Anti-cancer potential of dodoneine study

PROJECT PROPOSAL

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Title of the project: Comparative study of anti-cancer potential of Dodoneine and its derivative.

SUMMARY:

Dodoneine [(R)-6-[(S)-2-hydroxy-4-(4-hydroxyphenyl)butyl]-5, 6-dihydropyran-2-one] is a naturally occurring dihydropyranone which is isolated from *Tapinanthus dodoneinfolius* DC Danser, also known as African mistletoe. *Tapinanthus dodoneinfolius* is a parasitic plant that feeds from the sheanut tree in Burkina Faso. The unique, challenging structural and functional features of Dodoneine created great interest among the scientific community to find more possible activities of this compound. The current study focuses on comparative study of the anti-cancer potential of this compound and one of its derivative i. e methoxy Dodoneine. The compound was checked for its cyto – toxicity on three different but closely related cell lines : A431 (squamous carcinoma cells), A549 (human lung adenocarcinoma cell line) and MCF7 (human breast cancer cells). Cyto-toxicity assay (in this study MTT assay) was carried out on the above cell lines along with a normal human cell line INT-407. Results of the above study showed that the compound had cyto – toxic activity against the three cancer cell lines but was found to be non – toxic against the normal human cell line INT-407. The IC50 value of the compound against the cell lines was also determined. After the initial cyto-toxic assay further studies were carried out only on A431 cells because translation of in-vitro results into a skin carcinoma animal model is

easier. Clonogenic assay is performed to study the colony characteristic of the cells. This assay also helps to study the reproducibility characteristic of the cells after addition of compound. The chromatin condensation in cells reflects the late stages of apoptosis. Microscopic studies of the Hoechst 33342 stained cells was done to study chromatin condensation in cells due to compound addition. Studies have demonstrated that apoptosis induction plays the most vital role in the cancer treatment by chemo and radiation therapies. Study of apoptosis was confirmed by quantification of SubG1 population in the cells, Annexin V/PI staining and assessing the cell viability in the presence of a pan – caspase inhibitor. Cancer cells are generally more active than normal cells in metabolic reactive oxygen species generation. There are strong evidences that this reactive oxygen species can induce the apoptotic process. Hence reactive oxygen species level in the cells treated with the compound was estimated from the reactive oxygen species mediated fluorescence enhancement of the cell permeable oxidation sensitive probe DCFH – DA. The positive control used in this study was hydrogen peroxide. External supplementation of anti – oxidants like N – acetyl cysteine and glutathione is known to protect target cells against cyto – toxic effects that are mediated through the participation of reactive oxygen species. Hence to investigate the involvment of reactive oxygen species in the Dodoneine induced apoptosis the cells were exposed to N – acetyl cysteine and glutathione separately before incubation with the compound and the subG1 population was assessed by flow cytometry. The future part of research lies in finding out the functional group of compound responsible for its biological activities. For this purpose, the derivatives of the compound

have to be tested for its biological activities and a comparative study have to be conducted.

INTRODUCTION:

Most of the currently available anti-cancer agents exhibit numerous side effects, and are too expensive for a major share of the world population. This warrants search for alternative, target-selective anti-neoplastic molecules, which are less expensive, efficacious and exhibit minimum toxicity to normal cells. Hence the cyto – toxic activity of Dodoneine against the cancer cells were examined. Dodoneine is a naturally occurring dihydropyranone which is isolated from *Tapinanthus dodoneinfolius* in West Africa (1, 2). Since many dihydropyran – 2- ones exhibited potential biological activities like HIV protease inhibition, apoptosis induction and anti – leukemic effect, Dodoneine was also expected to possess this activities. Recent biological research on (+)-Dodoneine revealed its potent relaxing effect on pre – constricted rat aortic rings (3, 4). Many research studies reveal that Dodoneine acts as an inhibitor of several human carbonic anhydrase isoforms (5). Carbonic anhydrase forms a family of enzymes that catalyse the rapid interconversion of carbon dioxide and water to bi – carbonates and protons. This is a reversible reaction that occurs at a very slow rate in the absence of a catalyst (6). The carbonic acid inhibitors are shown to inhibit the growth of several tumor cell lines in – vitro as well as in in – vivo studies. Such results obtained forms interesting leads for development of novel anti – tumor therapies (7).

The current research started with a cyto – toxicity assay i. e MTT assay. The compound was checked for its cyto – toxicity on three different but closely related cell lines: A431 (squamous carcinoma cells), A549 (human lung adeno – carcinoma cell line) and MCF7 (human breast cancer cells). In order to verify that the compound selectively acts only against cancer cell lines and was ineffective against normal human cells, a normal human cell line INT – 407 was also used in cyto – toxicity assay of the compound. Results of this study revealed that the compound showed impressive anti – proliferative activity to human lung carcinoma cells, breast cancer cells and skin carcinoma cells and was found to be non – toxic to normal human cell line even upto 75µm. The IC50 values of the compound were determined to be 23. 34µm, 22. 1µm and 26. 1µm against A549, MCF-7 and A431 cells respectively. Further studies were carried out only on A431 cells because translation of in-vitro results into a skin carcinoma animal model is easier. Clonogenic assay which is generally performed to study the colony characteristic of the cells showed significant reduction in colonies after twelth day suggesting that the drug caused loss of reproducibility in the cells. Apoptosis induction plays the most vital role in the cancer treatment by chemo and radiation therapies (8). Defective and / or reduced apoptosis has been implicated in the development and progression of malignant tumors as well as occurrence of chemo-resistant phenotypes (9, 10). Study of apoptosis was confirmed by quantification of SubG1 population in the cells. The time and dose dependent effect of the compound on the cell cycle revealed a G1 phase arrest in A431 cells. The cell viability was assessed in the presence of pan – caspase inhibitor Z-DEVD-FMK and the results confirmed that the compound induces robust apoptosis in A431 cells. Cancer cells generate

more metabolic reactive oxygen species than the normal cells and are constantly under oxidative stress. The apoptotic process can be induced by reactive oxygen species. The natural phenolics abundant in our daily diets can perturb the cellular redox conditions specifically in cancer cells leading to elevated levels of reactive oxygen species and subsequent cell death (11, 12, 13). The pro-oxidant properties of several natural phenolics and anti – cancer drugs forms the basis of their therapeutic roles. The reactive oxygen species mediated fluorescence enhancement of the cell permeable oxidation sensitive probe DCFH – DA was used to estimate the reactive oxygen species in the cells treated with the compound. In order to study the effect of presence of any anti – oxidants on apoptosis of the cells induced by the compound through participation of reactive oxygen species the cells were exposed to N – acetyl cysteine and glutathione separately before incubation with the compound and the subG1 population was assessed by flow cytometry.

Further a comparative study has to be done between Dodoneine and its derivatives to find the functional group in the compound responsible for its biological activities.

OBJECTIVES:

1. To search for target-selective anti-neoplastic molecules, which are less expensive, efficacious and exhibit minimum toxicity to normal cells.
2. To study the potency of the available compound Dodoneine as an anti – cancer agent.
3. To study its mode of action on cancer cells.

4. To find the functional group in the compound responsible for its anti – cancer property.

DURATION OF THE PROJECT:

The current study requires a time duration of about five months which is subjected to extension based on the availability of the compound Dodoneine and its derivatives.

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