Chapter 8
Antioxidant & Cytotoxicity of Zanthonitrile
Free-radicals are able to increase or decrease the risk of cancer based on diverse situations. Actually, reactive oxygen species (ROS) can act as a trigger for carcinogenesis by permanent damage of DNA, causing mutations of p53 gene (the tumor suppressor gene) which is frequently mutated up to 50%. The ROS also modulates the activity of several transcription factors like nuclear factor kappa B (NF-κB) and activator protein 1 (AP-1) which play regulatory roles for the Jun and c-Fos as two nuclear oncoproteins. The above processes are participated in initiation, promotion and progression of cancer. However, interestingly, adequate levels of ROS may show opposite effects to inhibit carcinogenesis by enhancing p53 expression and inducing apoptosis in the tumor cells (Alawadi et al., 2011; Liu et al., 2008).

Interestingly, “the antioxidant network theory” has been raised because many clinical evaluations of antioxidant supplements to prevent cancer have been unsatisfactory. For example, lung cancer in smokers can progress by using high doses of beta carotene or unexpected activity of vitamin E (Bagchi and Preuss, 2005), thus, besides the unstable position of antioxidants in cancer therapy, another argument comes up as “Do typical antioxidants really prevent occurrence of cancer?” It seems that, the answer to this question is complicated by the stage of cancer progress and also diverse role of ordinary antioxidants.

Now, the hypothesis of “beneficial role of antioxidant in cancer chemoprevention” is really premature in the fast-evolving area of research. Moreover, understanding the mechanism of action and advantageous doses of antioxidants for consumption as a chemopreventive medicine is essential (Saeidnia and Abdollahi, 2013). In order to reach a comprehensive discussion, main examples of these compounds are as follows:

Protein kinase C (PKC) is introduced as a critical enzyme in tumor progression in some types of cancer. Another side, α-tocopherol or vitamin E has been reported to suppress PKC activity in different cell lines. (Saeidnia and Abdollahi, 2013).
On the basis of above literature, this chapter deals with the evaluation of antioxidant and cytotoxic properties of Zanthonitrile isolated from *Zanthoxylum alatum*.

**Materials and Methods**

**Plant collection, Extraction and Isolation**

All the procedure in this part are described in the chapter 3. Here the aim of the present study was antioxidant and cytotoxic activity of isolated Zanthonitrile.

**Chemicals used**

1. 1-Diphenyl-2-picryl-hydrazyl (DPPH) was obtained from Sigma Chemicals, USA.
2. Penicillin, Streptomycin, MTT (3-(4,5-dimethyl-2-thiazolyl) 2,5 diphenyl-2H-tetrazolium bromide), cell culture grade DMSO, cell culture media RPMI 1640 and all analytical grade chemicals were from HiMedia (Mumbai, India).

**DPPH radical scavenging activity**

DPPH radical scavenging activity was measured using the described method (Karmakar *et al.*, 2011). 2.8 ml of test solution or standard ascorbic acid (in methanol), at different concentrations (µg/ml) and 0.2 ml of DPPH (100 µM in methanol) were mixed and incubated at 37°C for 30 min. Absorbance of the resulting solution was measured at 517 nm using spectrophotometer. The percentage inhibition of DPPH radical was calculated by comparing the results of the test with the control (not treated with extract) using the following formula.

\[
\text{Percentage inhibition} = \left[ \frac{(C - T)}{C} \right] \times 100
\]

Where,

- C = Absorbance of the control
- T = Absorbance of the test

**In vitro cytotoxic activity**

*In vitro* cytotoxicity of Zanthonitrile was determined using standard MTT assay with some modification (Nikhil *et al.*, 2014). In brief, EAC cells (3×10^5 ml^-1) were seeded into 96-well flat microtiter plates in enriched RPMI 1640 medium (200 µl) supplemented with penicillin (100 IU/ml) and streptomycin (100 µg/ml). Different concentrations of Zanthonitrile (10, 25,
50, 100 µg/ml) was added to the cells in the volume of 100 µl/well. All samples were incubated for 24 h at 37 °C in a humidified incubator with 5% of CO$_2$. After 24 h, the medium was removed and cell cultures were incubated with 20 µl MTT reagent (5 mg/ml) for 4 h at 37° C. DMSO (150 µl) was added to remove the formazan produced by the viable cells. The suspension was placed on micro-vibrator for 5 min and absorbance was recorded at 570 nm by the ELISA reader. The experiment was performed in triplicate. The percentage of inhibition was calculated using the following formula.

The % inhibition = 100 - [(Mean OD of treated cell × 100) / mean OD of negative control.

The IC$_{50}$ values were calculated by plotting % inhibition vs drug concentration.

Statistical analysis

Statistical analysis was performed using Graph Pad Prism (version 5.00, San Diego, California) Software. All data are expressed as mean ± standard error of mean (SEM).

Results and discussion

Antioxidant

Free radicals are atoms or groups of atoms with an odd (unpaired) number of electrons and can be formed when oxygen interacts with certain molecule. These can significantly contribute to disease states like cancer, arthritis and neurological disorder by producing oxidative damage to the cells (Saeidnia and Abdollahi, 2013). DPPH assay is the most widely reported in vitro method for screening of antioxidant activity. A stable violet colored nitrogen-centred free radical produced by DPPH in methanol solution was reduced to yellow colored diphenylpicryl hydrazine by Zanthonitrile in a concentration-dependent manner (Karmakar et al., 2011). The inhibitory ability of Zanthonitrile was compared to standard ascorbic acid. The IC$_{50}$ values of Zanthonitrile and ascorbic acid were found to be 7.86 ± 0.23 µg/ml and 9.17 ± 0.39 µg/ml (Figure 1) respectively.
Cytotoxic activity

EAC has a resemblance with human tumors which are the most sensitive to chemotherapy due to the fact that it is undifferentiated and that it has a rapid growth rate (Ozaslan et al., 2011). There is no doubt that the MTT assay has great potential as a rapid method of screening for drug responsiveness of cell lines. Reduction of MTT in isolated cells is regarded as an indicator of cell redox activity and the reaction is attributed mainly to mitochondrial enzymes and electron carriers (Bernas and Dobrucki, 2000).

**Figure 1:** The IC₅₀ values of Zanthonitrile and Ascorbic acid for DPPH. The results are mean ± SEM of three experiments.

**Figure 2:** Cytotoxic effect of Zanthonitrile on *in vitro* EAC cell. Values are mean ± S.E.M.; where n = 3.
In vitro cytotoxicity of Zanthonitrile was evaluated by the MTT reduction assay, after 24 h of exposure in culture. The assay showed that cytotoxic effect of Zanthonitrile on the EAC cell was in a concentration dependent manner. The IC$_{50}$ value was found to be 57.28 ± 0.64 μg/ml by plotting the graph of concentration versus percentage inhibition (Fig. 2).

**Conclusion**

The isolate Zanthonitrile, an alkaloid, proves to a potent antioxidant as is evidenced from the DPPH radical scavenging activity. Zanthonitrile also showed a good cytotoxic effect that may posh elated of its antitumor property. Further research is required of this compound to investigate its anticancer activity.
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References


