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EVALUATION OF BENEFICIAL EFFECTS OF *MORINDA CITRIFOLIA* L. IN PRESENCE OF CISPLATIN ON EHRLICH'S ASCITES CARCINOMA BEARING MICE

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ABSTRACT: A medicinal plant known as Morinda citrifolia L. (Rubeaceae) is generally referred to as Noni. Inflammation, cancer, high blood pressure, diabetes, bacterial infections, and arthritis have all traditionally been treated using the whole plant. Noni Juice (NJ) and Divine Noni are being investigated for their potential anticancer effects in this current research.Gold (DNG) predominates in mice with Ehrlich ascites cancer (EAC). A single dose of cisplatin (CP) at a dose of 5.0 mg/kg b.w. i.p. on day 1 in each cluster was administered to animals before half an hour of NJ and DNG administration, respectively, for 14 days. The mice bearing EAC were then tested for NJ and DNG's anticancer activity. Mean Survival Time (MST), percentage Increase in Life Span (%ILS), body weight, tumor volume, viable cell count, and other biochemical markers and hematological parameters were used to assess the antitumor activity of NJ and DNG. Tumor volume, viable cell count, body weight, and MST/%ILS were all significantly decreased in the NJ group and increased in the DNG group.Mice subjected to CP had a return to normal or near-normal levels of hemoglobin and blood biochemical markers. The results show that NJ and DNG have anti-angiogenic and antioxidant effects, which may explain their antineoplastic efficacy. Finally, it seems that NJ and DNG might be useful additives to CP chemotherapy, increasing its anticancer effectiveness while reducing its toxicity.

Keywords: NJ, DNG, EAC cell, Anticancer, Anti-angiogenesis antioxidant

INTRODUCTION: Different medical systems, such as Ayurveda, Siddha, and Unani, use unproven remedies derived from many plants to treat a wide range of illnesses. For their cytotoxic and cancer chemopreventive effects, some phytoconstituents found in herbal plants, such as phenolic compounds, flavonoids, terpenes, alkaloids, and anthraquinones, have garnered a lot of interest in recent years.

A number of well-known anticancer medications have their roots in plants; they include vincristine, vinblastine, taxol, and etoposide. In order to find herbs that may treat cancer, the best strategy is to follow ethnomedical leads when selecting plants, and then to evaluate the effectiveness and safety of such herbs in light of modern scientific knowledge. Thoughtful ethnomedical usage of NJ for tumor treatment in many places of India has been examined at ancient therapeutic systems.

Traditional medical practices from long ago, such as Ayurveda, refer to the Noni plant as an antineoplastic agent. 5. Noni, the commercial name for the Morinda citrifolia L. (Rubeaceae) plant. It is a little, well-liked medicinal plant native to South Asia that has longer leaves and a white tubular blossom.

A whitish-yellow hue develops on the mature bunches and fruits, which are initially green. Worldwide, people have relied on Noni products for the treatment of a wide variety of illnesses, including cancer, for over three centuries.

Not only does the noni fruit contain over 160 different phytoconstituents, but over 120 of them are nutraceuticals with biological and pharmacological activity. 6, 7. The evergreen ligneous Noni plant, which is native to tropical forests around the Pacific Ocean, is the source of the delicious noni fruit. The ancient Indian medical practice of Ayurveda revered the noni plant; its name, Ashyuka, means "longevity" in Sanskrit, appears in many manuscripts from that time. Herbal treatments are now being used in combination therapy with anticancer pharmaceuticals because of their influence on the disease.

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The anti-cancer effects of the phytoconstituents found in Noni fruit include alkaloids and flavonoids, among others.

9. Glycosides and iridoid glycosides extracted from Noni fruit that showed inhibitory effects on AP-1 transactivation and

In the JB6 cell line of mouse epidermis, cell transformation occurs between 10 and 14.

Among the many well-documented traditional medicinal uses for NJ are its antioxidant, antihypertensive, antibacterial, anti-inflammatory, antiangiogenic, and anticancer effects. New evidence suggests that NJ may have a protective function in chemically generated malignancies by inhibiting the production of 7, 12-dimethylbenz [a] anthracene (DMBA)-DNA adducts. The antitumor effects of NJ precipitates are cell-specific and rely on macrophages, NK cells, and T cells; moreover, they extend the synergistic effects of anticancer medications 16, 17.A Noni formulation that includes Garcinia cambogia fruit extract and Noni fruit juice is DNG concentrate. There is DNG concentrate on the market, and individuals are using it as a nutraceutical and in cancer treatments. Incorporating phytoconstituents like garcinol into Garcinia cambogia fruit was shown to have antioxidant and anticarcinogenic properties (18, 19). The present investigation aims to test the hypothesis that NJ and DNG, when administered to EAC-bearing mice in the context of CP, will have antineoplastic effects.

MATERIALS AND METHODS:

Animals: Inbred Swiss albino mice weighing 25– 30 g has been obtained from central animal house facility, JSS Medical College, Mysuru, India, to use for the experiments. Before start the experiment we obtained permission from Institutional Animal Ethical Committee (IAEC) of JSS College of Pharmacy, JSS University, Mysuru, and followed CPCSEA guidelines for animal care and handling. The obtained IAEC number of the present study is 161/2016.

Chemicals and Drug: Sodium chloride, trypan blue, propylene glycol, methyl violate, methylene blue and various kits were used to perform this experiment and supplementary chemicals and reagents used were of highest analytical grade. Cisplatin hydrochloride injection 50 mg/50 ml vial were procured from JSS Hospital, Mysuru, India.

Noni Samples: The ripe Noni fruits and DNG concentrate were procured from Noni Biotech Pvt. Ltd. Tamil Nadu, India. Fresh NJ was prepared from fully ripped Noni fruit by hand squeezing method and both the samples were kept in freeze throughout the experiment.

Transplantation of Tumor: The EAC fluid derived from a spontaneous murine mammary adenocarcinoma were maintained in the ascetic form by consecutive passage in Swiss albino mice by means of weekly i.p. transplantations of $10x10^6$ tumor cells. From this stock suspension 0.1 ml of tumor cell suspension containing $2.5x10^6$ tumor cells were injected /mouse to obtain ascitic tumor on day 0 to all the prescribed groups of experimental animals [20, 21]. After 24hr of tumor inoculation, animals were treated as follows.

Treatment Schedule: Mice were divided into 6 clusters and each cluster contained 16 mice, subjected to various daily treatment regimens. After 14 days of treatment blood samples were collected from 6 mice for the estimation of hematological parameters and serum biomarkers from respective cluster and other 10 mice were kept for the assessment of tumor parameters i.e. MST, body weight, tumor volume and viable cell count.

Group-I represented as control which was untreated. Group-II received single dose of CP (5.0 mg/kg b.w. i.p.) [22] on day 1. Group-III received NJ (0.35 ml/mouse p.o.) once daily for 14 days. Group-IV received DNG (0.35 ml/mouse p.o.) oncedaily for 14 days. Group-V received CP (5.0 mg/kg
b.w. i.p.) on day 1, after half an hour received NJ (0.35 ml/mouse p.o.) once daily for 14 days. Group-VI received CP (5.0 mg/kg b.w. i.p.) on day 1, after half an hour received DNG (0.35 ml/mouse p.o.) once daily for 14 days.

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Body Weight Analysis: Body weight were started to record from day 0 of the experiment and successively on every 3 day for 15 days. Average body weight and percentage decrease in body weight was calculated using the following formula [26].

% change in body weight =

$$\frac{\text{Animal weight on respective day}}{\text{Animal weight on day 0}} - 1 \times 100$$

The dose of NJ and DNG were calculated according to the following formula: Human adult dose * body surface area ratio convertible factor of mouse =133 ml * 0.0026 = 0.3458 ml [23].

Measurement of Antitumor Activity: Antitumor activity was evaluated with respect to the followingparameters mentioned below.

Determination of MST and %ILS: At the end of the experiment, the effect of NJ and DNG on tumor growth was monitored by recording the mortality daily for five weeks. MST and %ILS were calculated by the following formula mentioned below [24].

$$MST^{*} = \frac{\text{Day of first death} + \text{Day of last death}}{2}$$

*Time denoted by days

$$\%ILS = \left[\frac{\text{MST of the treated group}}{\text{MST of the control group}} - 1\right] \times 100$$

Measurement of Tumor Volume: The ascitic liquid was collected from the peritoneal cavity of mice by 1 ml disposable syringe. The volume of tumor liquid was measured in graduated centrifuge tube.

Measurement of Viable Cell Count: Viable cells were checked by trypan blue assay. The cells were stained trypan blue (0.4% in normal saline) dye. The cells which not received the stain were viable and those received the stain were non-viable. In this manuscript we have represented only viable cells count. The viable cells were counted by the following formula.

Viable cell count = (Number of cells x Dilution factor)/ (Area x Thickness of liquid film) [25].

Estimation of Hematological Parameters and Biochemical Markers: After 14 day's of treatment, on day 15th blood was collected from carotid vein and heart puncture routes were used for the estimation of hematological parameters i.e. RBC estimated according to the D'Armour *et al.*,[27] method, WBC and Hb are estimated according to the Wintrobe *et al.*,[28] method. Biochemical markers aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were estimated according to the Reitman and Frankel method [29].

Statistical analysis: Statistical analysis was performed by using graph prism - 6 followed by one way and two way ANOVA. All the data were expressed as mean±S.E.M. Statistical significance was considered at $p<0.05$, $p<0.01$ and $p<0.001$.

RESULTS:

Effect of NJ and DNG on MST and % ILS: In EAC control group observed that MST and %ILS were simultaneously decreased which significantly augmented by co-administration of NJ and DNG as adjuvant therapy in CP challenged mice (**Fig. 1**).

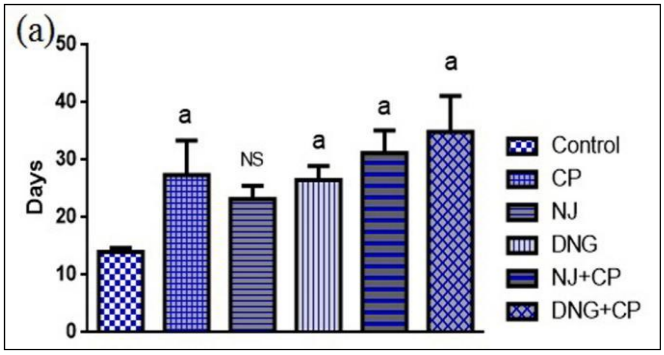

FIG. 1: MST (1a), ALL VALUES WERE CONSIDEREDAS MEAN±S.E.M. OF TEN MICE, $p<0.05$ compared to control

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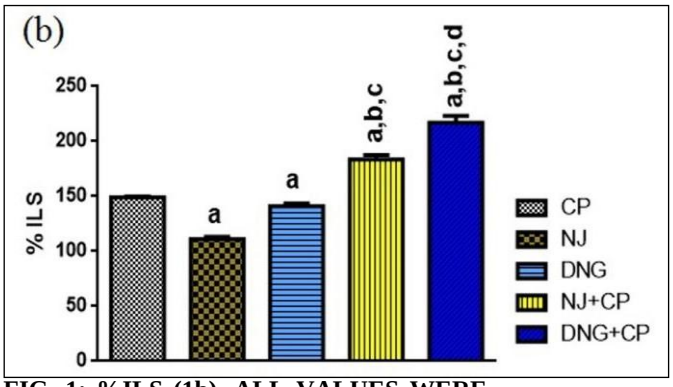
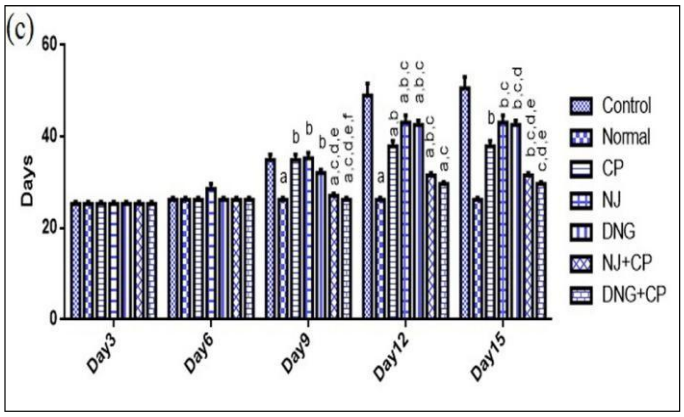


FIG. 1: %ILS (1b), ALL VALUES WERE CONSIDERED
AS MEAN±S.E.M. OF TEN MICE [a]p<0.01 compared to CP,
[b]p<0.01 compared to NJ, [c]p<0.01 compared to DNG, [d]p<0.01
compared to NJ+CP

Effects of NJ and DNG on Tumor Volume: Adjuvant therapy of NJ and DNG significantly (p<0.01) decreased tumor volume, viable cancer cell count, body weight in CP challenged mice in comparison to control (**Fig. 2**).



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DISCUSSION: Present *in-vivo* study exhibited that NJ as well as DNG significantly enhanced the life span in comparison to EAC control mice. Animals treated with DNG showed more MST and %ILS than NJ may be because of containing the extract of *Garcinia cambogia* fruit **(Fig. 1a)** and **(Fig. 1b)**. The reliable criteria for arbitrating the value of any antitumor drug extend life span in addition to decline WBC count [30]. Furthermore, the abridged volume of EAC and augmented survival time of mice suggests the deterring effect of NJ and DNG on cell proliferation.

This result support the recent study which suggested crude extract of *Morinda citrifolia* fruit exhibited antiproliferative activity against breast cancer (MCF-7) and neuroblastoma (LAN5) cell line at 29% and 36% respectively [31].

CONCLUSION: Overall results indicates that DNG showed more protective effect than NJ. This effect may be due to containing the fruit extract of *Garcinia cambogia*. Moreover, NJ is having bad odour and unpleasant taste so patient may reluctant to consume it. Therefore, this fact concluded that DNG may be a useful supplement in CP chemotherapy for augmenting the antitumorefficacy and dropping the toxic effects of CP.

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