statistical analysis

The results are expressed as the mean ± SD obtained from at least triplicate of experiment. Significance was evaluated statistically using analysis of variance i.e. ANOVA (Dunnett's or Tukey's test wherever required). The difference with p value less than 0.05 was taken as significant.

Results

The polyphenolic content in ethyl acetate fraction of alcoholic extract of *Chrysophyllum cainito* was found to be 30.1 ± 0.18 mg GAE/g. The antioxidant capacity in the ethyl acetate fraction of alcoholic extract of *C. cainito*, was found to be 213.2 ± 12.4 mM Fe^{2+} equivalent/g extract or 7.1 ± 0.4 mM Fe^{2+} equivalent/ mg GAE and DPPH assay which showed 85.6 ± 4.9 TE/g.

The effect of extract concentration on production of ROS was found to be increased with increase in concentration of extract (Figure 1). The extract at concentration 10 µg GAE mL^{-1} was showing increase but insignificant in light intensity i.e. ROS concentration in U-2 osteosarcoma cells. While treatment of extract at concentration 50 µg GAE/mL and above were showing ROS concentration significantly higher as compared to control i.e. no treatment. In another viewpoint, extract at 50 µg GAE/mL concentration was statistically equivalent to positive control i.e. 100 µM H_2O_2 while higher concentrations of extract were significantly more effective than that of positive control. When the production of ROS with concentrations of 100 and 200 µg/mL were compared, the two were found to be statistically same.

In Figure 2, there was clearly observable difference in the growth of the cells between untreated and treated (100 and 200 µg GAE/mL). The percentage of viable cells was also determined by taking absorbance of extracted cell associated dye at 590 nm. Results showed that extract caused no cell death at concentration 5 and 10 GAE µg/mL (represented as 'ns' as non significant) taking control (no extract) as 100% cell viability. While the higher concentrations from 25 to 200 GAE µg/mL showed significant (Figure 3) and linear relations with cell death (Figure 4). From the linear relation EC_{50} extract was also determined (133 GAE µg/mL).

The effect of extract concentration on apoptotic cell count has been shown in Figure 5. The lowest concentration used for extract (10 µg GAE/mL) was not significant as compared to control in terms of annexin V positive cell counts but all the higher concentrations were showing significantly higher number of annexin positive cell counts. In comparison to standard positive control (vincristine 10 µg/mL) only extract of 100 and 200 µg GAE/mL was statistically equivalent.

Results showed that highest effect as increased caspase specific activity was observed at extract concentration 300 µg GAE/mL (43.8 ± 6.6 µmol/min/mg in comparison to 5.7 ± 1 µmol/min/mg untreated control i.e. approximately 8-fold) as shown in Figure 6. Moreover, caspase specific activity at this extract

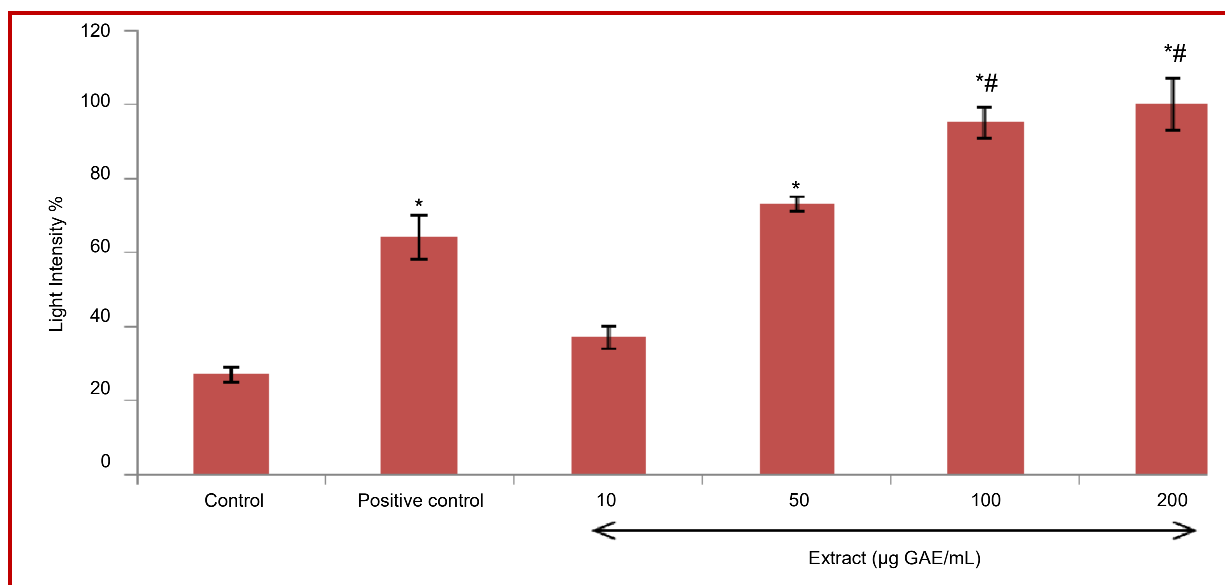

Figure 1: Effect of extract of different GAE concentrations on amount of reactive oxygen species in terms of intensity of fluorescence by DCF taking maximum (extract 200 µg GAE/mL) as 100%. Results represent the mean DCF fluorescence with error bars representing SD from three experiments. Positive control represented the treatment of the cells with 100 µM H_2O_2. *represents significant difference at $p<0.05$ from the untreated control and #represents the significance in comparison to standard (H_2O_2 100 µM) positive control

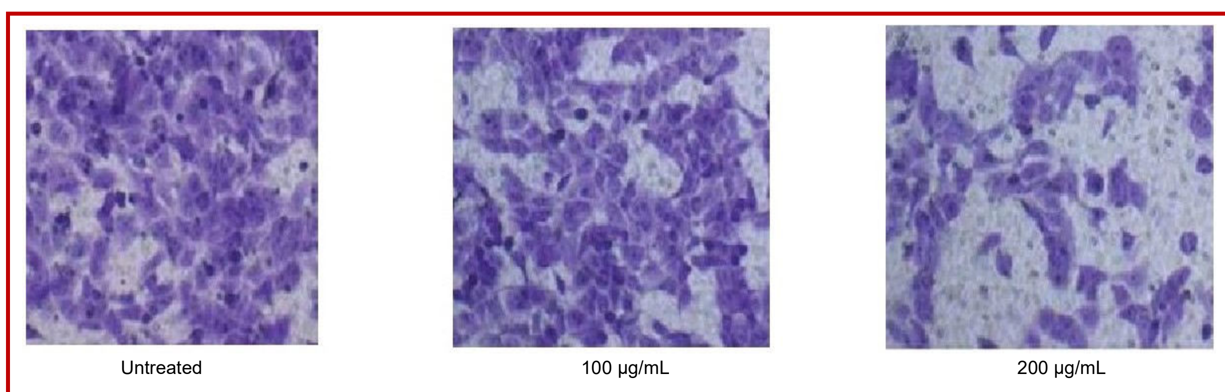

Figure 2: Images of U-2 osteosarcoma cells after 24 hours of untreated cells and treatment with extract of 100 and 200 µg/mL. Images of cells were taken with inverted microscope in bright field at 400x magnification (Olympus, Japan)

concentration i.e. 300 µg GAE/mL was significantly higher (43.8 ± 6.6 µmol/min/mg) than that of the positive control (32.5 ± 3.5 µmol/min/mg).

Discussion

Clinical reports have shown the relation of polyphenolic compounds intake through food with prevention of particular cancers, decreased risk in terms of different cancer types (Arts et al., 2002; Su and Arab, 2002) or a reduced recurrence (Le Marchand et al., 2000). There are many polyphenolic compounds such as gallocatechins, isoflavones, stilbenes, tannins and curcuminoids have been shown to have strong chemopreventive properties. Studying the mechanism of action as anticancer of these compound is an uncovered area of considerable interest (Yar Khan et al., 2012).

The effect of extract concentration on production of ROS which can be correlated with apoptosis. Oxidative stress usually cause the cells to enter apoptosis and ultimately to cell death. In contrast, one research showed the stress caused by H_2O_2 (an oxidative stress) in presence of mammalian p38α caused up-regulation gene responsible for antioxidant defenses in the cells viz. superoxide dismutase 1 (SOD-1), SOD-2 and catalase which naturally protects the cell from ROS accumulation and leads to cell survival (Gutierrez-Uzquiza et al., 2012). Similarly, antioxidants are thought to protect the cells from oxidative stress (Dhamija et al., 2014; Lowes et al., 2013) but gallic acid, a phenolic compound with antioxidative activity, has been

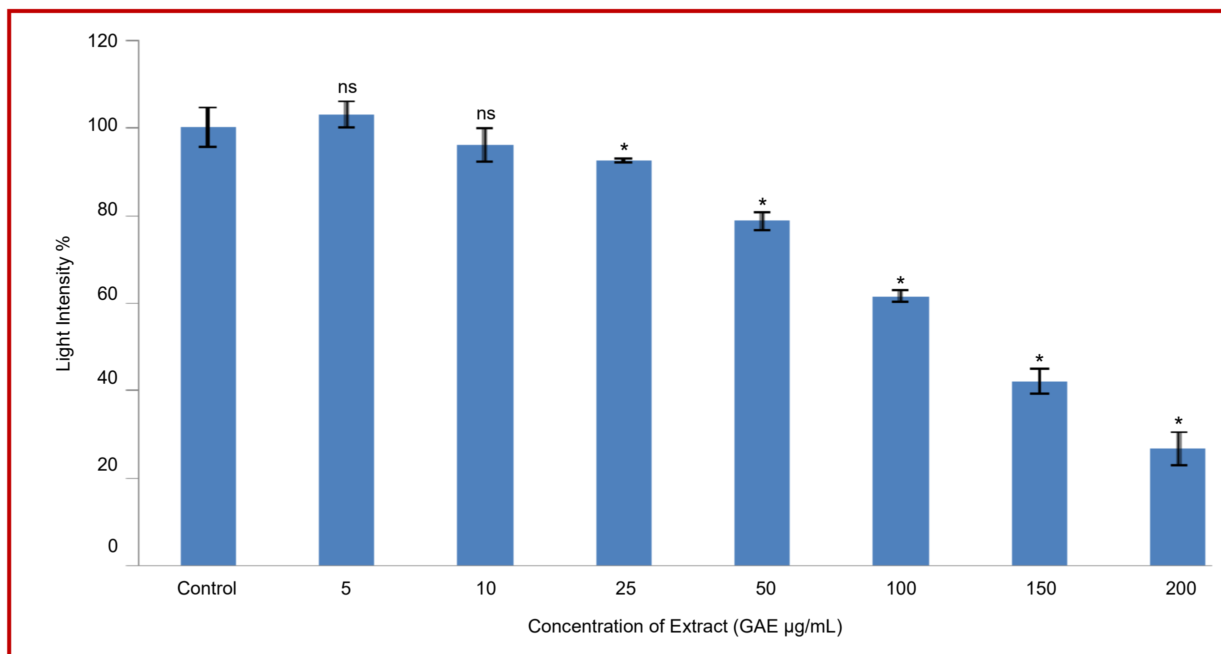

Figure 3: Effect of different concentration of extract in terms of GAE on U-2 osteosarcoma cell viability. The value was average of three samples with standard deviation as error bars. *represents the significant difference and ns means non-significant compared to control with $p<0.05$ using Dunnet multiple comparison test

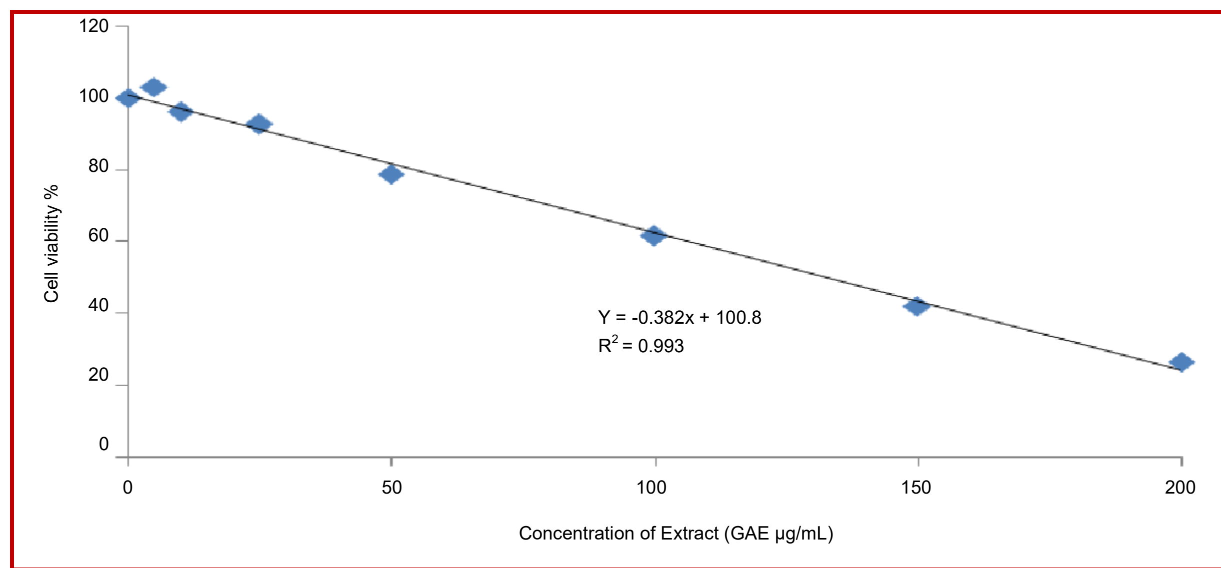

Figure 4: Linear relation between extract concentration in terms of GAE on U-2 osteosarcoma cell viability

reported to cause apoptosis in promyelocytic leukemia cells. This assay of cell death/inhibition was the proof of involvement of reactive oxygen species such as H_2O_2, superoxide anion in addition to Ca^{2+} ion and calmodulin-dependent enzymes. Its structure activity relationship studies gave the idea of apoptosis induction in this particular cell line was depending on apoptosis activity was derived from the structure, not from its antioxidative property (Inoue et al., 1994). Similar to cytotoxicity study done in this research, the cytotoxicity of curcumin, a well-known representative natural polyphenolic compound, was checked at 5, 10, 25, 50, 75, and 100 µM concentration and comparison was established between healthy human osteoblast cells and osteosarcoma (MG-63 cell line). As a consequence of curcumin treatment, result showed the 10 µM concentration retaining less than 50% viability of osteosarcoma cell compared to the control while healthy osteoblast cells retained at least 80% viability at all concentrations of curcumin (Chang et al., 2014).

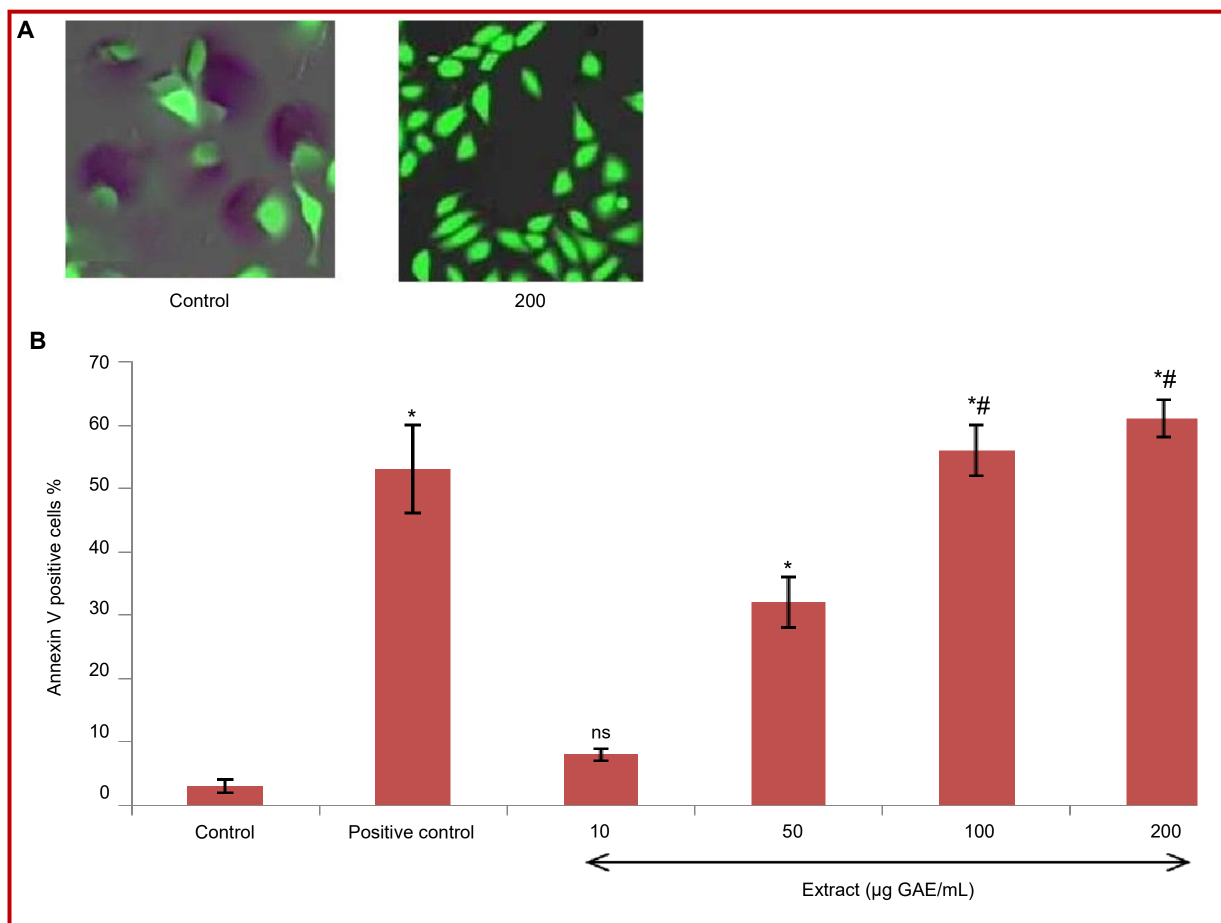

Figure 5: A. Fluorescent image of U-2 osteosarcoma cells. B. Effect of extract of different GAE concentrations on apoptotic cells in terms of annexin V positive cells. Results are represented as the mean of %annexin V positive cells with error bars representing SD from three experiments. Positive control represented the treatment of the cells with 10 µg/mL vincristine. *represents significant difference and 'ns' represented non significant difference at $p<0.05$ from the untreated control and #represents the non significance i.e. equivalence in comparison to standard (vincristine 10 µg/mL) positive control

The phosphatidylserine (PS) translocation to the outer membrane from cytosolic membrane is one of important signature of apoptosis at very early stage. The assay of PS over outer membrane has been used to evaluate the potential drugs or extracts. Its assay exploits the affinity of annexin V which is labeled with FITC and this FITC attached cells cause green fluorescence and separation in flow-cytometer for calculation of cell count which are annexin V positive which means PS positive or apoptotic cells (Tedesco et al., 2013) while the propidium iodide gives red fluorescence and is representative of late apoptotic cells or necrotic cells were omitted from the analysis. It was also noteworthy that the pattern of result of extract on apoptotic cells was similar to that obtained from production of ROS. This relation suggested that apoptosis by ROS is the mechanism used by the compounds in the extract.

Increase in caspase activity has also been one of path line of the cell's pathways to undergo apoptosis. In a study by Tedesco et al. (2013) the caspase activity was found to be significantly decreased (unexpectedly) at 200 µg lyophilized dealcoholated red wine mL^{-1} concentration than lower concentrations. The interpretation behind this unexpected was excessive killing of cells at higher concentrations while optimal time for increase in caspase activity had already been achieved (Tedesco et al., 2013).

Conclusion

The plant *Chrysophyllum cainito* has a great potential in aurvedic medicines and could be great source of lead novel molecule for allopathic medicines for treatment of osteosarcoma. The significance in each result obtained in this study clearly depicts its candidature for further exploration.

Acknowledgement

The research was supported by the Youth Research Institute

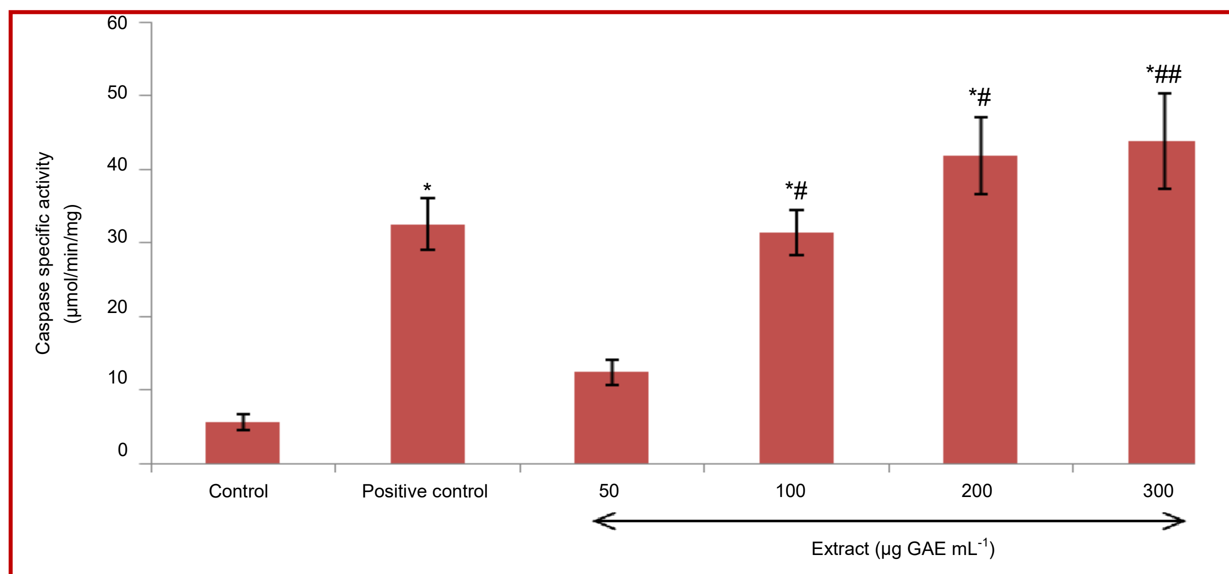

Figure 6: Effect of extract of different GAE concentrations on apoptotic cells in terms of caspase specific activity in cells. Results are represented as the mean of caspase specific activity (µmol/min/mg) with error bars representing SD from three repeats. Positive control represented the treatment of the cells with 10 µg/mL vincristine. *represents significant difference at $p<0.05$ from the untreated control and #represents the non significance i.e. equivalence in comparison to standard (vincristine 10 µg/mL) positive control while ##represents the significantly higher caspase specific activity than that from standard or positive control

Foundation Of the Second Affiliated Hospital of Soochow University (No. SDFEYQN1401 and 1411), and Sports Scientific Research Bureau Program of Suzhou Sports Bureau (No. TY2014-202). This project is subject to the second affiliated hospital of Soochow university preponderant clinic discipline group project funding.

References

Abdullah ASH, Mohammed AS, Rasedee A, Mirghani MES. Oxidative stress-mediated apoptosis induced by ethanolic mango seed extract in cultured estrogen receptor positive breast cancer MCF-7 Cells. Int J Mol Sci. 2015; 16: 3528-36.

Alomar S. Cadmium sulfide nanoparticle induces oxidative stress and pro-inflammatory effects in human lung adenocarcinoma epithelial cells. Toxicol Environ Chem. 2015; 97: 619-33.

Antonio MA, Brito ARMS. Oral anti-inflammatory and anti-ulcerogenic activities of a hydroalcoholic extract and partitioned fractions of *Turnera ulmifolia* (Turneraceae). J Ethnopharmacol. 1998; 61: 215-28.

Arts I C W, Jacobs Jr, D R, Gross, M, Harnack, L J,Folsom, A R. Dietary catechins and cancer incidence among postmenopausal women: The Iowa Women's Health Study (United States). Cancer Causes Control. 2002; 13: 373-82.

Ayoub N, Singab, AN, El-Naggar, M, Lindequist U. Investigation of phenolic leaf extract of *Heimia myrtifolia* (*Lythraceae*): Pharmacological properties (stimulation of mineralization of SaOS-2 osteosarcoma cells) and identification of polyphenols. Drug Discov Ther. 2010; 4: 341-48.

Bautista-Banos S, Barrera-Necha LL, Bravo-Luna L, Bermudez-Torres K. Antifungal activity of leaf and stem extracts from various plant species on the incidence of *Colletotrichum gloeosporioides* of papaya and mango fruit after storage. Rev Mex Fitopat. 2002; 20: 8-12.

Chang R, Sun, L,Webster, T J. Selective cytotoxicity of curcumin on osteosarcoma cells compared to healthy osteoblasts. Int J Nanomed. 2014; 9: 461.

Dhamija I, Kumar N, Manjula SN, Parihar V, Setty MM, Pai KSR. Preliminary evaluation of *in vitro* cytotoxicity and *in vivo* antitumor activity of *Premna herbacea* Roxb. in Ehrlich ascites carcinoma model and Dalton's lymphoma ascites model. Exp Toxicol Pathol. 2013; 65: 235-42.

Dhamija I, Kumar N, Pai KSR, Setty MM, Kumar S, Jana AK. Exploration of antioxidant and antimicrobial potential of methanolic extract of root stock of *Premna herbacea* Roxb. Bangladesh J Pharmacol. 2014; 9: 663-64.

Einbond LS, Reynertson KA, Luo XD, Basile MJ, Kennelly EJ. Anthocyanin antioxidants from edible fruits. Food Chem. 2004; 84: 23-28.

Gutierrez-Uzquiza A, Arechederra M, Bragado P, Aguirre-Ghiso JA, Porras A. p38a Mediates cell survival in response to oxidative stress via induction of antioxidant genes effect on the p70S6K pathway. J Biol Chem. 2012; 287: 2632-42.

Hafeez BB, Ahmed S, Wang N, Gupta S, Zhang A, Haqqi TM. Green tea polyphenols-induced apoptosis in human osteosarcoma SAOS-2 cells involves a caspase-dependent mechanism with down-regulation of nuclear factor kB. Toxicol Appl Pharmacol. 2006; 216: 11-19.

Hsu SC, Lin JH, Weng SW, Chueh FS, Yu CC, Lu KW, Wood WG, Chung JG. Crude extract of *Rheum palmatum* inhibits migration and invasion of U-2 OS human osteosarcoma cells by suppression of matrix metalloproteinase-2 and -9. Biomedicine 2013; 3: 120-29.

Huang J, Ni J, Liu K, Yu Y, Xie M, Kang R, Vernon P, Cao L, Tang D. HMGB1 promotes drug resistance in osteosarcoma. Cancer Res. 2012; 72: 230-38.

Ichimura T, Yamanaka, A, Ichiba, T, Toyokawa, T, Kamada Y, Tamamura T, Maruyama S. Antihypertensive effect of an extract of *Passiflora edulis* rind in spontaneously hypertensive rats. Biosci Biotechnol Biochem. 2006; 70: 718-21.

Inoue M, Suzuki, R, Koide, T, Sakaguchi, N, Ogihara, Y, Yabu Y. Antioxidant, gallic acid, induces apoptosis in HL-60RG cells. Biochem Biophys Res Commun. 1994; 204: 898-904.

Jones KB, Salah Z, Del Mare S, Galasso M, Gaudio E, Nuovo G J, Lovat F, LeBlanc K, Palatini J, Randall RL. miRNA signatures associate with pathogenesis and progression of osteosarcoma. Cancer Res. 2012; 72: 1865-77.

Lawal IO, Uzokwe NE, Igboanugo ABI, Adio AF, Awosan EA, Nwogwugwu JO, Faloye B, Olatunji BP, Adesoga AA. Ethno medicinal information on collation and identification of some medicinal plants in Research Institutes of South-West Nigeria. Afr J Pharma Pharmacol. 2010; 4: 1-7.

Le Marchand LC, Murphy SP, Hankin JH, Wilkens LR, Kolonel LN. Intake of flavonoids and lung cancer. J Natl Cancer Inst. 2000; 92: 154-60.

Lowes DA, Webster NR, Murphy MP, Galley HF. Antioxidants that protect mitochondria reduce interleukin-6 and oxidative stress, improve mitochondrial function, and reduce biochemical markers of organ dysfunction in a rat model of acute sepsis. Br J Anaesth. 2013; 110: 472-80.

Luo XD, Basile MJ, Kennelly EJ. Polyphenolic antioxidants from the fruits of *Chrysophyllum cainito* L. (star apple). J Agric Food Chem. 2002; 50: 1379-82.

Martin MA, Goya L, Ramos S. Potential for preventive effects of cocoa and cocoa polyphenols in cancer. Food Chem Toxicol. 2013; 56: 336-51.

Meira NA, Klein Jr LC, Rocha LW, Quintal ZM, Monache FD, Cechinel Filho V, Quintao NLM. Anti-inflammatory and anti -hypersensitive effects of the crude extract, fractions and triterpenes obtained from *Chrysophyllum cainito* leaves in mice. J Ethnopharmacol. 2014; 151: 975-83.

Nguessan K, Amoikon KE, Tiebre MS, Kadja B, Zirihi GN. Effect of aqueous extract of *Chrysophyllum cainito* leaves on the glycaemia of diabetic rabbits. Afr J Pharma Pharmacol. 2009; 3: 501-06.

Oboh G, Ademiluyi AO, Akinyemi AJ, Henle T, Saliu JA, Schwarzenbolz U. Inhibitory effect of polyphenol-rich extracts of jute leaf (*Corchorus olitorius*) on key enzyme linked to type 2 diabetes (a-amylase and a-glucosidase) and hypertension (angiotensin I converting) *in vitro*. J Funct Foods. 2012; 4: 450-58.

Parker IM, Lopez I, Petersen JJ, Anaya N, Cubilla-Rios L, Potter D. Domestication syndrome in Caimito (*Chrysophyllum cainito* L.): Fruit and seed characteristics. Econ Bot. 2010; 64: 161-75.

Ramasamy S, Wahab NA, Abidin NZ, Manickam S. Effect of extracts from *Phyllanthus watsonii* Airy Shaw on cell apoptosis in cultured human breast cancer MCF-7 cells. Exp Toxicol Pathol. 2013; 65: 341-49.

Saeed N, Khan MR, Shabbir M. Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts *Torilis leptophylla* L. BMC Complement Altern Med. 2012; 12: 221.

Su LJ, Arab L. Tea consumption and the reduced risk of colon cancer: Results from a national prospective cohort study. Public Health Nutr. 2002; 5: 419-25.

Szliszka E, Zydowicz G, Janoszka B, Dobosz C, Kowalczyk-Ziomek G, Krol W. Ethanolic extract of Brazilian green propolis sensitizes prostate cancer cells to TRAIL-induced apoptosis. Int J Oncol. 2011; 38: 941-53.

Tedesco I, Russo, M, Bilotto S, Spagnuolo C, Scognamiglio A, Palumbo R, Nappo A, Iacomino G, Moio L, Russo GL. Dealcoholated red wine induces autophagic and apoptotic cell death in an osteosarcoma cell line. Food Chem Toxicol. 2013; 60: 377-84.

Wang J, Ho L, Zhao W, Ono K, Rosensweig C, Chen L, Humala N, Teplow DB, Pasinetti GM. Grape-derived polyphenolics prevent Ab oligomerization and attenuate cognitive deterioration in a mouse model of Alzheimer's disease. J Neurosci. 2008; 28: 6388-92.

Yar Khan H, Zubair H, Fahad Ullah M, Ahmad A, Mumtaz Hadi S. A prooxidant mechanism for the anticancer and chemopreventive properties of plant polyphenols. Curr Drug Targets. 2012; 13: 1738-49.

Author Info
Jian-Zhong Qin and Zong-Gang Xie (Principal contact)
e-mail: qinjianzhong1978@hotmail.com; zonggangxie@hotmail.com

First two authors contributed equally