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Cancer preventive Effect of *Morinda citrifolia* (Noni) fruit juice against the AflatoxinB1-induced genotoxicity in human peripheral lymphocytes in vitro.

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ABSTRACT
Morinda citrifolia (Noni) has been used in folk medicine by Polynesians for over 2,000 years and is reported to have a broad range of therapeutic effects, including anticancer activity. The exact mechanism of action is unknown. The hypothesis is generated from the experiment that *Morinda citrifolia* possesses a cancer preventive effect at the initiative stage of carcinogenesis. The antigenotoxic potential of Noni juice (NJ) was demonstrated on the aflatoxin B1 induced genotoxicity. *In vitro* studies were carried on human lymphocyte culture. We have used chromosomal aberration (CA), sister chromatid exchange (SCE) and cell cycle kinetics (CCK) with and without S_9 mix. as markers in this experiments. Four doses viz., 200, 250, 300, 350 µl/ml per culture were selected and found that NJ significantly reduces the frequencies of chromosomal aberration, sister chromatid exchanges and enhances RI *in vitro* .It was also noticed that the antigenotoxic potential of NJ shows dose – response relationship. The results suggest that NJ was a potent anticarcinogen may contribute to the cancer prevention.

Key words: *Morinda citrifolia* (Noni), *Noni Fruit juice (NJ), chromosomal aberration, Sister Chromatid Exchange, anticarcinogen.*

INTRODUCTION:

Noni is the common name for *Morinda citrifolia.* It is a medicinal plant called *Indian mulberry* in India, *bajitian* in China, *Nono* in Tahiti, and *noni* in Hawaii (Morton J, 1992.). In India it is distributed throughout Tamil Nadu and Kerala in South India, especially coastal region and also in the Mangalore area of Karnataka. Among the medicinal plants discovered by the ancestors of Polynesians, *Morinda citrifolia* L(Noni) is one of the traditional folk medicinal plants that has been used for over 2000 years in Polynesia. It has been reported to have a broad range of therapeutic effects like anti-cancerous, anti-tumor, and have nutritional value. Noni has an abundance of micronutrients. It has also been used by the native Tahitians for over 2000 years as a nutritional supplement to treat diseases and promote general good health (Abbott & Shimazu, 1985; Degener, O. 1973).

Noni plants have 160 identified chemicals, the major components are terpene, scopoletin, octoanoic acid, potassium, vitamin C, terpenoids, alkaloids anthraquinones (such as nordamnacanthal, morindone, rubiadin, and rubiadin-□-methyl ether anthraquinone glycosides), sitosterol, carotene, vitamin A, flavone glycosides, linoleic acid, alizarin, amino acids, cacubin, *L*-asperuloside, caproic acid, caprylicacid, ursolic acid, rutin, and a putative proxeronine polysaccharides, and alkaloids. Dr Neil Solomon summarized 15,000 cases of NJ users, and found the total effective rate of NJ on various health problems, including cancer to be 78% effective. NJ may possess a cancer preventive effect at the initiation stage of carcinogen and /or antioxidant activity.

The anticancer activity from alcohol-precipitate of Noni fruit juice (Noni-ppt on to lung cancer in c57 B1/6 mice has been presented in the 83 Annual Meeting of American Association for Cancer Research. The NJ significantly increased the life of mice up to 75% with implanted Lewis lung carcinoma as compared with the control mice (Hirazumi *et al.*, 1994). It was concluded that the NJ seems to suppress tumor growth directly by stimulating the immune system (Hirazumi *et al.*, 1996). Improved survival and curative effects occurred when Noni-ppt was combined with sub optimal doses of the standard chemotherapeutic agents such as adriamycin (Adria), cisplatin(CDDP), 5-flourouracil (5-FU) and vincristine (VCR), suggesting important clinical application of Noni-ppt as a supplemental agent in cancer treatment (Hirazumi and Furusawa, 1999). These results indicated that the Noni-ppt might enhance the therapeutic effect of anticancer drugs. Therefore, it may be a benefit to cancer patients by enabling them to use lower doses of anticancer drugs to achieve the same or even better results. Wang *et al.* (2002) demonstrated that the cytotoxic effect of Tahitian Noni Juice (TNJ) on cultured leukemia cell line at various concentrations. They also observed the synergistic effects of TNJ with known anticancer drugs. At a sub-optimal dose, both prednisolone and TNJ could induce apoptosis. When the dose of prednisolone was fixed, the dose of TNJ increased. Therefore TNJ is able to enhance the efficacy of anticancer drugs such as predinosolone. When a single dose of taxol induced a lower percentage of apoptosis in leukemia cells, TNJ enhanced the rate of apoptosis. Hiramatsu *et al.* (1993) reported effects of over 500 extracts from tropical plants on the K-Ras-NRK cells. The Ras oncogene is believed to be associated with the signal transduction in several human cancers such as lung, colon, pancreas, and leukemia. Two glycosides extracted from Noni-ppt were effective in inhibiting cell transformation induced by TPA or EGF in the mouse epidermal JB6 cell line. The inhibition was found to be associated with the inhibitory effects of these compounds on AP1 activity. The

compounds also blocked the phosphorylation of c-Jun, a substrate of JNKs, suggesting that JNKs are the critical target for the compounds in mediating AP1 activity and cell information (Liu *et al.,* 2001).

MATERIALS AND METHODS:

Experiment was performed using the technique of Moorehead *et.al* (1960), for metaphase chromosome analysis and for detection of chromosomal aberration analysis (CAs). Human lymphocyte cultures were set by adding 0.5 ml of whole blood (from two adult and healthy donors, occupationally not exposed to mutagens) to 4.5 ml of RPMI 1640 (Gibco, USA), antibiotics (Penicillin and streptomycin 100 IU/ml each; Hoechst) and L. Glutamine (1 mM; Gibco, USA). Lymphocytes were stimulated to divide by adding 0.1 ml of phytohaemagglutinin– M (PHA– M, Gibco). The cultures were incubated at 37^{o}C with 5% CO_2 for 72 hours in dark. Aflatoxin B1 at a final concentration of 50 µg was added at 0 hour and kept for 24, 48 and 72 hours of duration, which served as positive control. Subsequently, desired test chemical were added along with Aflatoxin B1 and the cultures were kept for 24, 48 and 72 hours. Noni juice and Aflatoxin B1 were prepared in DMSO. In the metabolic activation experiments cultures were treated with S_9 mix (0.8 ml.), the S_9 mix was freshly prepared as per the standard procedures of Maron and Ames (1983). The S_9 fraction was complemented by the addition of 5 µM NADP and 10 µM glucose –6- phosphate just before use. Colchicines (0.20 µg/ml, Micro lab) were added to the cultures 2.5 hours prior to harvesting. The cells were collected by centrifugation (10 min, 1200 rpm), hypotonic treatment (0.075M KCl) was given for 10-12 min at 37^{0}C and the recollected cells after centrifugation were fixed in methanol: acetic acid (3:1). DMSO and Aflatoxin B1 were uses as negative and positive controls, respectively. Preparation of slides, staining and scanning was done under code. A total of 300 well - spread metaphases were analyzed per treatment per duration for all types of chromatid and chromosome type of aberrations. Aberrations were scored as per Hundal, *et al* (1997). Analysis of SCE was carried out following the fluorescent plus Giemsa technique of Perry and Wolfs (1974). The cells in the cultures were exposes to 5-bromo-2-de oxyuridine (BrdU 2 µg/ml; Sigma) after 24 hours of initiation of culture. The test compounds with same concentrations as in the case of CA analysis were added together with the BrdU. To minimize photolysis of BrdU another 48 hours cultures were maintained in the dark. After 90 min. of this pulse treatment the cells were spun down and the supernatant discarded. The cells were washed twice to remove any traces of the drug, phytoproducts and the liver metabolites. Finally the cell pellets were re-suspended in fresh medium supplemented with fetal calf serum, antibiotics and BrdU, and kept for another 24 hours in the dark at 37^{o}C.

One day old slides were stained in Hoechst 33258 stain (Sigma 0.5 µg/ml), exposed to UV lamp (254 nm) for 30 min. and incubated in 2X SSC (0.3 M NaCl, 0.03M Sodium citrate; pH 7.0) at 60^{o}C for 90 min and stain for sister chromatid. The slides were coded prior to scoring and 50 well- spread metaphase cells were scanned per concentration and the number of exchanges scored (Hundal, *et al.*, 1997). Cells undergoing 1st (M_1), 2nd (M_2) and 3rd (M_3) metaphase divisions were detected with BrdU – Harlequin technique for differential staining of metaphase chromosome by studying 200 metaphases for each combination and duration. The replication index (RI), an indirect measure of studying cell cycle progression, was calculated by applying the following formula (Tice, *et al.*, 1976).

$$RI = \frac{M_{1x1}+M_{2x2}+M_{3\,x3}}{100}$$

RESULT AND DISCUSSION:

Noni is a medicinal plant that has antioxidant activity, may protect individuals from free oxygen radicals and consequent lipid peroxidation. In this experiment we have notices that Aflatoxin B1 treatment caused aberrations from 30.50 to 60.0% as the durations increases, when NJ are used it reduces from 19.75 to 50.0% at lower concentration and 19.0 to 46.5% at the highest concentration (Table-1).When culture were augmented with S_9 mix, effects of NJ has shown more effective in reducing the percent of aberration. The range and mean values of SCE get reduced with NJ in the absence as well in the presence of metabolic activation (Table-2). The replication indices, which are reduced due to Aflatoxin B1 treatment level (1.44), is elevated to 1.58, thus bringing the cell proliferation nearly back to near about normal of 1.60. The S_9 activation has follow the above trend (Table-3).

In general, consuming fruits and vegetables reduces free radicals induced oxidative damage and the consequent lipid peroxidation and therefore reduce the cancer risk (Wang and Leiher, 1995; Diplock *et al*., 1998). It is believed that fruits and vegetables are major sources for antioxidants (Weisburer *et al.,* 1997; Nishikimi *et al*., 1972). Noni is a medicinal plant that helps the human in different health conditions. It was believed that the Noni fruit juice contained significant level of antioxidants. This has been proved scientifically by the analysis of NJ. The study was designed to measure how the NJ scavenged superoxide anion radicals (SAR) and quenched lipid peroxides (LPO) by TNB assay and LMB assay, respectively (Auerbach *et al*., 1992; Wang and Su, 2001). SAR scavenging activity was examined *in vitro* by Tetrazolium nitroblue (TNB) assay. The SAR scavenging activity of NJ was compared to that of three known antioxidants; vitamins C, grape seed powder, and pyncogenol at the daily dose per serving level recommended by US RDA's or manufacturer's recommendations. Under the experimental conditions the SAR scavenging activity of NJ was shown to be 2.8 times that of vitamin C, 1.4 times

that of pyncogenol and1.1 times that of grape seed powder. Therefore NJ has a great potential to scavenge reactive oxygen free radicals (Wang and Su, 2001).

The major ingredient in NJ and fruit has been safely consumed in other parts of the world for several hundred years. The interactions of carcinogens, free oxygen radicals, and LPO may be changed by NJ. The mechanism of the cancer preventive effect of NJ needs further study.

In conclusion, on the basis of our preliminary data, NJ may possess a cancer preventive effect at the initiation stage of chemical carcinogenesis. It serves as a good liquid nutritional supplement. NJ may help to prevent cancer and other diseases, and maintaining overall good health.

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Table-1. Analysis of C.A. after treatment of Aflatoxin B1 and NONI JUICE (NJ)in absence as well as presence of S_9 – mix *in vitro*.

Treatment	Metabolic Activation	Metaphase scanned	Percent Aberration Metaphase		Types of aberration(%)			Aberration/Cell ± SE
			Including Gap	Excluding Gap	Chromatid	Chromosome	Total	
AflatoxinB1(50µg/ml)								
	$-S_9$	300	30.00	28.00	25.50	12.50	38.00	0.38 ± 0.02
	$+S_9$s	200	36.25	32.50	30.20	20.30	50.50	0.50 ± 0.02
Aflatoxin B1 + NJ								
	$-S_9$	300	25.35	23.22	20.10	15.20	35.30	0. 35 ± 0.02
Af B1+ NJ0	$+S_9$	200	32.50	30.35	25.00	23.50	48.50	0 .48 ± 0.02
	$-S_9$	300	22.30	20.19	20.50	12.30	32.80	0.32 ± 0.02
Af B1+ NJ1	$+S_9$	200	28.00	25.35	24.50	21.20	45.70	0.45 ± 0.02
	$-S_9$	300	20.50	19.25	18.30	12.20	30.50	0.30 ± 0.02
Af B1+ NJ2	$+S_9$	200	27.32	23.30	23.70	18.50	42.20	0.42 ± 0.02
	$-S_9$	300	18.50	17.24	17.30	10.20	27.50	0.27 ± 0.02
Af B1+ NJ3	$+S_9$	200	25.50	21.35	22.50	18.50	41.00	0.41 ± 0.02
Control								
	$-S_9$	300	2.50	2.00	2.50	0.75	3.25	0.03 ± 0.02
Normal + F2	$+S_9$	200	3.00	2.50	2.25	1.00	3.25	0.03 ± 0.02
Normal	$-S_9$	300	3.50	2.00	2.75	0.35	3.10	0.03 ± 0.02
	$+S_9$	200	4.20	3.70	3.00	0.50	3.50	0.03 ± 0.02
DMSO(5 µ g/ml)	$-S_9$	300	4.00	1.75	3.50	0.75	4.25	0.04 ± 0.02
	$+S_9$	200	4.50	2.00	3.00	1.00	4.00	0.04 ± 0.02

Table-2. Analysis of SCE, after treatment of Aflatoxin B1 and Noni juice (NJ) *in vitro* with and without metabolic activation.

Treatment	Duration (h)	Metabolic activation	Metaphase scanned	Total SCE	Range	SCE/Cell ± SE
Aflatoxin B1(50 µ g/ml)	48	$-S_9$	50	175	1-10	3.50 ± 0.50
		$+S_9$	50	210	1- 9	4.20 ± 0.80
Aflatoxin B1 + NJ						
Af B1+ NJ0		$-S_9$	50	152	1-10	3.04 ± 0.50
	48	$+S_9$	50	170	2-12	3.40 ± 0.70
	48	$-S_9$	50	135	0 - 9	2.70 ± 0.40
Af B1+ NJ1		$+S_9$	50	165	1 - 9	3.30 ± 0.50
		$-S_9$	50	121	0 - 7	2.42 ± 0.30
	48	$+S_9$	50	141	0 - 9	2.82 ± 0.40
Af B1+ NJ2	48	$-S_9$	50	110	0- 6	2.20± 0.20
Af B1+ NJ3		$+S_9$	50	130	0- 9	2.60 ± 0.40
Control						
		$-S_9$	50	57	0-6	1.14 ± 0.14
Normal + F2	48	$+S_9$	50	70	0-6	1.40 ± 0.14
		$-S_9$	50	65	0-5	1.30 ± 0.12
Normal	48	$+S_9$	50	85	0-7	1.70 ± 0.20
		$-S_9$	50	70	0-5	1.40 ± 0.14
DMSO(5 µ g/ml)	48	$+S_9$	50	95	0-6	1.90 ± 0.20

Table-3. Analysis of Cell cycle kinetic after treatment of Aflatoxin B1 and Noni juice(NJ) *in vitro* with and without metabolic activation.

Treatment	Cell scored	Metabolic activation	Percent Aberration Metaphase			Replication Index (RI)	2*3 Chi square test
			M1	M2	M3		
Aflatoxin B1(50 µ g/ml)	200	$-S_9$	54	39	04	1.44	Significant
	200	$+S_9$	57	35	05	1.42	Significant
Aflatoxin B1 + Flavonoids							
Af B1+ NJ0	200	$-S_9$	51	38	08	1.51	Signficant
	200	$+S_9$	53	36	07	1.46	Signficant
		$-S_9$	52	37	09	1.53	Signficant
Af B1+ NJ1	200	$+S_9$	54	38	12	1.66	Signficant
	200						
		$-S_9$	48	40	13	1.67	Not Signficant
Af B1+ NJ2	200	$+S_9$	50	42	08	1.58	Signficant
	200						
Af B1+ NJ3	200	$-S_9$	49	36	15	1.66	Signficant
	200	$+S_9$	51	40	09	1.58	Signficant
Control							
Normal + NJ	200		40	35	18	1.64	
Normal	200		41	37	15	1.60	Not Signficant
DMSO(5 µ g/ml)	200		42	36	14	1.56	normal