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Morinda Citrifolia (*Noni*) Reduces Cancer Risk in Current Smokers by Decreasing Aromatic DNA Adducts

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Quantitative determination of aromatic DNA adducts in peripheral blood lymphocytes (PBLs) of current smokers is an useful surrogate biomarker for the evaluation of environmental carcinogen exposure or chemopreventive intervention. In this study, we examined the impact of Tahitian Noni Juice (TNJ) on the aromatic DNA adducts of PBLs, before and after a 1-mo intervention, using ^{32}P postlabeling assay. Of 283 enrolled, 203 smokers completed the trial. Aromatic DNA adducts levels in all participants were significantly reduced by 44.9% ($P < 0.001$) after drinking 1 to 4 oz of TNJ for 1 mo. Dose-dependent analyses of aromatic DNA adduct levels showed reductions of 49.7% ($P < 0.001$) in the 1-oz TNJ group and 37.6% ($P < 0.001$) in the 4-oz TNJ group. Gender-specific analyses resulted in no significant differences in the 4-oz TNJ groups. Interestingly, the 1-oz TNJ group showed a reduction of 43.1% ($P < 0.001$) in females compared with 56.1% ($P < 0.001$) in males. The results suggest that drinking 1 to 4 oz of TNJ daily may reduce the cancer risk in heavy cigarette smokers by blocking carcinogen-DNA binding or excising DNA adducts from genomic DNA.

INTRODUCTION

Cigarette smoke is the single known, leading preventable cause of lung cancer. In 2006, an estimated 170,000 cancer deaths in the United States were caused by tobacco smoke. Approximately 45 million Americans are current smokers, of which nearly 11 million, or 24%, are between the ages of 18 and 44. If this current trend continues, the 2030 global death toll due to smoking will exceed 9 million (1). On average, adults who smoke cigarettes die 14 years earlier than nonsmokers.

It is known that chemical carcinogenesis is a multistage process involving initiation, promotion, and progression (2). Initiation, the critical first step in carcinogenesis**,** requires the binding of active chemical carcinogens to genomic DNA and forming DNA adducts, which result in mutation and consequent cancer (3). Therefore, the detection and quantification of DNA adducts is an important, fundamental, early indicator of cancer risk (4).

Tobacco smoke contains approximately 4,700 compounds and more than 68 carcinogens. The most important carcinogens in tobacco smoke are polycyclic aromatic hydrocarbons (PAHs) such as benzo(a)pyrene (5,6). Aromatic DNA adducts have been detected in lung, breast, and pancreatic cancer tissues and their surrounding normal tissues as well as in peripheral blood lymphocytes (PBLs) (7–9). Molecular epidemiological studies have demonstrated that aromatic DNA-adduct levels in smokers are significantly higher than in nonsmokers (10,11). The smoking-related aromatic DNA adduct levels decrease on smoking cessation and are positively correlated with pack years (packages of cigarettes smoked per day × years smoked) (12–14). Furthermore, epidemiological studies have also demonstrated that the aromatic DNA-adduct levels in PBLs are a good surrogate biomarker for lung-tissue adducts and for procarcinogenic damage (15). A recent study indicated that peripheral blood aromatic DNA-adduct levels predicted lung-cancer risk in current smokers. Male smokers with higher aromatic DNA adducts, detected by ^{32}P postlabeling assay, had a threefold increased risk of lung cancer compared with males with low aromatic adduct levels (16). Additionally, a smoking-specific ^{32}P-postlabeling DNA adduct pattern called Diagonal Radioactive Zone (DRZ) has been successfully detected in the PBLs of current smokers, which represents the complex mixture of carcinogens from cigarette smoke and the major components of the aromatic DNA adducts in smokers (17). Meta-analysis has revealed a significant association between the elevated, tobacco smoke-induced, aromatic DNA-adduct levels and cancer risk (18). Thus, measuring smoking-related aromatic DNA adducts may serve as a tool for monitoring cigarette smoke exposure, assessing subsequent risk and the efficacy of chemopreventive strategies (19–24). The variance in aromatic DNA adduct patterns and levels in the PBLs of current smokers before and after preventive intervention can act as a surrogate biomarker for evaluating the ability of preventive agents to reduce PAH carcinogen-induced DNA damage, reducing cancer risk.

Morinda citrifolia (*Noni*) has been used by Polynesians for over 2,000 years as a tropical folk medicine. It is reported to

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have a broad range of health benefits including preventive properties. Due to its medicinal and nutritional value, it is considered the "Queen" of the other 80 species belonging to the Rubiaceae family (25). Recent scientific studies have supported the Polynesians' claim of its unusual healing power. These studies have implicated noni as a natural remedy that lowers blood pressure, reduces joint swelling, stops internal and external infection, clears up congestion, and prevents precancer cells from growing (26). It has been reported that damnacanthal from noni roots induces normal phenotypes in *ras*-transformed tumor cells (27). Also, noni fruit extract inhibits the proliferation of breast cancer cells (28). Our previous studies have demonstrated that juice made from Tahitian noni fruits (Tahitian Noni® juice or TNJ) is a strong antioxidant, anti-inflammatory, antiangiogenic, and antiproliferative nutritional supplement (29). More specifically, TNJ has been demonstrated to significantly reduce an aromatic carcinogen, dimethylbenzo(a)anthrancene (DMBA), and to induce DNA adduct formation in different organs of male C57/BL6 mice and female SD rats (30). The latest study in our laboratory indicated that TNJ was able to prevent DMBA-induced mammary carcinogenesis at the initiation and postinitiation stages in female SD rats. The reduction of the DMBA adduct level in the mammary gland tissues might be 1 of the preventive mechanisms of TNJ (31).

In this study, we examined whether TNJ was able to reduce the cancer risk by decreasing the aromatic DNA adduct levels in PBLs of current smokers after 1 mo TNJ intervention. The TNJ used in this trial was donated by Morinda Holding Inc. TNJ is a blend of Noni juice, formulated with blueberry and grape juice. Noni juice is the dominate component of TNJ (32) We hypothesized that TNJ was a good chemopreventative nutritional supplement and predicted that the aromatic DNA level in PBLs of current smokers would be reduced after 1 mo of chemopreventive intervention with TNJ. Our results are very promising.

METHODS

The trial received human subject's approval from the Institutional Review Board of University of Illinois College of Medicine at Rockford.

Enrollment

Intervention participants for this study met the following criteria: 1) healthy adult male or female, 2) 18 to 65 yr in age, 3) smoke more than 20 cigarettes per day, 4) a smoking history exceeding 1 yr, 5) no usage of prescribed medicine or antioxidant vitamins in the last 3 mo, and 6) willingness to complete a 1-mo trial. In addition, a control group of 20 male and 22 female nonsmokers were also recruited to this study to compare the baseline level of the aromatic DNA adducts between smokers and nonsmokers.

Participants were randomly assigned to either a 1- or 4-oz TNJ group on a 1-to-1 male to female ratio. Study participants in the 1-oz group were asked to drink their assigned TNJ dose in the morning on an empty stomach. Those in the 4-oz group were asked to drink a 2-oz dose twice daily, 1 in the morning on an empty stomach and 1 before bedtime. Additionally, all participants were asked to drink 1 cup of pure water after consuming the TNJ.

Immediately after enrollment, 10 ml of whole blood were drawn from each participant. These pretest blood samples were used to determine baseline level of the aromatic DNA-adduct levels in both current smokers and nonsmokers. From these blood samples, we were able to assess DNA damage caused by cigarette smoking compared with nonsmokers. At the conclusion of the trial, 10 ml of blood sample was again drawn from each participant. These posttest blood samples were analyzed for aromatic DNA adduct levels after the 1-mo intervention. The comparison of prearomatic and postaromatic DNA adduct level was performed in current smokers. We predicted that the reduced aromatic DNA adduct level in current smokers would be observed if TNJ was an active chemopreventive nutritional supplement.

Preparation of PBLs and DNA Isolation

The blood samples (pretrial and posttrial) were drawn into vacationers (green top tubes from BD, CITY, STATE) containing heparin. The tubes were centrifuged at 1,500 *g* for 20 min to remove plasma. The rest part of blood was transferred into a 50 ml tube mixed with 15 ml of red blood cell lysis buffer [150 mM NH4Cl, 10 mM sodium hydrogen carbonate, 1 mM ethylenediamine tetraacetic acid (EDTA), pH 7]. The tube was then inverted several times followed by incubation at room temperature for 5 min. Samples were then centrifuged at 300 *g* for 10 min at 4°C to isolate PBLs, and the supernatant was discarded. The PBL pellet was washed with 5 ml of red blood cell lysis buffer, incubated at room temperature, and centrifuged at 300 *g* for 10 min at 4°C, and the supernatant was discarded. This procedure was repeated 3 times. The final PBLs pellet was resuspended with 0.5 ml of buffer 1 (150 mM sodium chloride and 10 mM EDTA, pH 8.0), and cells were lysed by Vortex for 2 min at full speed. DNA was then isolated by following the standard procedure of instruction (Fast DNA-kit from Q-Biogene, CITY, STATE) (33). The purified DNA samples were stored at −80°C until DNA adduct analysis was performed using ^{32}P-postlabeling assay.

^{32}P-Postlabeling Assay

Aromatic DNA adduct levels were measured by the nuclease P1 procedure of ^{32}P-postlabeling assay. DNA (5–10 μg) was digested with micrococcal endonuclease (Sigma Chemicals Inc, CITY, STATE) and spleen phosphodiesterase (Sigma Chemicals Inc) to 3′ mononucleotides. The unmodified normal nucleotides were dephosphorylated with nuclease P1 (USBiological, CITY, STATE), and the adducted nucleotides were labeled with [$\gamma-^{32}$P]adenosine-5′-triphosphate (ICN Pharmaceuticals,

Inc., Irvine, CA) and T4 polynucleotide kinase (Sigma Chemicals Inc) (34). The labeled products were purified and separated by thin layer chromatography using homemade polyethylenimine (PEI) cellulose thin layer chromatography (TLC) sheets. After the first development overnight, the chromatogram was cut into 1.0 × 2.4 cm strips above the origin point and transferred to a fresh PEI-cellulose TLC sheet by a magnet transfer technique. Two-dimensional chromatography was completed in different solvents as described in previous publications (35). Adducts were detected by autoradiography and quantified by scintillation counting. Adduct levels were expressed as a relative adduct labeling value, which is a count ratio of adducted nucleotides to total nucleotides used as a standard in this assay (36).

Preparation of the PEI Cellulose TLC Sheets

The PEI cellulose TLC plate was homemade using IONAC Corcat P-600 PEI (Sybron Chemicals Inc., Wellford, SC) and Cellulosepulver MN 301 (Cat #816250 from Macherey Nagel, Germany; Carrier Alltech Associations, Deerfield, IL). 54.0 g of cellulose plus 5% PEI 42 ml (pH 6.0) and ddH2O 270 ml to make 4 sheets by following the original procedure developed by Randerath (37). The procedures of the preparation of PEI cellulose TLC sheets and/or ^{32}P-postlabeling assay is discussed elsewhere (38).

To avoid systemic errors, the aromatic DNA adduct analysis for all of the DNA samples (before and after intervention) and DNA samples from nonsmokers were analyzed at the same time using the same batch number of reagents and the same preparation of PEI cellulose TLC sheets. The ^{32}P postlabeling assay was carried out by an expert. To overcome possible bias, all the DNA samples were coded and blinded.

Statistical Analysis and Data Interpretation

A power analysis was performed to estimate the number of cases needed to detect significant effects (39). All coded adduct data were entered into a predesigned database by a research assistant in a blinded manner. All data analyses were completed by an experienced and independent epidemiologist. Because the study was designed to compare the predate and postdata, all analyses were conducted on the paired cases of each group. The code number and the results were not released until all data analyses were completed. To assess the modification of TNJ on the aromatic DNA adduct levels, the averaged mean of the DNA adduct levels was compared before and after the trial in each group using a paired Student's *t*-test (40).

RESULTS

A typical aromatic DNA adduct profile from the PBLs were detected before and after 1 mo TNJ intervention (see Fig. 1). In Fig. 1, panel A represents a typical aromatic DNA adduct profile, whereas panel B shows significant TNJ-induced reductions in size and density of the aromatic DNA adduct spots 1 to 4. Additionally, the characteristic DRZ, which contained a complex mixture of aromatic and/or hydrophobic adducts with a variety of chemicals, was greatly diminished in size and pigmentation. The aromatic DNA adduct level in current smokers was 3.5 times higher than nonsmokers (155.1×10^{-9} in smokers vs. 34.7×10^{-9} in nonsmokers); this finding was consistent with other reports (41).

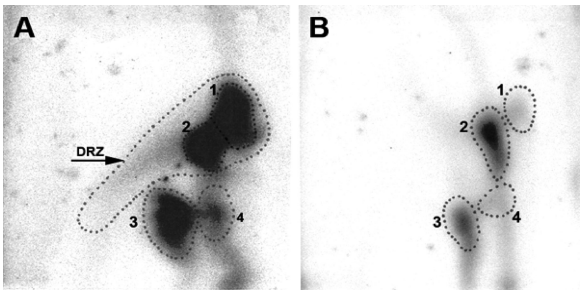

FIG. 1. Typical profile of the blood aromatic DNA adduct pattern in a current smoker before (panel A) and after (panel B) consumption of 1-oz Tahitian Noni Juice. Numbers 1 to 4 represent adduct spots in the blood aromatic adduct patterns. The smoking-specific Diagonal Radioactive Zone (DRZs) are represented by the dotted-line areas.

To further test our hypothesis that TNJ reduces aromatic DNA adducts, we analyzed the predate and postdata from the pooled 1- and 4-oz TNJ-treated groups (Table 1). This analysis showed a aromatic DNA adduct-level decrease of 44.9% ($P < 0.001$) after drinking 1- to 4-oz TNJ doses daily for 1 mo. Additional stratified analyses of the combined 1- and 4-oz TNJ data suggested that the aromatic DNA adduct level in participants' PBLs were significantly reduced ($P < 0.001$) by 49% in male smokers and 40.8% in female smokers, suggesting that males respond better to TNJ than females, although the difference of the reductions between male and female was not statistically significant.

Table 2 displays separate TNJ dose and gender effects on reducing aromatic DNA adduct levels among participant smokers. The 1-oz TNJ treatment produced a reduction of 49.7% among combined genders. However, when genders were evaluated separately, males showed a 56.1% reduction compared with 43.1% for the females. All of the 1-oz TNJ-induced reductions

TABLE 1
Aromatic DNA adduct levels in current smokers before and after consuming 1 or 4 oz of TNJ[a]

Gender	Time	RAL × 10^{-9}	Cases (*n*)	Changes (%)	*P* Value
	Before	155.1 ± 9.0	203		
	After	85.5 ± 4.7	203	44.9 ↓	<0.001
Males	Before	156.9 ± 13.4	101		
	After	80.1 ± 6.6	101	49.0 ↓	<0.001
Females	Before	153.3 ± 12.0	102		
	After	90.8 ± 6.8	102	40.8 ↓	<0.001

[a] Abbreviations are as follows: TNJ, Tahitian Noni Juice; RAL, relative adduct labeling.

TABLE 2
Aromatic DNA adduct levels in different groups before and after consuming TNJ[a]

		Adduct Levels ($\times 10^{-9}$)	
Gender	Trial Time	1 oz TNJ	4 oz TNJ
	Before	161.4 ± 11.8	146.3 ± 9.0
	After	81.3 ± 6.3	91.3 ± 65.9
	Changes	49.7% reduction	37.6% reduction
	N	118	85
	P Value	<0.001	<0.001
Male	Before	162.9 ± 17.1	148.5 ± 21.8
	After	71.5 ± 8.0	92.1 ± 10.9
	Changes	56.1% reduction	38% reduction
	N	59	42
	P Value	<0.001	0.003
Female	Before	159.9 ± 16.3	144.2 ± 17.4
	After	91.3 ± 9.6	90.5 ± 9.5
	Changes	43.1% reduction	37.3% reduction
	N	59	43
	P Value	<0.001	0.002

[a]Abbreviation is as follows: TNJ, Tahitian Noni Juice.

were significant at $P < 0.001$. The 4-oz treatment produced a significant ($P < 0.001$) reduction of 37.6% among combined genders; but when genders were analyzed separately, the reductions were 38% ($P = 0.003$) and 37.3% ($P = 0.002$) for males and females, respectively.

DISCUSSION

This study was designed to examine whether consuming TNJ, during a 1-mo intervention, would reduce blood aromatic DNA damage in current smokers. Our analyses revealed 5 important findings. First, current heavy smokers have 3.5-fold higher aromatic DNA adduct level than nonsmokers, indicating that aromatic carcinogens in the cigarette smoke are responsible for the elevated level of DNA damage. Second, when examining pretest and posttest aromatic DNA adduct levels of pooled genders, but separate TNJ-dose levels, the data revealed that TNJ produced highly significant reductions ($P < 0.001$). Third, separate data analyses by gender and TNJ dose also produced significant reductions ($P \leq 0.003$) of aromatic adduct levels. Fourth, irrespective of separate or pooled genders, the 1-oz TNJ dose produced a highly significant ($P < 0.001$) reduction in the aromatic DNA adduct levels. Fifth, although reductions from the 4-oz TNJ dose were also significant ($P \leq 0.003$), they were less so than for the 1-oz TNJ participants ($P < 0.001$).

In our study, TNJ showed a more positive impact on reducing the aromatic DNA adduct levels in male smokers than female smokers. Whether this finding is correlated with women being more prone to cigarette-related lung cancer remains unknown (42).

Individual susceptibility to DNA damage may be indicated by the higher DNA adduct levels in PBLs of current smokers. Reducing the aromatic DNA adduct levels by consumption of 1 oz of TNJ suggests that at this dose level, the efficacy may reach a plateau such that greater reduction in DNA adduct levels with higher doses of TNJ may not occur or may do so with extended use.

Additionally, the results of this study suggest that the efficacy of TNJ on the reduction of aromatic DNA adduct levels may be extremely high in current smokers. If smokers consume 1 to 4 oz of TNJ daily, their bodies might detoxify, thus reducing the harmful impact on aromatic DNA adduct levels in cigarette smoke and possibly reducing the risk of cancer.

These findings are important because the DNA adduct levels are a cancer-risk marker and as such represent the genotoxicity, or DNA damage, smoking causes (43). If TNJ consumption reduces aromatic DNA adduct levels, cancer risk may also be reduced. In addition to cancer risk reduction, DNA adduct levels are also good biomarkers for all degenerative diseases associated with smoking (44). As noted earlier, molecular epidemiologic studies have demonstrated that aromatic DNA adduct levels in smokers are significantly higher than in nonsmokers; our data has conformed this observation. Furthermore, these types of smoking-related DNA adducts decreased with cessation of smoking and were positively correlated with pack years (packages of cigarettes smoked per day × years smoked) (45). Additionally, findings from 1 study indicated that blood-aromatic DNA adduct levels predicted lung-cancer risk in current smokers and that male smokers with higher aromatic DNA adduct levels detected by ^{32}P postlabeling assay had a threefold increased risk of lung cancer compared with males with low aromatic adduct levels (16). The mechanisms for the reduction of aromatic DNA adduct in current smokers by consuming TNJ needs further study to determine if TNJ inhibits Phase 1 enzyme activities and enhances Phase 2 and DNA repair enzyme activities (46). The impact of TNJ on gene susceptibility of current smokers will be investigated soon (47).

Finally, based on the response of study participants to a questionnaire, no adverse events were observed during the trial. Approximately 14% of the study participants reported that the number of cigarettes smoked daily was slightly reduced and they are willing to continue drinking TNJ. The study results suggest that TNJ may have a cancer preventive effect in current smokers. The significant reduction of aromatic DNA adduct level by consuming TNJ might be associated with the reduced number of cigarettes they smoked during this trial. Long-term impacts of TNJ on the aromatic DNA adduct level in current smokers needs further study.

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