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Comparative Cytotoxic Assessment of Hydro-methanolic Extracts Derived from Ripe *Morinda citrifolia* L. Fruit (Fresh, Dried, Pasteurized Juice) and Dried Seeds on Eukaryotic Normal and Carcinogenic Cellular Models

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Abstract: Noni *(Morinda citrifolia* L.*)* is utilized for wellness drinks, puree, and nutraceuticals, while its seeds are a source of vegetable oil, but misconceptions persist due to limited scientific research in Sri Lanka. This study evaluated the cytotoxic effects of hydro-methanolic extracts from fresh and dried noni fruits, pasteurized juice, and seeds on normal (BHK) and cancer (Hep2) cells using the MTT assay. Results indicated dose-dependent toxicity on cancer cells, while normal cells were less affected. Processing methods influenced the cytotoxicity, with dried seeds showing the least toxicity. These findings suggest the potential of noni extracts as cytotoxic agents against cancer, influenced by processing conditions. Further research is needed to identify the specific bioactive compounds and their mechanisms.

Keywords: Noni (*Morinda citrifolia* L.), Cytotoxicity; Nutraceuticals

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1. Introduction

Noni (Morinda citrifolia L.) fruit, known as 'Ahu' locally, is a traditional medicinal plant in Sri Lanka. However, it is not widely consumed or processed due to perceived myths(Samarasinghe et al., 2023a). Whereas, due to its remarkable ability to thrive in harsh environments, even in drought conditions, it is commonly referred to as the 'Starvation fruit,' consequently, it will contribute to the development of new applications and products with minimal adverse effects(Patil et al., 2022). Noni fruit juice has been recognized as a novel food product by the European Union(European Commission, Scientific Committee for Food, 2002). Meanwhile, approval has been granted to expand the utilization of Polynesian noni fruit puree and fruit juice concentrate as novel food ingredients across a range of food categories (European Commission, 2010). Therefore, several dietary supplements made from noni, such as juice, capsules, powder, concentrates, and tea, are currently available in the market(Kulathunga & LDAM, 2017). M. citrifolia has been used as a medicine to maintain good health and prevent various diseases like those affecting the skin, brain, gastrointestinal tract (GIT), heart, liver, and cancer. Currently, the only recommended daily oral dose of M. citrifolia is 2 grams(Abou Assi et al., 2017). Noni seeds are also a potential source of functional food(West et al., 2008), and Noni seed oil (NSO) is non-toxic(West et al., 2011) and possesses a unique fatty acid composition, rendering it an attractive option for utilization in the food, pharmaceutical, and cosmetic industries.

This offers potential benefits in terms of enhancing nutritional quality(Pazos et al., 2011), structural attributes, aroma, and stability in various formulated food products(Fontes et al., 2023; Jahurul et al., 2021).

Publications highlight the health benefits of noni components (fruit, leaf, stem, and seeds), but safety reports are limited. Regular noni juice consumption reportedly doesn't cause toxic effects, such as acute toxicity, hepatotoxicity, or sub-chronic toxicity(Shin et al., 2022). Despite the worldwide use and pharmacological significance of noni fruit and seeds, the effect of this fresh fruit flesh, dried fruit flesh, pasteurized juice, and dried seeds on cancer and also on normal cells has not yet been examined. This in vitro study was undertaken to demonstrate the effect of the extract of M. citrifolia on human laryngeal carcinoma (Hep2) cells and baby hamster kidney (BHK) cells. The purpose of the study was to determine whether this compound had a selective cytotoxic effect against cancer cells. An MTT-based cytotoxic assay was carried out using a cancer cell line and a normal cell line.

2. Methodology

Ripened Noni fruits were obtained from trees grown in the Katugathota area of the Kandy district, Sri Lanka. The selected fruits, based on color and shape, were vacuum-packaged in polyethylene bags and stored at -18 ℃ until further analysis.

Methanolic extracts from the fresh fruit flesh, dried fruit flesh, pasteurized juice, and dried seeds were prepared using a modified methodology as described by (Samarsinghe et al., 2023b). Each sample of ground fruit flesh (1.00 g), dried fruit flesh (1.00 g), dried seeds (1.00 g), and pasteurized juice (1.00 ml) was combined with 15 mL of an 80% methanol/water mixture (v/v) to create the extracts. These mixtures were allowed to soak overnight, followed by high-speed vortexing for 5 minutes and centrifugation at 2600 g for 10 minutes at room temperature using an EBA 20 centrifuge from Hettich, Tuttlingen, Germany. The obtained extracts were then filtered using Whatman No. 42 filter paper from Whatman Paper Ltd., Maidstone, UK. Subsequently, the filtered extracts were evaporated in a rotary evaporator (HAHNVAPOR, Model HS-2005 V, HAHNSHIN Scientific, Korea) under vacuum conditions at 40°C. The resulting evaporated extracts were stored at -18°C until analysis, within a period of 1 week.

HepG2 (human hepatoma) cells and baby hamster kidney (BHK) cells were harvested by following the methodology explained in the study conducted by (Samarakoon et al., 2010) to evaluate cell viability. The cells were harvested by trypsinization, and then plated at a density of 5 x 10^3 cells per well in a 96-well cell culture plate. They were maintained in Dulbecco's Modified Eagle Medium (DMEM) for 24 hours at 37°C in an atmosphere of 95% air and 5% CO2, with 95% humidity. Cultures were exposed only to medium (1% DMSO, controls) or medium containing different concentrations of methanolic extracts dissolved in 1% DMSO (ranging from 300 μg/ml to 4800 μg/ml) and incubated for 24 hours. At the end of this incubation period, cells were briefly washed with Phosphate Buffered Saline (PBS).

The method described by (Ruhomally et al., 2016) was employed to assess cell viability using the MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. After pre-treating with different concentration series for each of noni extracts (including fresh fruit, dried fruit, pasteurized juice, and dried seeds) for 24 hours, cells were exposed to 20 mL of PBS for 30 minutes. Next, 5 mg/mL of MTT was added to each well and incubated for an additional 2 hours. The medium was then removed by aspiration. Finally, 150 μl DMSO was added per well, and the absorbance was read at 595 nm and 690 nm using a microplate reader (ELx800 Universal Microplate Reader, BIO-TEK INSTRUMENTS, USA). The results were expressed as a percentage of the control values.

3. Results

Table 1. In vitro Cytotoxicity of Crude Extract of M. citrifolia Fruit (Fresh, Dried, Pasteurized Juice) and Dried Seeds on BHK and Hep 2 Cell Lines**.**

Concentration	Percentage of Inhibition(%)							
	Fresh fruit flesh		Dried fruit flesh		Pasteurized juice		Dried Seed	
(mg/mL)	BHK cell	Hep2 cell	BHK cell	Hep2 cell	BHK cell	Hep2 cell	BHK cell	Hep2 cell
0.1	4 ± 0.12	17 ± 0.11	2 ± 0.34	14 ± 0.45	3 ± 0.02	10 ± 0.59	1 ± 0.09	8 ± 0.35
0.2	9 ± 0.18	24 ± 0.39	5 ± 0.23	11 ± 0.61	4 ± 0.11	10 ± 0.86	6 ± 0.08	8 ± 0.09
0.4	11 ± 0.62	33 ± 0.77	8 ± 0.38	16 ± 0.72	4 ± 0.09	12 ± 0.12	4 ± 0.39	8 ± 0.77
0.8	23 ± 0.34	45 ± 0.87	24 ± 0.57	25 ± 0.18	13 ± 0.19	15 ± 0.18	12 ± 0.14	17 ± 0.29
0.9	27 ± 0.57	49 ± 0.98	21 ± 0.48	31 ± 0.27	12 ± 0.88	28 ± 0.71	17 ± 0.21	24 ± 0.37
1	42 ± 1.12	59 ± 0.48	35 ± 0.97	40 ± 0.32	21 ± 0.68	30 ± 0.36	16 ± 0.33	23 ± 0.10
2	67 ± 0.88	64 ± 0.88	53 ± 0.66	58 ± 1.01	49 ± 0.19	49 ± 0.91	36 ± 1.03	43 ± 0.26

[1]Values are mean ± SD (n = 18).

The cytotoxic assessment of hydro-methanolic extracts derived from ripe Morinda citrifolia L. fruit (Fresh, Dried, Pasteurized Juice) and dried seeds on eukaryotic normal and carcinogenic cellular models was conducted using the MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. The viability of both normal and cancerous cells, with an initial seeding density of 5 x 10^3 cells per well in a 96-well cell culture plate, was evaluated after treatment with the respective extracts for 24 hours. The results, as presented in Table 1, indicated a general dose-response decrease in cell viability.

For normal cells, the viability ranged between (4-67) %, (2-53) %, (3-49) %, and (1-36) % when pretreated with methanolic-extracted ripe Morinda citrifolia L. fruit (Fresh, Dried, Pasteurized Juice), and Dried Seeds, respectively. The IC50 values for these extracts on normal baby hamster kidney (BHK) cells were IC50(BHK)-0.9759 mg/mL, IC50(BHK)-1.0409 mg/mL, IC50(BHK)-1.1824 mg/mL, and IC50(BHK)-1.6822 mg/mL, respectively.

In the case of carcinoma cells, the viability ranged between (17-64) %, (14-58) %, (10-49) %, and (8-43) % when pretreated with methanolic-extracted ripe Morinda citrifolia L. fruit (Fresh, Dried, Pasteurized Juice), and Dried Seeds, respectively. The IC50 values for these extracts on human laryngeal carcinoma (Hep2) cells were IC50(Hep2)-0.674 mg/mL, IC50(Hep2)-0.9537 mg/mL, IC50(Hep2)-0.9716 mg/mL, and IC50(Hep2)-1.08 mg/mL, respectively.

Furthermore, the effect on normal cells was compared to one cancer cell line (Hep2) to assess selectivity. The results indicated that, unlike normal cells, the extracts from both the fruit (Fresh, Dried, Pasteurized Juice) and Dried Seeds appeared to exhibit dose-dependent toxicity towards cancer cells. Additionally, the inhibition effect on normal cell lines' viability increased as follows: fresh noni fruit flesh, dried noni fruit flesh, pasteurized noni fruit juice, and dried noni seed. This pattern was also observed in the inhibition effect on cancer cell lines' viability. Notably, the reduction in the proliferation rate of Hep2 cells was significantly higher for each crude extract compared to the reduction observed in eukaryotic normal cells (baby hamster kidney (BHK) cells). This observation suggests that the cytotoxic effects may have implications for human eukaryotic cells as well.

4. Conclusion

The results of this study underscore the remarkable potential of fresh *Morinda citrifolia* L. fruit, dried fruit, pasteurized fruit juice, and dried Morinda citrifolia seeds, as powerful anti-proliferative food supplements. The observed dose-dependent inhibitory effect on cancer cell growth, along with the selectivity exhibited towards malignant cells compared to normal cells, highlights the promising role of these extracts in potential cancer therapies. The heightened impact on normal cell lines, coupled with the significant reduction in cancer cell viability, suggests a potential avenue for natural anti-cancer strategies. Further research should focus on unraveling the specific mechanisms behind this selectivity, alongside comprehensive investigations into the safety and bioavailability of these compounds, ultimately aiming to integrate these findings into the development of effective and safe anti-proliferative food supplements for potential oncological interventions.

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