

## RESEARCH ARTICLE

# Morinda citrifolia (Noni) Alters Oxidative Stress Marker and Antioxidant Activity in Cervical Cancer Cell Lines

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## Abstract

**Background:** Cervical cancer, the second most common cancer in women, has a high mortality rate. Cisplatin, an antitumor agent, is generally used for its treatment. However, the administration of cisplatin is associated with side effects and intrinsic resistance. *Morinda citrifolia* (Noni), a natural plant product, has been shown to have antioxidant activities *in vitro* and *in vivo*. **Materials and Methods:** Both HeLa and SiHa cervical cancer cell lines were treated with 10% Noni, 10 mg/dl cisplatin, or a combination of both 10% Noni and 10 mg/dl cisplatin for 24 hours. Post culturing, the cells were pelleted and stored at -70°C for malondialdehyde and catalase assays. **Results:** On treatment with Noni, CP, and their combination, the level of MDA decreased by 0.76 fold, 0.49 fold, and 0.68 fold respectively in HeLa cells; and by 0.93 fold, 0.67 fold, and 0.79 fold respectively in SiHa cells, as compared to their controls; whereas catalase activity increased by 1.61 fold, 0.54 fold, and 2.35 fold, respectively in HeLa cells; and by 0.98 fold, 0.39 fold, and 1.85 fold respectively in SiHa cells. **Conclusions:** A decrease in level of lipid peroxidation and an increase in catalase activity were observed with Noni by itself and the effect ameliorated changes observed with cisplatin when given in combination.

**Keywords:** *Morinda citrifolia* (Noni) - cisplatin - malondialdehyde - catalase - HeLa - SiHa

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## Introduction

Cervical cancer is a major health problem worldwide and is the most frequent cause of cancer in women in India (Jemal et al., 2011; Pathak et al., 2012). In majority of the cases, it is associated with the presence of human papilloma virus (HPV) infection, with high-risk HPV types 16 and 18 being responsible for more than 70% of cases of cervical cancer (Gupta et al., 2013).

The antitumor drug Cisplatin (CP) with clinical and experimental efficiency is employed as a first-line chemotherapeutic modality in the treatment of epithelial malignancies, including lung, ovarian, testicular, cervix cancer and others. Unfortunately, the chemotherapy's efficiency is far from satisfactory due to the side effects and to the resistance of tumor cells. Recent publications open the possibility of increasing the efficiency of chemotherapy (Hernandez-Flores et al., 2011). Therefore, new agents or new regimens in combination with Cisplatin or Cisplatin analogs are being sought in order to alter oxidative stress and antioxidant activity and hence to decrease its adverse effects (Kostova, 2006).

Antioxidants nutrients have been shown to be involved in detoxification of the Reactive oxygen species (ROS) (Peerapatdit et al., 2006). Simone II et al. (2007) wrote a review which showed that antioxidants and other nutrients do not interfere in therapeutic modalities of cancer.

Furthermore, they enhance the killing of therapeutic modalities of cancer, decrease their side effects, and protect normal tissue; while few showed increased survival (Simon II et al., 2007).

Previous research has established that fruits and vegetables are major sources of dietary antioxidants. Epidemiological studies have demonstrated that consumption of fruits and vegetables may reduce free-radical-induced oxidative damage and lipid peroxidation in cigarette smokers (Mian-Yang et al., 2009; Bullo'M et al., 2011).

*Morinda citrifolia*, also known as Great Morinda, Indian Mulberry, or Noni, is an evergreen or small tree, belonging to the family Rubiaceae, and grows in many tropical regions of the world. A number of major chemical compounds and antioxidants have been identified in the leaves, roots, and fruits of Noni plant (Mohd-Zin et al., 2002; Su et al., 2005; Alitheen et al., 2010). Several reports have described various potential health benefits of Noni fruit (Ma et al., 2007) including immune modulation and antioxidant activities *in vitro* and *in vivo* (Wang et al., 2009; Serafini et al., 2011).

While clinical studies on the effect of antioxidants in modulating cancer treatment are limited in number and size, experimental studies show that antioxidant vitamins and some phytochemicals selectively induce apoptosis in cancer cells but not in normal cells and

prevent angiogenesis and metastatic spread, suggesting a potential role for antioxidants as adjuvants in cancer therapy (Borek, 2004b). Even though some studies have examined the antioxidant effects of Noni on cancer and have elucidated some of the mechanisms involved, there is still very little information available on the usefulness of Noni in the field of cancer, especially in terms of its antioxidant effects in cervical cancer. Hence, the aim of this study was to study the antioxidant properties of Noni by itself and in combination with Cisplatin (a commonly used chemotherapeutic agent for treatment of cervical cancer) in human cervical cancer HeLa and SiHa cell lines.

## Materials and Methods

### Cell line (s)

Two cell lines, i.e. HeLa (HPV18+) and SiHa (HPV16+) cervical cancer cell lines were used in this study. These cell lines were cultured in DMEM (Sigma), supplemented with 10% fetal bovine serum (FBS), 100 U/ml Penicillin, and 100 µg/ml Streptomycin. Cultures were maintained at 37°C in 5% CO<sub>2</sub> and 95% humidified atmosphere.

### Cell culture and treatments

Noni juice was obtained from Health India Laboratories (a unit of Noni BioTech Pvt. Ltd., Chennai, India). Cisplatin was purchased from Sigma, USA. The cells were treated with different concentrations of CP and Noni and incubated at 37°C for 24 hrs.

Both the cervical cancer HeLa and SiHa cell lines were treated with 10% Noni (v/v), 10 µg/ml Cisplatin, and combination of both 10% Noni (v/v), 10 µg/ml Cisplatin for 24 hours at 37°C in 5%CO<sub>2</sub> and 95% humidified atmosphere. Post culturing, the cells were pelleted and stored at -70°C for various assays (Gupta et al., 2013).

### Measurement of malondialdehyde (MDA) level

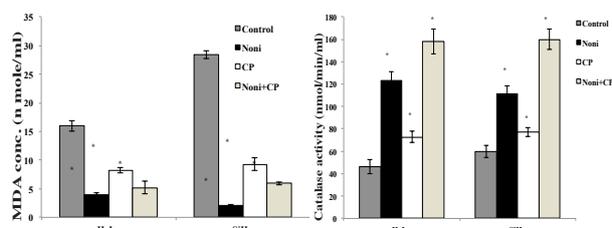
The cells were collected and re-suspended in 1 ml of DMEM culture medium. The samples were then sonicated and used for MDA measurement as described in the protocol. The Thiobarbituric Acid Reactive Substances (TBARS) assay was performed using protocol provided in the TBARS Assay Kit, Cayman Chemical Company.

### Assay of catalase activity

The cells were collected by centrifugation and the cell pellet was then sonicated on ice in 1-2 ml of cold buffer (i.e. 50 mM potassium phosphate, pH 7.0, containing 1 mM EDTA). After sonication, the product was centrifuged at 10,000× g for 15 min at 40°C. The supernatant was removed for assay and stored on ice. The assay was performed with Catalase Assay Kit, Cayman Chemical Company.

### Statistical analysis

Results of each experiment represent the mean±standard deviation (SD) of three independent experiments carried out in triplicate. All the data were analysed using Student's t-test. Statistical analysis showing a value of p<0.05 was considered significant.



**Figure 1. Effect of Noni (10%), CP (10 µg/ml), and their Combination on A) MDA Level and B) Catalase Activity.** The results shown in the bar diagram are mean±SD of the three individual experiments. Statistical analysis showing a value of p<0.05 was considered significant

## Results

### Effect of noni and cisplatin on the level of malondialdehyde

Increased levels of Malondialdehyde (MDA) indicate upsurge in lipid peroxidation which is a consequence of increased free radical generation. It causes profound alterations in the function of the cell membrane and structural organization of DNA leading to mutations. Therefore, it can be stated that lipid peroxidation may possibly be involved in cervical carcinogenesis. On treatment with Noni, CP, and their combination, the level of MDA decreased by 0.76 fold, 0.49 fold, and 0.68 fold respectively in HeLa cells; and by 0.93 fold, 0.67 fold, and 0.79 fold respectively in SiHa cells, as compared to their controls (Figure 1A).

### Effect of noni and cisplatin on catalase activity

Catalase is an ubiquitous antioxidant enzyme that is present in most aerobic cells. Catalase is involved in the detoxification of hydrogen peroxide, a reactive oxygen species (ROS), which is a toxic product of both normal aerobic metabolism and pathogenic ROS production. On treatment with Noni, CP, and their combination, Catalase activity was found to be increased by 1.61 folds, 0.54 fold, and 2.35 folds respectively in HeLa cells; and by 0.98 fold, 0.39 fold, and 1.85 folds respectively in SiHa cells, as compared to their controls (Figure 1B).

## Discussion

Search for new chemopreventive and antioxidant agents that are more effective but less toxic has kindled great interest in phytochemicals. Noni, fruit juice derived from the plant *Morinda citrifolia*, is one such compound which was used in this study. Noni is a herbal remedy with promising antioxidant properties (Wang et al., 2009; Serafini et al., 2011).

We studied the antioxidant properties of Noni by itself, Cisplatin by itself, and their combination, on two cervical cancer cell lines (HeLa and SiHa). These cell lines were chosen for the study because they harbor the human papilloma virus (HPV) type 18 (HeLa) and type 16 (SiHa) genotypes. These HPV's have been shown in multi-institutional studies as etiological agents in the development of cervical cancer and these genotypes account for >70% of all HPV DNA positive invasive cervical cancers (Gupta et al., 2013).

Reactive oxygen species (ROS) such as hydrogen peroxide, superoxide anions, and hydroxyl radicals promote apoptosis. However, the hydroxyl radicals are capable of abstracting a hydrogen atom from polyunsaturated fatty acids in membrane lipids to initiate lipid peroxidation. These free radicals can evoke extensive tissue damage, reacting with macromolecules, such as membrane lipids, proteins, and nucleic acids (Gupta et al., 2012). Thus, an alteration in enzymatic antioxidant status with increase in lipid peroxidation indicates that the enzymes play an important role in combating free radical-induced oxidative stress. Hence, in this study, we measured the ability of Noni/Cisplatin to inhibit lipid peroxidation and also studied the activity of antioxidant enzymes.

The level of Malondialdehyde (MDA), a marker for lipid peroxidation, was found to be maximum in controls as compared to the treated cells ( $p < 0.001$ ), in both HeLa and SiHa cells, therefore reflecting insufficient antioxidant potential in both the cervical cancer cells. The level of MDA decreased significantly on treatment with Noni/Cisplatin alone and with their combination, in both HeLa and SiHa cells; with Noni showing greater decrease in lipid peroxidation. This suggests that antioxidants present in Noni, mainly the lipid soluble ones, might exert a profound effect on inhibition of lipid peroxidation and free radical generation. However, the exact mechanism is not clear.

Catalase is a free radical scavenging enzyme, present in all oxygen metabolizing cells and provides defense against potentially damaging entities of hydrogen peroxide. Our study showed increased level of Catalase on various treatments, indicating their protective role. The activity of Catalase increased significantly with Noni alone ( $p < 0.05$ ), but on Cisplatin treatment, it was found to decrease as compared to Noni alone ( $p < 0.01$ ). This implies that Noni is a better enhancer of Catalase activity. The activity of Catalase increased significantly with their combination as compared to control ( $p < 0.01$ ) and as compared to either Noni alone ( $p < 0.001$ ) or Cisplatin alone ( $p < 0.001$ ). This could be due to the ability of Noni to scavenge free radicals more effectively, magnifying the  $H_2O_2$  level when treated in the combination, and hence, magnified Catalase activity could be a part of the defense system specific to the epithelial cells, designed to eliminate  $H_2O_2$  produced. The increase in Catalase activity could also be due to decrease in lipid peroxidation with Noni/Cisplatin alone and with their combination, thus enhancing Catalase activity.

Based on the results obtained, it is highly possible that several compounds of different polarity may contribute to the antioxidant activity of *M. citrifolia* fruit juice (Noni). Part of the antioxidant activity may be due to lipid soluble polyphenols, anthraquinones,  $\alpha$ -tocopherol, and  $\beta$ -carotene present in Noni, and many natural phytochemicals have been demonstrated to promote antioxidant activity in cancer cells, including phenolics, alkaloids, damnacanthal, and flavonoids (Alshawi et al., 2011; Wong and Kadir, 2012). It has also been reported that most natural antioxidant compounds often work synergistically with each other to produce a broad

spectrum of effects against free radical attack. Our data also demonstrate the presence of possible interrelationship and crosstalk between Noni and Cisplatin which may be related, at least, partly to the antioxidant status in cervical cancer cell lines.

Taken together, this study showed that Noni/Cisplatin by themselves and their combination were able to decrease lipid peroxidation and enhance Catalase activity in both the cervical cancer HeLa and SiHa cell lines. Hence, Noni offers potential to be used as a chemo adjuvant, as an antioxidant, especially for the treatment of cervical cancer. Although further studies are needed to conclude that Noni, as antioxidants, do not conflict with the use of chemotherapy in the treatment of a cervical cancer and that, it may significantly mitigate the adverse effects of that treatment.

## References

- Alitheen NB, Manaf AA, Yeap SK, et al (2010). Immunomodulatory effects of damnacanthol isolated from roots of *Morinda elliptica*. *Pharm Biol*, **48**, 446-52.
- Alshawi AA, Shafi G, Hasan TN, et al (2011). Apoptosis-mediated inhibition of human breast cancer cell proliferation by lemon citrus extract. *Asian Pac J Cancer Prev*, **12**, 1555-9.
- Borek C (2004b). Dietary antioxidants and human cancer. *Integr Cancer Ther*, **3**, 333-41.
- Bulló M, Lamuela-Raventós R, Salas-Salvadó J (2011). Mediterranean diet and oxidation: nuts and olive oil as important sources of fat and antioxidants. *Curr Top Med Chem*, **11**, 1797-810.
- Gupta RK, Banerjee A, Pathak S, et al (2013). Induction of mitochondrial-mediated apoptosis by morinda citrifolia (noni) in human cervical cancer cells. *Asian Pac J Cancer Prev*, **14**, 237-42.
- Gupta RK, Patel AK, Kumari R, et al (2012). Interaction between oxidative stress, lipid profile and antioxidants in breast cancer: a case-control study. *Asian Pac J Cancer Prev*, **13**, 6295-8.
- Jemal A, Bray F, Center MM, et al (2011). Global cancer statistics. *CA Cancer J Clin*, **61**, 69-90.
- Hernandez-Flores G, Ortiz-Lazareno PC, Lerma-Diaz JM, et al (2011). Pentoxifylline sensitizes human cervical tumor cells to cisplatin-induced apoptosis by suppressing NF- $\kappa$ B and decreased cell senescence. *BMC Cancer*, **11**, 483.
- Kostova I (2006). Platinum complexes as anticancer agents. recent patents on anti-cancer. *Drug Discovery*, **1**, 1-22.
- Ma DL, West BJ, Su CX, et al (2007). Evaluation of the ergogenic potential of noni juice. *Phytother Res*, **21**, 1100-1.
- Mohd-Zin Z, Abdul-Hamid A, Osman A (2002). Antioxidative activity of extracts from Mengkudu (*Morinda citrifolia* L.) root, fruit and leaf. *Food Chem*, **78**, 227-31.
- Pathak S, Bhatla N, Singh N (2012). Cervical cancer pathogenesis is associated with one carbon metabolism. *Mol Cell Biochem*, **369**, 1-7.
- Peerapatdit T, Patchanans N, Likidilid A, Poldee S, Sriratanasathavorn C (2006). Plasma lipid peroxidation and antioxidant nutrients in Type 2 Diabetic patients. *J Med Assoc Thai*, **89**, 147-55.
- Serafini MR, Santos RC, Guimarães AG, et al (2011). *Morinda citrifolia* Linn leaf extract possesses antioxidant activities and reduces nociceptive behavior and leukocyte migration. *J Med Food*, **14**, 1159-66.
- Simone CB 2<sup>nd</sup>, Simone NL, Simone V, Simone CB (2007). Antioxidants and other nutrients do not interfere with

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- chemotherapy or radioation therapy and can increase kill and increase survival. Part 1. *Altern Ther Health Med*, **13**, 22-8.
- Su BN, Pawlus AD, Jung HA, et al (2005). Chemical constituents of the fruits of morinda citrifolia (Noni) and their antioxidant activity. *J Nat Prod*, **68**, 592-5.
- Wang MY, Lutfiyya MN, Weidenbacher-Hoper V, et al (2009). Antioxidant activity of Noni juice in heavy smokers. *Chem Cent J*, **3**, 13.
- Wong YH, Abdul Kadir H (2012). Induction of mitochondria-mediated apoptosis in ca ski human cervical cancer cells triggered by mollic acid arabinoside isolated from leea indica. *Evid Based Complement Alternat Med*, **2012**, 684740