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Nelumbo nucifera Leaf Extract Induces Cytotoxicity in Osteosarcoma Saos-2 Cells

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Abstract

Background

Osteosarcoma is the eighth most common cancer and its prevalence in children makes it a global concern. Existing medications and treatments like high-dose methotrexate possess harmful side effects. Therefore, novel herbal drugs like *Nelumbo nucifera* are of utmost importance.

Aim

To analyze a novel anticancer herbal drug, *Nelumbo nucifera* leaf extract for its cytotoxic potential against osteosarcoma.

Materials and method

Nelumbo nucifera leaf extract was prepared. Saos-2 Cells (human osteosarcoma cell line) were treated with *Nelumbo nucifera* leaf extract (25, 50, 75, 100, 125, and 150 µg/ml) for 24 hours which were then subjected to MTT assay, morphological analysis and DAPI staining.

Results

The results suggested that *Nelumbo nucifera* leaf extract had a concentration-dependent cytotoxic effect on Saos-2 cell line. The extract significantly reduced the number of viable cells, inhibited proliferation and induced morphological changes in Saos-2 cells.

Conclusion

Nelumbo nucifera has the potential to induce cytotoxicity against osteosarcoma cell lines and hence, this study provides a novel therapeutic regimen for the treatment of osteosarcoma.

Categories: Oncology, Therapeutics
Keywords: saos-2 cells, osteosarcoma, nelumbo nucifera, herbal drug, cytotoxicity, cancer

Introduction

Osteosarcoma, which is also called osteogenic sarcoma, is the most common type of cancer originating in the bones. It is the eighth most common form of childhood cancer accounting for 2.4% of all pediatric cancers and 20% of all primary bone cancers. Long-term survival of osteosarcoma patients can be expected in less than 20% of all other patients who present with or develop overt metastatic disease [1,2]. Osteosarcoma that occurs without any underlying bone pathology or cancer refers to primary osteosarcoma, whereas secondary osteosarcoma is characterized by any underlying bone pathologies. The diagnosis of osteosarcoma involves imaging of the tumor site by radiographs like Magnetic Resonance Imaging, Computed Tomography, nuclear imaging like positron emission imaging and radionuclide bone scans [3,4].

Combined use of high-dose methotrexate, cisplatin and doxorubicin is widely used as the treatment for osteosarcoma [5]. Further, the malignancies associated with osteosarcoma are treated with preoperative and postoperative chemotherapy [6]. Drugs like vincristine, doxorubicin and cyclophosphamide [7] are employed with alternatives of ifosfamide and etoposide [8]. However, the side effects of existing treatment involve risk of infection, nausea, vomiting, hair loss, loss of appetite, kidney damage, myelosuppression, peripheral neuropathy and hypomagnesemia [8] which affects the physical and mental health of osteosarcoma patients under treatment. Moreover, the increased doses of alkylating agents may possibly increase the risk of second malignancies (leukemia) [9].

Nelumbo nucifera is also called Indian lotus or sacred lotus. It is widely cultivated and extensively used in eastern Asia. Lotus is used as a traditional medicine to treat cough, fever, insomnia, and diarrhea and to

balance the body heat [10]. It has several biological properties like antioxidant, antidiarrheal, antiviral, anti-obesity, and hepatoprotective activities. *Nelumbo nucifera,* India's national flower, can be used for its therapeutic properties because of its cost and availability [11]. The principal bioactive components present in *Nelumo nucifera* are liensinine and nuciferine, which have been proven to show many medicinal properties with the ability to treat diseases including cancer [12]. There are various reports exhibiting the anticancer property of *Nelumbo nucifera*. *Nelumbo nucifera* has been demonstrated to inhibit the proliferation and metastasis of breast, colon and non-small cell lung cancer cell lines. Particularly, a recent *in vitro* study on HCT-116 (a human colorectal cancer cell line) proved that *Nelumbo nucifera* stamen extract promoted apoptosis of the cancer cells [13]. Therefore, the objectives of this study are to analyze the antiproliferative activity of *Nelumbo nucifera* leaf extract against Saos-2 cells and to examine *Nelumbo nucifera* leaf extract-induced cellular changes in Saos-2 cells for the treatment of osteosarcoma.

Materials And Methods

Reagents and chemicals

Dulbecco's minimum-low glucose medium (DMEM), penicillin, streptomycin, trypsin-ethylenediaminetetraacetic acid (EDTA), fetal bovine serum (FBS) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Gibco (Billings, MT, USA). All other necessary chemicals were of analytical grade and purchased locally.

Plant extract preparation

Leaf powder of *Nelumbo nucifera* was obtained from IMPCOPS (Chennai, India). To prepare the extract, 50 g of *Nelumbo nucifera* leaf powder was soaked in 95% ethanol for three days at room temperature. The solution was subjected to two steps of filtration using a crude filter paper and a Whatman paper. The filtrate was then placed in a rotary evaporator. About 3g of material was obtained after evaporation. Concentration of the extract was carried out in a vacuum evaporator and stored at 4˚C for further use.

Cell culture

Saos-2 (Sarcoma osteogenic) cell line was procured from the National Centre for Cell Science, Pune, India. The cells were cultured in DMEM with 10% FBS, penicillin (100 units/mL), and streptomycin (100 µg/mL) at 5% CO_2 and 37^{O}C. The cells were treated with 25, 50, 75, 100, 125, and 150 µg/ml of *Nelumbo nucifera* extract for 24 hours.

MTT assay

Following treatment with *Nelumbo nucifera* leaf extract of different concentrations, the cells were treated with MTT reagent, and the cells were incubated in a humidified incubator (5% CO_2) at 37°C to allow the MTT to be metabolized. After four hours of incubation, the formazan crystals formed were dissolved with dimethyl sulfoxide. Absorbance of the samples was measured at a wavelength of 540 nm using a microplate reader.

DAPI staining

According to Dmitrieva and Burg, 2008 [14], the cells treated with *Nelumbo nucifera* leaf extract at different concentrations for 24 hours were stained with 4,6-diamidino-2-phenylindole (DAPI). The fluorescence was measured at 405 nm, the cells were observed under a fluorescent microscope and images were captured.

Statistical analysis

Data were expressed as mean ± SD and analyzed by Dunnett's test following one-way ANOVA. A p value lower than 0.05 was considered to be significant.

Results

The cytotoxic effect of *Nelumbo nucifera* leaf extract on the fibroblast cells was determined by MTT assay. Cells were treated with 10, 20, 30, 50, 100, 125, and 150 µg/ml of *Nelumbo nucifera* leaf extract for 24 hours. The extract was observed to reduce the viability of Saos-2 cells with increasing concentrations. The proliferation of Saos-2 cells was decreased in a dose-dependent manner with maximum cytotoxicity observed at 150 µg/ml of extract (Figure *1*).

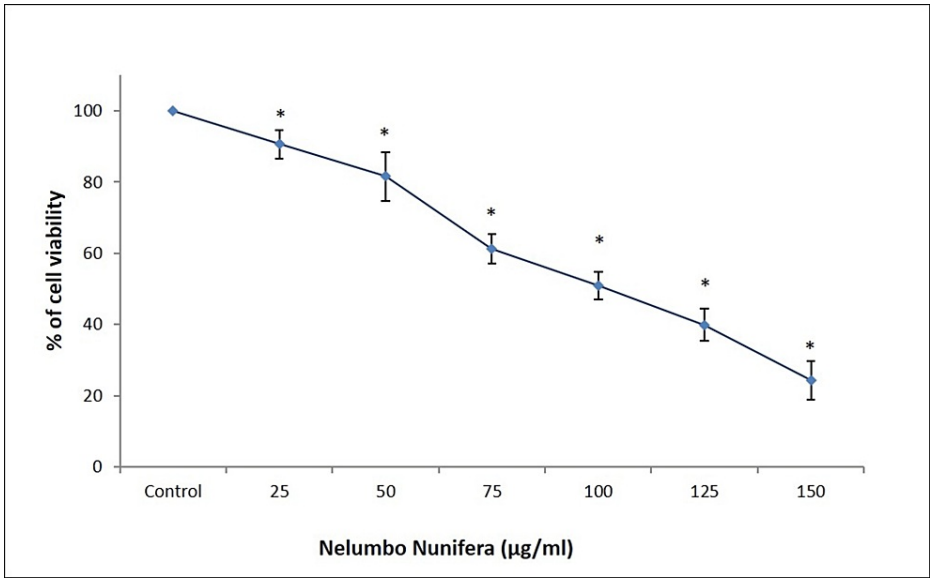

FIGURE 1: Effect of Nelumbo nucifera against Saos-2 cell viability by MTT assay

Dose-dependent decrease in Saos-2 cell proliferation after 24 hours of treatment with *Nelumbo nucifera* leaf extract. Data are shown as means ± SD (n = 3) compared with the control group, $^{*}p < 0.001$.

Saos-2: human osteosarcoma cell line, MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide

The effect of *Nelumbo nucifera* leaf extract on the cancer cell morphology and viability was analyzed by microscopic analysis. The normal epithelial morphology of Saos-2 cells treated with *Nelumbo nucifera* leaf extract was deformed as evidenced by microscopic observation. Further, a decrease in the number of Saos-2 cells after 24 hours of treatment of *Nelumbo nucifera* leaf extract was observed (Figure *2*).

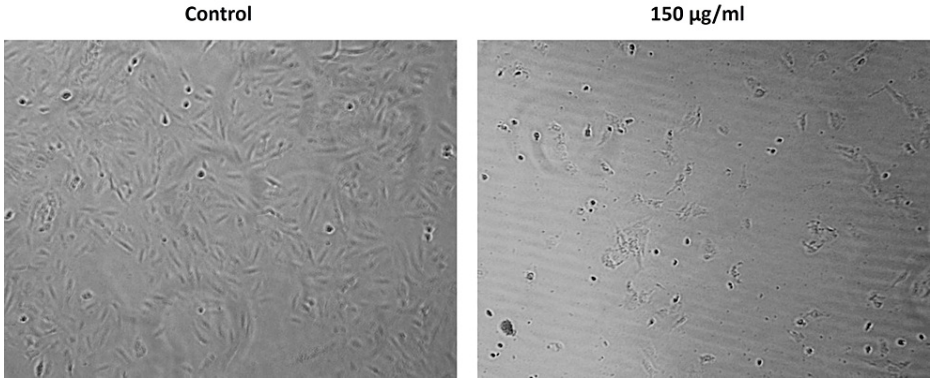

FIGURE 2: Effect of Nelumbo nucifera on morphological changes of Saos-2 cells

Altered morphology of Saos-2 cells and decreased number of viable cells upon 24 hours of treatment of*Nelumbo nucifera* leaf extract.

Saos-2 cells: human osteosarcoma cell line

DAPI staining was performed to analyze the *Nelumbo nucifera* leaf extract-induced morphological changes in the nucleus of Saos-2 cells. Chromatin condensation and nuclear fragmentation leading to cell death confirmed the cytotoxic effect of *Nelumbo nucifera* leaf extract on Saos-2 cell line (Figure *3*).

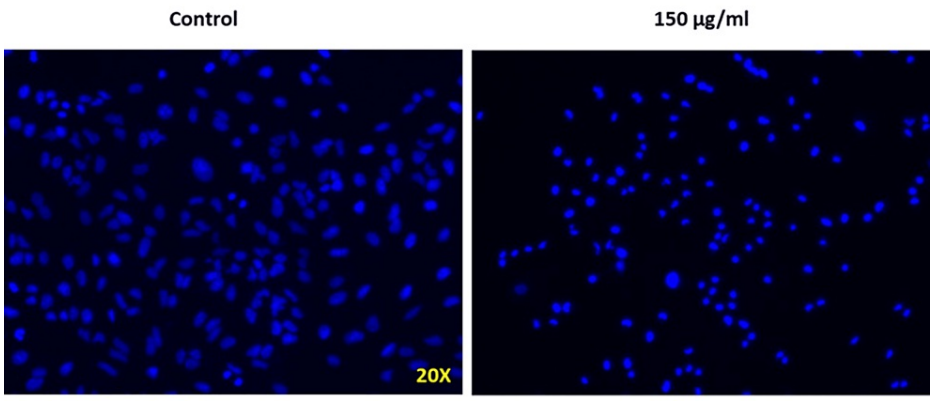

FIGURE 3: Effect of Nelumbo nucifera on nuclear changes of Saos-2 cells

Nelumbo nucifera treatment on Saos-2 cells resulted in fragmented nuclei which led to cell death.

Saos-2: human osteosarcoma cell line

Discussion

The prevalence of osteosarcoma is high, especially in children between the ages of 10 and 19, and in adults above the age of 65, which makes it a global threat. Although therapeutic medications are prescribed and implemented for the treatment of osteosarcoma, the limitations of these medications and novel therapeutic approaches are widely studied. Accordingly, various herbs and herbal compounds were examined for their anticancer properties against osteosarcoma. Herbs such as *Rabdosia rubescens,* Baikal skullcap (*Scutellaria baicalensis Georgi*)*, Olea europaea L., and Evodia rutaecarpa* and their components have been investigated for anti-cancer effects [15]. Furthermore, all the parts of *Nelumbo nucifera* like the leaves, rhizome, flowers and seeds were considered to be of medicinal importance traditionally [16]. On that account, *Nelumbo nucifera* leaf was analyzed in this study against a human osteosarcoma cell line.

According to Zhao et al. [13], *Nelumbo nucifera* stamen extract may be a potent anticancer agent against colon cancer. The study revealed that *Nelumbo nucifera* stamen extract prevented cancer cell growth in a dose-dependent fashion. The extract induced apoptosis of HCT-116 cells by upregulating the apoptosis-associated mRNA expression of Fas, Fas ligand, death receptor-4 and 5, caspases-3,8 and 9. Consistent with the above reports, our study also showed that *Nelumbo nucifera* leaf extract exhibits concentration-dependent cytotoxicity against the Saos-2 osteosarcoma cell line, evidenced by the decreased viability of cells in the MTT assay. Further, the reduced number of viable Saos-2 cells under microscopic observation revealed the anti-proliferative effect of *Nelumbo nucifera* leaf extract.

Plant bioactive components such as curcumin, butein, berberine, quercetin, silibinin, galangin, resveratrol, etc. have been shown to suppress osteosarcoma [17]. There are two strategies bioactive substances employ to target cancer cells [18]. They function as cytotoxic substances that target macromolecules like DNA, enzymes, and microtubules within the cancer cells. Another anticancer strategy of bioactive compounds involves targeting the oncogenic signal transduction pathways that regulate the cancer progression. Correspondingly, alkaloids, flavonoids, phenols, tannins, steroids and glycosides are the major constituents responsible for the biological properties of *Nelumbo nucifera* [19]. Liensinine and nuciferine, present in *Nelumbo nucifera*, were reported to inhibit the receptor activator of nuclear factor kappa-B ligand-induced osteoclast differentiation in mouse bone marrow macrophage cells and mature osteoclast-mediated bone resorption which inhibits the growth of breast cancer cells and breast cancer-associated bone loss [20]. Among the various mechanisms of anticancer agents, apoptosis induction is known to play a major role. Several cellular, nuclear and molecular changes occur during apoptosis. Chromosomal fragmentation and condensation have been reported as crucial events of apoptosis. *Nelumbo nucifera* leaf extract induced DNA fragmentation in the rat aortic smooth muscle cell line A7r5 causing apoptotic cell death [21]. Similarly, DAPI staining in our present study demonstrated that *Nelumbo nucifera* leaf extract resulted in apoptosis of Saos-2 cells characterized by fragmentation and condensation of chromosomes.

Collectively, anticancer agents including herbs and herbal constituents predominantly target cancer cell proliferation, migration and angiogenesis [22]. The results of our study suggested that *Nelumbo nucifera* leaf extract potentially inhibits the cell survival and proliferation of Saos-2 cells by promoting apoptosis. These anticancer properties of *Nelumbo nucifera can be* attributed to the presence of bioactive components such as liensinine and nuciferine. Since herb- and plant-formulated drugs show better antitumor effects and lesser to no adverse effects when compared with chemotherapeutic drugs, our study provides a novel therapeutic

candidate for the treatment of osteosarcoma [23]. Additionally, *Nelumbo nucifera* leaf extract could be a potential anticancer agent, which requires extensive studies.

Limitations

This study demonstrates that *Nelumbo nucifera* leaf extract has the ability to inhibit proliferation, reduce viability and promote cell death of Saos-2 cells and further induce nuclear changes. However, a detailed phytochemical analysis of the components of the extract and the exact mechanism in which the extract and its components exert its anticancer effect is yet to be determined. Moreover, analyzing the molecular level changes induced by the extract will enhance the understanding and application of *Nelumbo nucifera* leaf extract in the osteosarcoma treatment.

Conclusions

The study concluded that *Nelumbo nucifera* leaf extract had a concentration-dependent cytotoxic effect on Saos2 cell lines. *Nelumbo nucifera* extract possesses cytotoxic and anti-proliferative properties against Saos-2 cells. Therefore, it might be used against osteosarcoma after necessary *in vivo* and clinical studies.

Additional Information

Author Contributions

All authors have reviewed the final version to be published and agreed to be accountable for all aspects of the work.

Concept and design: Devaraj Ezhilarasan, Gautam Britina

Acquisition, analysis, or interpretation of data: Devaraj Ezhilarasan, Gautam Britina, Karthik Shree Harini

Critical review of the manuscript for important intellectual content: Devaraj Ezhilarasan, Gautam Britina, Karthik Shree Harini

Supervision: Devaraj Ezhilarasan

Drafting of the manuscript: Gautam Britina, Karthik Shree Harini

Disclosures

Human subjects: All authors have confirmed that this study did not involve human participants or tissue. **Animal subjects:** All authors have confirmed that this study did not involve animal subjects or tissue. **Conflicts of interest:** In compliance with the ICMJE uniform disclosure form, all authors declare the following: **Payment/services info:** All authors have declared that no financial support was received from any organization for the submitted work. **Financial relationships:** All authors have declared that they have no financial relationships at present or within the previous three years with any organizations that might have an interest in the submitted work. **Other relationships:** All authors have declared that there are no other relationships or activities that could appear to have influenced the submitted work.

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