Cancer Preventive Effect of *Morinda citrifolia* (Noni)

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ABSTRACT: *Morinda citrifolia* (Noni) has been extensively used in folk medicine by Polynesians for over 2,000 years. It has been reported to have broad therapeutic effects, including anticancer activity, in both clinical practice and laboratory animal models. The mechanism for these effects remains unknown. The hypothesis that *Morinda citrifolia* possesses a cancer preventive effect at the initiation stage of carcinogenesis was studied. Our preliminary data indicated that 10% Tahitian Noni® Liquid Dietary Supplement or Tahitian Noni® Juice (TNJ), made from *Morinda citrifolia* fruit by Morinda Inc, in drinking water for one week was able to prevent DMBA-DNA adduct formation. The levels of DMBA-DNA adducts were reduced by 30% in the heart, 41% in the lung, 42% in the liver, and 80% in the kidney of female SD rats. Even more dramatic results were obtained in male C57 BL-6 mice: 10% TNJ was able to reduce DMBA-DNA adduct formation by 60% in the heart, 50% in the lung, 70% in the liver, and 90% in the kidney. In order to explore the mechanism of this preventive effect, the antioxidant activity of TNJ was examined *in vitro* by lipid hydroperoxide (LPO) and tetrazolium nitroblue (TNB) assays. In the LPO assay, LPO oxidizes leucomethylene blue to methylene blue in the presence of hemoglobin. The resultant blue color was quantified at 660 nm spectrophotometrically. In the TNB assay, superoxide anion radicals (SAR) reduce TNB into formazan blue that was also measured by absorption at 602 nm. TNJ showed a dose-dependent inhibition of both LPO and SAR in our system. The antioxidant activity of TNJ was compared to the effects of vitamin C, grape seed powder (GSP), and pycnogenol (PYC) at the daily dose per serving level recommended by U.S.RDAs or manufacturers. The results suggest that prevention of carcinogen-DNA adduct formation and the antioxidant activity of TNJ may contribute to the cancer preventive effect of *Morinda citrifolia*.

KEYWORDS: Tahitian Noni® liquid dietary supplement or Tahitian Noni®, Juice (TNJ); cancer prevention; 7,12-dimethylbenz(a)anthrancene (DMBA); DNA adducts; lipid hydroperoxide (LPO); superoxide anion radical (SAR), tetrazolium nitroblue (TNB), antioxidant

INTRODUCTION

Morinda citrifolia or noni is a medicinal plant called *Indian mulberry* in India, *ba ji tian* in China, *nono* in Tahiti, and *noni* in Hawaii.[1–3] It has been reported to have a

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broad range of therapeutic effects—anti-inflammatory, antihistamine, anti-fungal, antibiotic, antiviral, anticancer, and hypotensive—as well as functioning as a painkiller.[4–6] Tahitian Noni® Liquid Supplement or Tahitian Noni Juice (TNJ) is made from the fruits of *Morinda citrifolia* grown in virgin and tropical soils in the Tahitian Islands. Noni has an abundance of micronutrients and has been used by the native Tahitians for over 2000 years as a nutritional supplement to treat diseases and promote general good health. Among the more than 160 identified chemicals in noni, the major components are terpene compounds, anthraquinones, morindone, morindin, asperuloside, acubin, caproic acid, caprylic acid, damnacanthal, scopoletin, polysaccharide, and alkaloids.[7] Dr. Neil Solomon summarized 15,000 cases of TNJ users, and found the total effective rate of TNJ on various health problems, including cancer, to be 78%.[8] After 50 years of study, Dr. Ralph Heinicke proposed his new hypothesis—the xeronine system.[9] He has found an essential nutrient in *Morinda citrifolia* that he named proxeronine. According to his theory, a deficiency of this material can lead to various health problems. Proxeronine is the molecule that the cell uses to synthesize xeronine. Xeronine may be an alkaloid essential to life. He endorses TNJ as the best source of proxeronine. Our hypothesis is that TNJ may possess a cancer preventive effect at the initiation stage of chemical carcinogenesis, by preventing the carcinogen-DNA adduct formation and/or antioxidant activity.

MATERIALS AND METHODS

The Preventive Effect of TNJ on 7, 12-dimethylbenz(a)anthrancine (DMBA)-DNA Adduct Formation in Vivo

Six-week-old female SD rats were divided into two groups. Control animals were given water and the others 10% TNJ. On the 8th day, three animals from each group were intragastrically given 25 mg/kg DMBA in 1% DMSO in corn oil. The animals were sacrificed after 24 hours. The DMBA-DNA adducts were examined in various organs by ^{32}P-postlabeling assay. The materials and technique of ^{32}P-postlabeling were described in our previous study.[10–11]

Antioxidant Activity of TNJ

The lipid hydroperoxide (LPO) quenching activity of TNJ was examined *in vitro* by LPO assay.[12] In this assay, LPO oxidizes leucomethylene blue to methylene blue in the presence of hemoglobin. The resultant blue color can be quantified spectrophotometrically (660 nm). In this study, authentic cumene hydroperoxide was used as a standard to monitor the LPO quenching activity of TNJ. The SAR scavenging activity of TNJ was examined *in vitro* by tetrazolium nitroblue (TNB) assay.[13] In TNB assay, SAR reduces TNB into formazan blue, which absorbs at 602 nm. A SAR scavenger reduces the absorbence by reacting with SAR. In this assay, NADH generated SAR under aerobic conditions, where phenazine methosulfate (PMS) was used as a catalyst. The antioxidant activity of TNJ was compared to that of three known antioxidants against SAR *in vitro* at the recommended daily dose per serving by U.S.RDAs (U.S.Government Recommended Daily Allowances) or by manufacturers. Sixty mg of Vitamin C (Roche Vitamins Inc, Parsippany, NJ), 60 mg of PYC

(TwinLabortories Inc., Ronkonkoma, New York 11779), 100 mg of GSP (DNP International Co., Inc.-3035 Red Hat Lane, Whittier, CA 90601), and 32 ml of TNJ (Morinda, Inc.) were used to estimate the biological levels of various antioxidants.[14–16] Our calculations were based upon a proposed 100% bioavailability. The recommended dose of each antioxidant was divided by 4.5 liters of blood per person as our experimental concentration *in vitro*, which may be close to the biological level in the human body. Based upon our calculation, the concentrations of vitamin C, GSP, PYC, and TNJ were 13.3 µg, 22.2 µg, 13.3 µg, and 7.1 µl per ml, respectively.

RESULTS

The preventive effects of TNJ on DMBA-induced DNA adduct formation have been observed in female SD rats (see FIGURES 1 and 2). DMBA-DNA adduct formation was

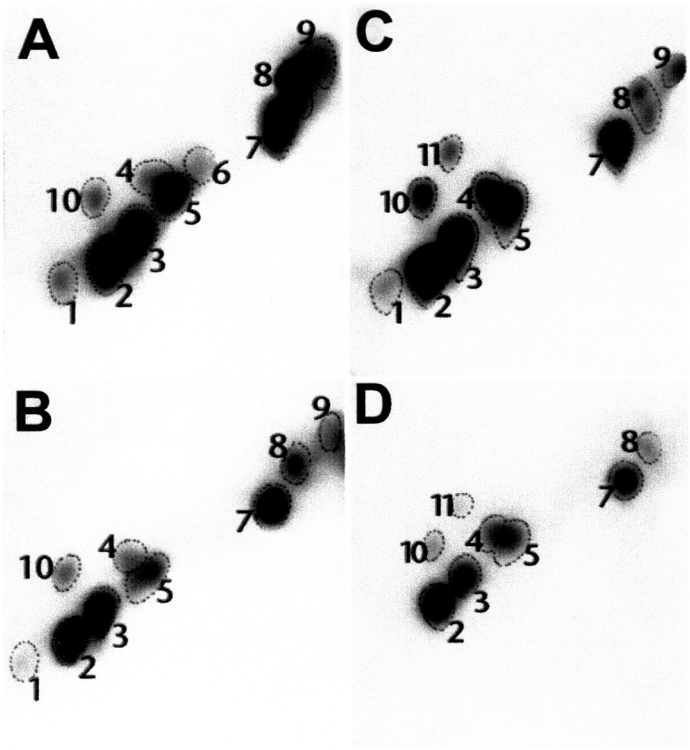

FIGURE 1. Typical profiles of DMBA-DNA adducts in control liver (**A**) and lung (**C**) of female SD rats were induced. The densities and the numbers of DNA adducts in liver (**B**) and lung (**D**) were reduced in 10% TNJ. The level of DMBA adducts was prevented by 42% in liver, 41% in lung, respectively, when compared to the control group. The films were exposed at −80°C for three hours.

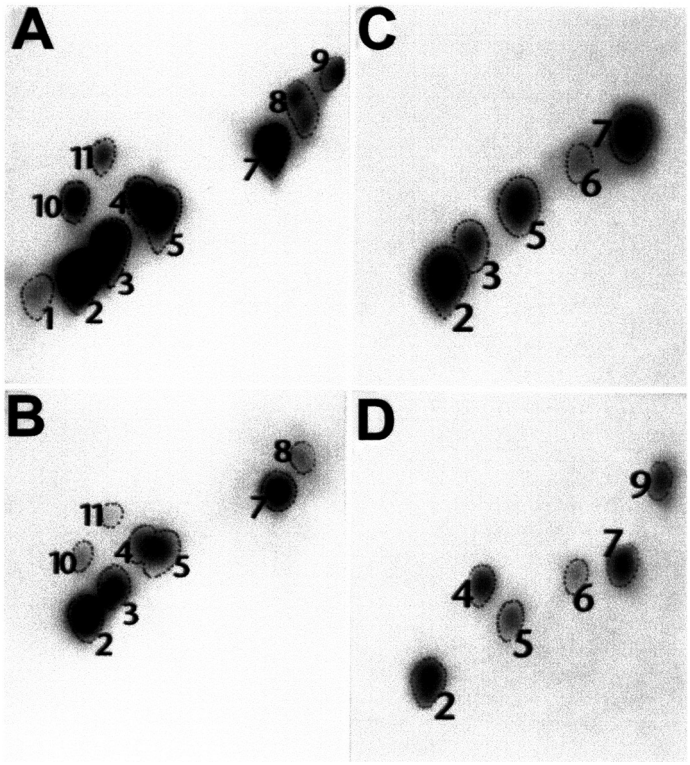

FIGURE 2. Typical profiles of DMBA-DNA adducts in control heart (**A**) and kidney (**C**) of female SD rats were induced. The densities and the numbers of DNA adducts in heart (**B**) and kidney (**D**) were reduced in 10% TNJ group. The level of DMBA adducts was prevented by 30% in heart, 80% in kidney, respectively, when compared to the control group. The films were exposed at −80°C for three hours.

reduced in female SD rats drinking 10% TNJ. The levels of DMBA-DNA adducts were reduced by 30% in heart, 41% in lung, 42% in liver, and 80% in kidney, respectively. Even more dramatic results were observed in male C57 BL-6 mice. Ten percent of TNJ was able to prevent DMBA-DNA adduct formation by 60% in heart, 50% in lung, 70% in liver, and 90% in kidney.

The antioxidant activity of TNJ was observed *in vitro*. A dose-dependent curve of the SAR scavenging activity of TNJ *in vitro* was obtained by TNB assay (see FIGURE 3). A dose-dependent curve of the LPO quenching activity of TNJ *in vitro* was also observed by LPO assay (see FIGURE 4).

The SAR scavenging activity of TNJ was compared to that of three known antioxidants: vitamin C, GSP, and PYC (see FIGURE 5). Under our experimental conditions, the SAR scavenging activity of TNJ was shown to be 2.8 times that of vitamin C, 1.4 times that of PYC, and 1.1 times that of GSP.

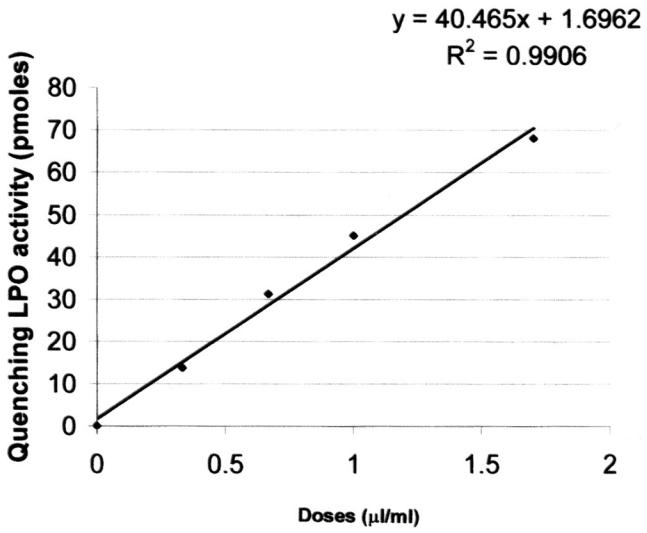

FIGURE 3. Dose response curve of TNJ quenching LPO activity *in vitro.* A linear relationship between the quenching LPO activity of TNJ and the selected doses has been observed. $y = 40.465x + 1.6962$ and $R^2 = 0.9906$.

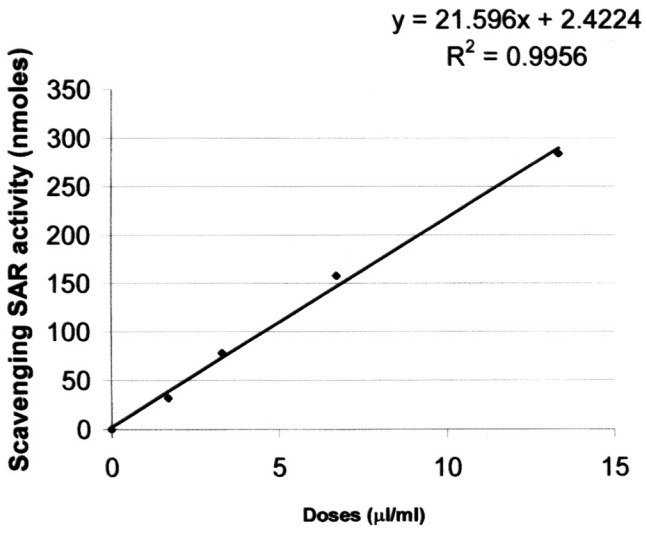

FIGURE 4. Dose response curve of TNJ scavenging LPO activity *in vitro.* A linear relationship between the scavenging SAR activity of TNJ and the selected doses has been observed. $y = 21.596x + 2.4224$ and $R^2 = 0.9956$.

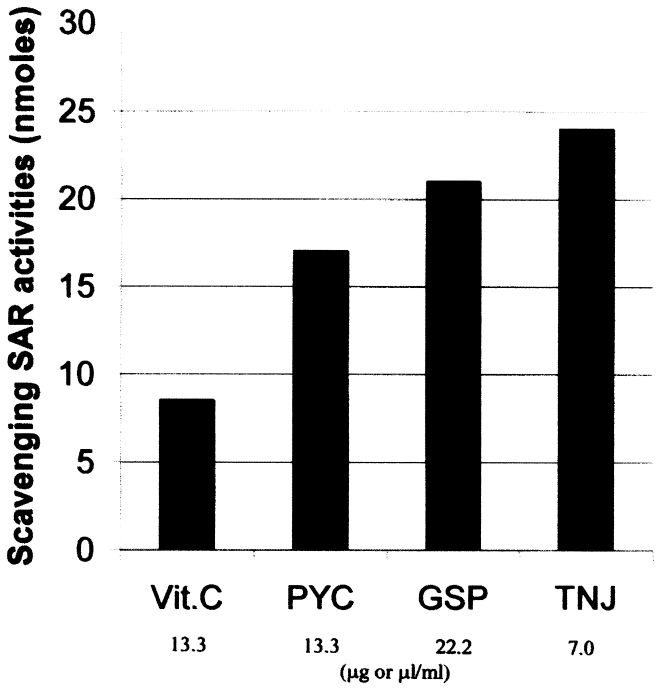

FIGURE 5. Comparison of scavenging SAR activities between Vit. C (vitamin C), PYC (pycnogenol), GSP (grape seed powder), and TNJ at the recommended daily dose per serving by U.S. RDAs or the manufacturers. Vit.C 13.3 μg, PYC 13.3 μg, GSP 22.2 μg, and TNJ 7.0 μl were added in the I ml reaction system. The SAR scavenging activity of TNJ was 2.8 times that of Vit. C, 1.4 times that of PYC, and 1.1 times that of GSP.

DISCUSSION

Our results indicated that 10% TNJ was able to significantly reduce the DMBA–DNA adduct formation in different organs of female SD rats and male C57 Bl-6 mice. Adducts were reduced the most in the kidney. Since DNA adduct formation is a critical initiation step in chemical carcinogenesis, the preventive effect of TNJ on DMBA adduct formation indicates that TNJ may prevent cancer at the initiation stage of chemical carcinogenesis. The strong antioxidant activities of TNJ against SAR and LPO were observed *in vitro* by TNB and LPO assays. The higher antioxidant activity shown by TNJ suggests that TNJ may possess great potential for protecting cells or lipids from oxidative modification mediated by SAR. Both the carcinogen-DNA adduct–prevention and the antioxidant properties may contribute to the cancer preventive effect of TNJ.

We hypothesized that the mechanism by which TNJ prevents the formation of DMBA-DNA adducts is as follows: TNJ may inhibit phase I enzyme activity while enhancing phase II enzyme and DNA repair enzyme activities.[17] TNJ may block the

redox-cycling between the carcinogen and their metabolites by interrupting the metabolic pathway, scavenging oxygen free radicals, and quenching the consequent LPO.[18] In Dr. Heinicke's hypothesis, the xeronine-system in TNJ may alter protein structure thereby modulating the activity of key enzymes and proteins involved in the metabolic pathways. TNJ may affect redox-sensitive signal transduction pathways and alter gene expression. Therefore, the interactions of carcinogens, oxygen free radicals, and LPO may be changed by TNJ. The mechanism of the cancer preventive effect of TNJ needs further study.

In conclusion, on the basis of our preliminary data, TNJ may possess a cancer preventive effect at the initiation stage of chemical carcinogenesis. It serves as a good liquid nutritional supplement. Including TNJ in five daily fruit and vegetable servings may help prevent cancer and other diseases, while maintaining overall good health.

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REFERENCES

1. MORTON, J. 1992. The ocean-going noni, or Indian mulberry (*Morinda citrifolia*, Rubiaceae) and some of its "colorfull" relatives. Econ. Bot. **46**(3): 241–256.
2. ABBOTT, I.A. & C. SHIMAZU. 1985. The geographic origin of the plants most commonly used for medicine by Hawaiians. J Ethnopharmacol. **14:** 213–222.
3. DEGENER, O. 1973. *In* Plants of Hawaii National Park Illustrative of Plants and Custom of South Seas. Photo lithoprint reproduction, Braun-Brumfield, Inc. Ann Arbor, MI.
4. HABRAH, F.L. & M.B. EVELETH. 1996. Evaluation of the effectiveness of ancient Hawaiian medicine. Hawaii Med. J. **25:**.
5. HIRAZUMI, A.Y. 1997. Antitumor Studies of a Traditional Hawaiian Medical Plant, *Morinda citrifolia* (Noni), in Vitro and in Vivo. University of Hawaii, Ph.D. Thesis, Dec.
6. HIRAZUMI, A., E. FURUSAWA, S.C. CHOW & Y. HOKAMA. 1994. Anticancer activity of *Morinda citrifolia* (noni) in intraperitoneally implanted Lewis lung carcinoma in syngeneic mice. Proc. West. Pharmacol. Soc. **37:** 145–146.
7. HEINICKE, R.M. 1985. The pharmacologically active ingredient of Noni. Bulletin of the National Tropical Botanical Garden.
8. SOLOMON, N. 1999. The Noni phenomenon. Direct Source Publishing.
9. HEINICKE, RM. 2001. The Xeronine System. A New Biological System, 2000. Direct Source Publishing.
10. RANDERATH, K. & E.A. RANDERATH. 1994. ^{32}P-postlabeling methods for DNA adduct detection: overview and critical evaluation. Drug Metab. Rev. **26:** 67–85.
11. WANG, M.Y. & J.G. LIEHR. 1995. Induction by estrogens of lipid peroxidation and lipid peroxide derived malondialdehyde-DNA adducts in male Syrian hamsters: role of lipid peroxidation in estrogen-induced kidney carcinogenesis. Carcinogenesis **16**(8): 1941–1945.
12. AUERBACH, B.J., J.S. KIELY & J.A. CORNICELLI. 1992. A spectrophotometric microtiter-based assay for the detection of hydroperoxy derivatives of linoleic acid. Anal. Biochem. **201:** 375–380.

13. NISHIKIMI, M., RAO, N.A. & K. YAGI. 1972. The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochem. Biophys. Res. Commun. **46**(2): 849–854.
14. PACKER, L., G. RIMBACH & F. VIRGILI. 1999. Antioxidant activity and biologic properties of a procyanidin-rich extract from pine *(Pinus martima)* bark, pycnogenol. Free Radic. Biol. Med. **27**(5-6): 704–724.
15. CHEN, L.H., G.A. BOISSONNEAULT & H.P. GLAUERT. 1988. Vitamin C, vitamin E and cancer. Anticancer Res. **8**(4): 739–748.
16. BROUILLARD, R., F. GEORG & A. FOUGEROUSSE. 1997. Polyphenols produced during red wine ageing. Biofactors **6**(4): 403–410.
17. CELOTTI, L., O. FERRARO, D. FURLAN, *et al*. 1993. DNA repair in human lymphocytes treated in vitro with anti- and syn-benzo(a)pyrene diolepoxide. Mutat. Res. **294:** 117–126.
18. THAKKER, D.R., H. YAGI, W. LEVIN, *et al*. 1985. Polycyclic aromatic hydrocarbons: metabolic activation to ultimate carcinogens. *In* Bioactivation of Foreign Compounds. M.W. Anders, Ed.: 177–192. Academic Press. New York.