SUMMARY AND CONCLUSION

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In recent years, a great deal of attention has been focused on the use of alternative methods in animal experimentation. This interest has arisen in part because of a concern for the animal's welfare and the increasing costs of animal purchase and care. However, the term "alternative" has caused a great deal of confusion, because it implies that there are replacements for animals in many experimental situations. In reality, there are few situations in which computer simulations, *In vitro* techniques, or other methods are suitable replacement for animals. The present study, on checking the cellular events of apoptosis with the treatment of plant extract on chick embryo fibroblasts as a model system, was done keeping the ethics concerning animal suffering in mind.

Apoptosis is the process of programmed cell death (PCD) that may occur in multicellular organisms. Programmed cell death involves a series of biochemical events leading to a characteristic cell morphology and death; a series of biochemical changes including blebbing, changes to the cell membrane asymmetry and attachment, cell shrinkage, nuclear fragmentation, chromatin condensation, and chromosomal DNA fragmentation are observed during apoptosis.

Research on apoptosis has increased substantially since the early 1990's. In addition to its importance as a biological phenomenon, defective apoptotic processes have been implicated in an extensive variety of diseases. Excessive apoptosis causes hypotrophy, such as in ischemic damage,

whereas an insufficient amount results in uncontrolled cell proliferation, such as cancer.

Plants have been a prime source of highly effective drugs for the treatment of many forms of cancer. The compound isolated from the plants lead to the development of potential novel agents. The molecules isolated from plants are proving to have the potential for development into selective anticancer agents.

The plant chosen for the present study was *Triticum aestivum*. Earlier studies conducted in our laboratory revealed *Triticum aestivum* to render significant protection against oxidative damage induced by H_2O_2 and etoposide to normal and induce apoptosis in cancer cells (goat liver cells, *Saccharomyces cerevisiae* and Hep2 cells) respectively.

The leaves were plucked from 4-day old plants from pot cultures maintained in our University. A methanolic extract of the leaves was prepared, dried and the residue obtained after the evaporation was dissolved in a known volume of DMSO and used for the treatment of chick embryo fibroblasts.

Etoposide, a standard anticancer chemotherapeutic agent, was used as a stress-inducing agent, which exerts its action by inducing apoptosis. The cellular events related to the process of apoptosis were followed in the presence and the absence of *Triticum aestivum* leaf extracts. In this regard, the present study was formulated to analyse the influence of *Triticum aestivum* leaf extract on minimizing the etoposide-induced events in chick embryo fibroblasts.

Various apoptotic related parameters like cell viability, morphological changes, nuclear changes, apoptotic index and DNA fragmentation were characterized.

The assay for cytotoxicity in the present work was done by the sulphorhodamine B assay, which estimates the amount of protein content, which is directly proportional to the cell viability, which was measured using the ELIZA reader. The results obtained after the calculations for cytotoxicity showed that the viability reduced drastically in the etoposide treated group of cells and significantly increased when the cells were treated with *Triticum aestivum* leaf extract, even in the presence of apoptosis-inducing stress. It can be deduced from these results that the plant possesses antiapoptotic property and prevents cell death caused by oxidative stress.

In order to quantify the extent of cell viability in the group exposed to etoposide, plant extract and their combination, the MTT assay was used. The results clearly showed a marked decrease in the viability of cells treated with etoposide. This stress was significantly reduced and the viability was increased when the cells were treated with *Triticum aestivum* leaf extract. These observations confirm that the plant extract exhibits very good antiapoptotic effect in chick embryo fibroblasts in the presence of oxidative stress- inducing agent, etoposide.

The apoptotic events studied in chick embryo fibroblasts with various parameters for morphological changes, DNA fragmentation and nuclear changes were quantified by using staining methods. In the present investigation, membrane blebbing was taken as a quantifiable parameter to measure apoptosis by giemsa staining. The results clearly indicated a steep rise in the number of apoptotic cells in the etoposide treated group. The plant extract, by itself, did not cause a significant increase in the extent of apoptosis and showed marked reduction in the number of apoptotic cells when administered along with etoposide.

EtBr and PI staining were used to study the nuclear changes that occur during apoptosis. The etoposide treated cells showed a marked increase in the number of apoptotic cells when stained with PI and EtBr, indicating etoposide- inducing apoptosis. The leaf extract treated groups showed a significant decrease in the number of apoptosing cells, indicating their protective effects against oxidative stress. DAPI staining was also used to assess the extent of apoptosis. The same result was observed in DAPI staining which indicated the good correlation.

Apoptosis is well characterized by DNA fragmentation. The extent of DNA fragmentation in chick embryo fibroblasts exposed to the different treatments was measured. The result showed that there was significant fragmentation in the DNA from chick embryo fibroblast cell of the etoposide treatment group. This was effectively counteracted by the presence of *Triticum aestivum* leaf extract. The present study, thus, provides conclusive evidences for the antiapoptotic activity of the leaf extract.

Thus, the various apoptosis-related events like cell survival, morphological changes, nuclear embryo fibroblast cells exposed to leaf extract, showed that a modulation of cytotoxicity was observed, indicating the antiapoptotic potential of the leaves. Thus, the leaves of *Triticum aestivum* have potential antiapoptotic activity implying that these extracts can be used to protect the non-cancerous cells in the body against etoposide-induced ill-effects during chemotherapy with any anticancer agent. It also justifies and validates the use of chick embryo fibroblasts as an alternative model to study apoptosis-related events.

SUGGESTIONS FOR FUTURE RESEARCH

- The effects of *Triticum aestivum* leaf extracts can be tested on various cancer cell lines in the presence and absence of various chemotherapeutic agents.
- The active components responsible for the antiapoptotic activity of *Triticum aestivum* leaf extracts can be identified and tested further.
- The antiapoptotic effect of the *Triticum aestivum* leaf extract can be studied in other lower organisms like *Drosophila melanogaster*.
- The molecular markers of apoptosis, like the affector (initiator) and efffector caspases, can be studied after exposure to the leaf extract.