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Antioxidant activity of noni juice in heavy smokers

Mian-Ying Wang*¹, M Nawal Lutfiyya^{2,3}, Vicki Weidenbacher-Hoper⁴, Gary Anderson⁵, Chen X Su⁶ and Brett J West⁶

Address: ¹Department of Pathology, UIC College of Medicine, 1601 Parkview Avenue, Rockford, IL 61107, USA, ²Department of Community Health Sciences, University of Manitoba, Faculty of Medicine, Winnipeg, MB, Canada, ³Division of Research and Learning, Winnipeg Regional Health Authority, Winnipeg Manitoba, 200-1155 Concordia Avenue, Winnipeg, MB, Canada, ⁴Department of Family & Community Medicine, UIC College of Medicine, 1601 Parkview Avenue, Rockford, IL 61107, USA, ⁵Department of Pathology, UIC College of Medicine, 1601 Parkview Avenue, Rockford, IL 61107, USA and ⁶Research and Development, Morinda Holdings Inc, 333 West River Park Dr, Provo, UT 84606, USA

Email: Mian-Ying Wang* - mianwang@uic.edu; M Nawal Lutfiyya - nlutfiyya@wrha.mb.ca; Vicki Weidenbacher-Hoper - VWeide1@uic.edu; Gary Anderson - GaryLA@uic.edu; Chen X Su - chen_su@tni.com; Brett J West - brett_west@tni.com

* Corresponding author

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Abstract

Background: Noni (*Morinda citrifolia*) juice has demonstrated antioxidant activity *in vitro* and *in vivo*. To evaluate this activity in humans, noni juice from Tahiti (TNJ) was evaluated in a 30 day, double-blind, and placebo controlled clinical trial with 285 current heavy smokers. Research participants were randomly assigned to three daily treatment groups: 118 mL placebo, 29.5 mL TNJ, and 118 mL TNJ. Plasma superoxide anion radicals (SAR) and lipid hydroperoxide (LOOH) levels were measured pre and post-intervention.

Results: After 30 days, mean SAR decreased from 0.26 ± 0.14 to 0.19 ± 0.10 $\mu\text{mol/mL}$ in the 29.5 mL dose group ($P < 0.01$) and from 0.26 ± 0.22 to 0.18 ± 0.11 $\mu\text{mol/mL}$ in the 118 mL dose group ($P < 0.001$). LOOH levels decreased from 0.53 ± 0.19 to 0.40 ± 0.10 $\mu\text{mol/mL}$ in the 29.5 mL dose group ($P < 0.001$) and from 0.55 ± 0.21 to 0.40 ± 0.14 $\mu\text{mol/mL}$ in the 118 mL dose group ($P < 0.001$). No significant reductions in SAR or LOOH levels were observed in the placebo group.

Conclusion: The results suggest an antioxidant activity from noni juice in humans exposed to tobacco smoke, thereby replicating the results found previous chemical and *in vivo* tests.

Background

Exposure to cigarette smoke results in the generation of oxygen free radicals that account, in part, for the pathogenesis of smoking related diseases [1-3]. It is estimated that about 10^{17} oxidant molecules are present in each puff of cigarette smoke [4]. Smoking results in a subsequent state of oxidative stress, due to the formation of circulating lipid peroxidation products in the blood [5-7], such as lipid hydroperoxides (LOOH), as well as superoxide anion radicals (SAR). Increases in LOOH and SAR have been measured in the blood following exposure to cigarette smoke [8-10]. The induction of lipid peroxidation is

largely due to free-radical reactions involving polyunsaturated fatty acids in biological membranes. The unsaturated bonds undergo autocatalytic or enzymatic processing to form harmful LOOH [11]. The active LOOH quickly decompose to aldehydes and alkenals. All of these are active in DNA binding and are responsible for major endogenous cell damage.

Previous research has established that fruits and vegetables are major sources of dietary antioxidants [12]. Epidemiological studies have demonstrated that consumption of fruits and vegetables may reduce free-radical-induced

oxidative damage and lipid peroxidation in cigarette smokers [13]. *Morinda citrifolia* (noni) is an evergreen or small tree that grows in many tropical regions of the world. The fruit of this tree has a history of use as both food and medicine among Pacific Islanders and in South-east Asia [14,15]. Several reports have described various potential health benefits of noni fruit [16,18], including immunomodulation [19,20] and antioxidant activities *in vitro* and *in vivo* [21-23]. The European Union-approved form of noni fruit juice from Tahiti (TNJ), has been found to exert an antioxidant effect in human athletes, thereby increasing exercise endurance [24]. This is also the mechanism by which TNJ provided protection against carbon-tetrachloride-induced liver damage in Sprague-Dawley rats [25]. Hence, TNJ may also mitigate oxidative damage and consequent lipid peroxidation in cigarette smokers. To further evaluate this potential effect, a 30-day, placebo-controlled, double-blinded, and randomized clinical trial was conducted with research participants who were current heavy smokers.

Results and discussion

There were no significant differences in demographics between the groups. There were no differences in a 1:1 gender ratio, with the ethnic compositions of the groups being primarily Caucasian, 80 - 89%, and African American, 9.4 - 18%. The mean age in each group ranged from 37 to 43 yr. There was no difference in the mean number of cigarettes smoked each day (26 - 29). During the course of the trial, no adverse health events due to TNJ or placebo ingestion were observed in any of the participants.

Mean pre and post-test SAR and LOOH values are compared in Table 1. No significant inter-group differences were observed in pre-test values. There was no significant change in SAR or LOOH levels in the placebo group. There was a trend for lower post-test SAR levels in both TNJ groups than in the placebo group, although this difference was not statistically significant. However, mean post-test SAR levels in both TNJ groups were significantly lower than their pre-test values. Percent reductions in SAR in the 29.5 and 118 mL TNJ groups were 26.9% ($P < 0.001$) and 30.8% ($P < 0.001$), respectively. The mean post-test

plasma LOOH levels in both TNJ groups were 32% lower than that of the placebo group ($P < 0.001$). Mean LOOH was reduced in the 29.5 mL TNJ group by 24.5% ($P < 0.001$), while a 27.3% reduction was observed in the 118 mL TNJ group ($P < 0.001$).

Gender-specific mean SAR and LOOH levels in the TNJ groups are compared in Table 2. Significant intra-group reductions in SAR and LOOH occurred in both the males and females of the TNJ groups. The degrees of LOOH reduction in males and females are essentially the same as observed in the combined gender analysis. A comparison of post-test values revealed no significant differences between males and females, as well as between TNJ dose groups. This latter observation suggests a threshold of antioxidant activity is reached by a daily dose of 29.5 mL.

It is important to note that the placebo contains grape and blueberry juices, the fruits of which have been shown to exhibit antioxidant activities in humans [26]. As such, the placebo also serves as a point of comparison between TNJ and other common fruit juices. The lack of a statistically significant difference in post-test SAR levels between the TNJ groups and the placebo may be explained by the fact that phytochemicals in grapes have the ability to scavenge SAR [27,28]. Although the SAR scavenging ability of the grape juice was not great enough to result in significant reductions in heavy smokers, there was enough activity to lower post-test levels in the placebo group to a point where the confidence interval of the mean overlapped those of the TNJ groups. The post-test means of the TNJ groups were, however, significantly less than their pre-test means, as well as the placebo pre-test mean. Therefore, the ability of TNJ to lower SAR in heavy smokers is demonstrated in this trial and is somewhat greater than the grape and blueberry juice blend of the placebo, although only marginally so.

The most significant effect of TNJ in this trial was on mean post-test plasma LOOH concentrations. In both TNJ groups, these were significantly less than pre-test values and the post-test mean of the placebo group. The differential effect on LOOH and SAR sheds some light on the potential target of the antioxidant action of noni juice. SAR is converted to H_2O_2 by superoxide dismutase, from which the hydroxyl radical is formed by Fenton-type reduction. The hydroxyl radical triggers chain peroxidation of unsaturated lipids to form LOOH [29]. As smoking generated plasma LOOH levels appear to be more significantly reduced than SAR, it is likely that the hydroxyl radical is a major target of the antioxidant activity of noni juice. Further research is required to elucidate the mechanisms responsible for the preferential lowering of LOOH over SAR.

Table 1: Serum free-radical levels in current heavy smokers.

	Placebo	29.5 mL TNJ	118 mL TNJ
Serum SAR ($\mu\text{mol/mL}$)			
Pre-test	0.23 \pm 0.15	0.26 \pm 0.14	0.26 \pm 0.22
Post-test	0.21 \pm 0.17	0.19 \pm 0.10*	0.18 \pm 0.11**
Serum LOOH ($\mu\text{mol/mL}$)			
Pre-test	0.58 \pm 0.22	0.53 \pm 0.19	0.55 \pm 0.21
Post-test	0.59 \pm 0.21	0.40 \pm 0.10***	0.40 \pm 0.14***

* $P < 0.01$, ** $P < 0.001$ compared to pre-test values

*** $P < 0.001$ compared to pre-test values and placebo

Table 2: Serum free-radicals by gender and TNJ dose.

	SAR ($\mu\text{mol/mL}$)		LOOH ($\mu\text{mol/mL}$)	
	29.5 mL TNJ	118 mL TNJ	29.5 mL TNJ	118 mL TNJ
		Female		
Pre-test	0.26 \pm 0.14	0.26 \pm 0.24	0.52 \pm 0.23	0.53 \pm 0.16
Post-test	0.19 \pm 0.10 **	0.18 \pm 0.12 *	0.39 \pm 0.11 ***	0.39 \pm 0.14 ***
		Male		
Pre-test	0.25 \pm 0.14	0.26 \pm 0.21	0.53 \pm 0.16	0.57 \pm 0.25
Post-test	0.19 \pm 0.10 **	0.19 \pm 0.12 *	0.40 \pm 0.10 ***	0.40 \pm 0.11 ***

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Experimental

The research protocol of this trial was approved by the Institutional Review Board Committee of the University of Illinois College of Medicine at Rockford, following an ethics review. The trial was conducted in accord with the Declaration of Helsinki and written informed consent was obtained from all participants.

Adult heavy smokers were recruited for enrollment in this study. The inclusion criteria were 18 to 65 years in age, smoker of more than 20 cigarettes per day, a smoking history exceeding one year, and no concurrent use, or use in the previous three months, of prescription medication or antioxidant vitamins. For ethics reasons, fruits and vegetables were not restricted from the diets of the subjects. Both males and females were enrolled in equal proportions.

For this trial noni juice from Tahiti (TNJ) was used (Tahitian Noni International Inc., Provo, Utah, USA). The placebo consisted of a blend of grape and blueberry juices and natural cheese flavor to mimic the flavor of TNJ. Study volunteers were randomly assigned to one of three daily treatment groups: 118 mL placebo ($n = 42$), 29.5 mL TNJ daily ($n = 121$), and 118 mL TNJ ($n = 122$). Participants and investigators were blinded as to which treatment group each volunteer was assigned to. The male to female ratio of each group was 1:1. Those in the 29.5 mL TNJ group were asked to drink the juice all at once in the morning and on an empty stomach. Those in the other two groups were asked to drink 59 mL twice daily, once in the morning on an empty stomach and once before bedtime. Additionally, volunteers were asked to drink one cup of water after consuming TNJ. The volunteers were asked to follow this schedule for 30 days.

Upon enrollment, 10 mL of whole blood was drawn from each volunteer and collected in tubes containing heparin, then centrifuged at $1,500 \times g$ to obtain plasma for the baseline determination of SAR and LOOH levels. Baseline plasma SAR and LOOH levels represented the initial oxidative status in current smokers. At the conclusion of the 30-day trial, a 10 mL blood sample was again drawn from each study participant, and plasma SAR and LOOH levels

were again determined to assess the impact of TNJ on the oxidative status of current smokers.

The plasma SAR level of subjects was determined by a Tetrazolium Nitro Blue (TNB) assay, as described previously [30]. In this assay, SAR reduces TNB into formazan blue, which absorbs light at 602 nm. An SAR scavenger reduces the absorbance by reacting with SAR, thus preventing formazan blue formation. Also in this assay, NADH generates SAR under aerobic conditions, with phenazine methosulfate (PMS) as the catalyst.

The plasma LOOH concentrations were determined by the leucomethylene blue (LMB) assay, as described previously [31,32]. Plasma samples were incubated for 5 minutes at room temperature with LMB reagent solution; 1.6 mmol LMB, 0.0055% hemoglobin (w/v), 1.4% (v/v) triton X-100, and 8% (v/v) dimethylformide in 0.05 M potassium phosphate buffer (pH 5). In the presence of hemoglobin, LOOH oxidizes leucomethylene blue to methylene blue, which absorbs at 660 nm. Plasma LOOH concentration was calculated from the absorbance at this wavelength, against a cumene hydroperoxide generated standard curve.

A power analysis was performed to estimate the number of cases needed to detect a significant effect [33]. The study was designed to compare pre- and post-test data. All analyses were conducted on unpaired cases of each group and included intent-to-treat analysis. The study completion rate among all groups was greater than 73%, with no significant differences between any of the groups (Pearson chi-square test $P > 0.5$). All data were expressed as a mean, \pm standard deviation (SD). To assess the effect of TNJ on plasma SAR and LOOH levels, the averaged means of each group were compared before and after the trial using Student's T-test. Analysis of variance (ANOVA) was used to compare intergroup differences in plasma SAR and LOOH levels.

Conclusion

SAR and LOOH levels are biomarkers for degenerative diseases associated with cigarette smoking [34-36]. As noted

earlier, molecular epidemiologic studies have demonstrated that SAR and LOOH levels in smokers are significantly higher than in nonsmokers [37]. The results of this trial substantiate the antioxidant properties of noni juice observed *in vitro* and *in vivo*, as well as demonstrate an effect under conditions of oxidative stress produced by heavy smoking.

Abbreviations

LMB: leucomethylene blue; LOOH: lipid hydroperoxide; PMS: phenazine methosulfate; SAR: superoxide anion radical; TNB: tetrazolium nitro blue; TNJ: noni juice from Tahiti.

Competing interests

MYW received funding through a grant made to the University of Illinois College of Medicine at Rockford by Morinda Holdings, Inc., a manufacturer of noni juice. CXS and BJW are employees of Tahitian Noni International, a division of Morinda Holdings, Inc. No competing interests exist for the remaining authors.

Authors' contributions

MYW generated study design, collected data, performed data analysis, and drafted manuscript. MNL conducted statistical analyses and GA was responsible for IRB preparation and research organization. VWH was the clinical coordinator, conducted patient interviews, and setup database used to collect and organize data. CXS was involved in analytical method development and data collection. BJW was involved in placebo preparation, data collection and analyses, and in drafting of manuscript. All authors have read and approved the final manuscript.

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