The Effect of Consumption of a Nopal Cactus Fruit Juice on C-Reactive Protein Levels in Healthy Adults: Results from a Randomized, Double-Blind, Controlled Clinical Pilot Study

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Author’s contribution
Author GSJ performed the data analysis, literature search, and wrote the manuscript.

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ABSTRACT

The Nopal cactus grows widespread in many parts of the world, and in some areas is considered a noxious weed. In addition to the use of the stems and leaves (also called pads or cladodes) and fruits (prickly pears) as food, Nopal has a long use in traditional folk medicine. The fruit shares some constituents with the stems, and contains additional unique polyphenols and betalain pigments with antioxidant and anti-inflammatory activities. A randomized, double-blind, controlled, parallel-arm human study was performed to compare consumption of Nopal fruit juice (NFJ) to control (apricot) juice. Serum C-reactive protein (CRP) and uric acid were measured at baseline and after 8 and 12 weeks. Reduced CRP levels were seen in both the NFJ and control groups. The 21.0% reduction at 8 weeks in the NFJ group was significantly more robust than the 6.6% reduction in the control group ($P<.05$). The reduced CRP level within the NFJ group was highly significant at 8 weeks ($P<.0001$), and remained reduced by 10.6% at 12 weeks compared to baseline ($P<.065$). The CRP reduction was not significant within the control group. There was no significant difference in uric acid levels between the groups at either 8 or 12 weeks. The 5.2% increase in uric acid levels within the control group was highly significant at 8 weeks ($P<.0003$), remaining significant at 12 weeks ($P<.04$). In contrast, a transient 3.6% increase in uric acid in the NFJ group at 8 weeks ($P<.02$) returned almost back to baseline levels at 12 weeks. Consumption of NFJ was associated with significant reduction in the CRP inflammatory biomarker while maintaining uric acid well within healthy range.
Keywords: Nopal fruit juice; apricot juice; C-reactive protein; uric acid.

1. INTRODUCTION

Globally, health is increasingly and negatively affected by oxidative stress, as it is associated with chronic illnesses, infectious diseases, environmental pollution, and malnutrition including obesity. Research into natural products has changed over the past decades from alleviating nutrient deficiencies to prevention of chronic disease development, in part through scientific exploration of traditional foods and folk medicine. There is an exponentially growing industrial interest in botanical products with known historical uses in health maintenance.

Cacti have a historical use for industrial and nutritional purposes, and offer unique compounds with health benefits. Specifically, the Opuntia genus of the cactus family, also known as Nopal or prickly pear cactus, has a long-standing traditional use as a functional food because of its antioxidant activity and ability to improve biomarkers associated with metabolic syndrome [1]. A volume of research has focused on the succulent stems called cladodes, regarding the effects on maintaining healthy blood sugar levels and insulin response. Nopal cladodes are traditionally consumed as a vegetable, and cladodes at different stages of maturity were tested with regard to their hypoglycemic properties, showing that consumption of young cladodes reduced postprandial blood glucose levels, whereas larger cladodes had no significant effect [2]. Dried powder of the cladodes significantly inhibited acid- and NSAIDs-induced gastric lesions, without affecting gastric juice secretion or pH [3]. Consumption of an extract was found to have a moderate effect on reducing hangover symptoms such as nausea, dry mouth, anorexia, and the risk of a severe hangover was reduced by half, [4] and both cladodes and fruits were found to be associated with hepatoprotection [5].

The Nopal fruit share some similarities to the stem in terms of content of mucilage and pectin, but in addition is rich in water-soluble betalain pigments. The pigment profile varies with the color of the fruit, which can be yellow, orange, pink, red, and purple, dependent on the Opuntia species [6]. Betalains are divided into two classes, the betaxanthins and betacyanins, which produce yellow to orange or violet colors, respectively. Betalains are structurally and chemically unlike anthocyanins, and the two have never been found in the same plant together in nature; [7,8] thus purple Nopal fruits contain only betalains, not anthocyanins. The highest antioxidant properties and protection from environmental toxin damage was observed in the red-purple variety of the Nopal fruit [9]. Betalains protected endothelial cells from cytokine-induced redox state alterations, [10] and inhibit the production of lipid hydroperoxides when human low-density lipoproteins were exposed to myeloperoxidase/nitrile-induced oxidation [11]. Oral consumption of the betaxanthin Indicaxanthin from Nopal fruit was found to inhibit release of PGE2, NO, IL-1β and TNF-α in carrageenan-induced pleurisy in a rat model, suggesting a use in the prevention of inflammation-based disorders [12].

Nopal cactus fruit has a unique profile of bioactive pigments, including the high content of water-soluble antioxidant, anti-inflammatory, and cellular protective compounds. Consuming Nopal fruit juice (NFJ) exerted gastroprotective activity in rats with stress-induced acute gastric lesions by significantly suppressing the levels of gastric mucosal tumor necrosis factor-α and myeloperoxidase, [13] and short-term supplementation with cactus pear fruit pulp has positively affected redox balance, decreased oxidative damage to lipids and improved antioxidant status [14].

The purpose of the study reported here was to conduct a controlled human clinical trial to examine selected biomarkers for antioxidant and anti-inflammatory properties in a healthy population, as defined by C-reactive protein (CRP) levels at or below 3.0 mg/L throughout the study. Although uric acid can act as an antioxidant, excess serum accumulation is often associated with cardiovascular disease. Since it is known that beverages containing fructose may elevate blood levels of uric acid, [15,16] levels of uric acid were also recorded in this study.

2. MATERIALS AND METHODS

2.1 Study Design

A randomized, double-blind, controlled clinical study design was used. Potential study participants went through screening to establish serum C-reactive protein levels at or below 3.0 mg/L, and enrolled into the 12-week study upon signing written informed consent, as approved by
Participants were instructed to ingest six ounces of juice daily. Beverages, Pleasanton CA), blending with agave, pineapple, pear, and apple, flavor added, pH adjusted to 3.93 +/- 0.02, and Brix 0 adjusted to 10o-12o by adjusting the final water content, and bottled. Product remained refrigerated during shipment until delivery to the study site. The control juice was produced for the study by obtaining Kern’s apricot nectar (Kern’s Fruit Juice, Scottsdale AZ) was produced from the puree of fully ripe fruits extracted from the Ruby Red variety of Opuntia ficus indica, harvested in the Mexican State of Zacatecas. The fruit is sensitive to heat, light, and oxidation, and the harvested fruit was removed from light within 4 hours of harvest, and either processed or refrigerated within 24 hours. The fruit was sanitized and pureed through a 0.5 mm screen, where air was removed by vacuum, and the resulting puree was acidified to pH 4.1 to preserve flavor and color. The puree was shipped to the bottling plant, where the puree was blended with syrups from agave, pineapple, pear, and apple, flavor added, and bottled at Flavours Inc, Yorba Linda, CA. Subjects were instructed to ingest six fluid ounces of juice daily. Subjects returned to provide blood samples at 8 and 12 weeks. During each follow-up visit, the subjects were asked to report any changes to their health.

Table 1. Comparison of composition of the two fruit juices

<table>
<thead>
<tr>
<th></th>
<th>Nopal fruit juice</th>
<th>Apricot juice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calories</td>
<td>80</td>
<td>105</td>
</tr>
<tr>
<td>Sugars*</td>
<td>14</td>
<td>24</td>
</tr>
<tr>
<td>Fat*</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Fiber*</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sodium*</td>
<td>0.001</td>
<td>0.005</td>
</tr>
<tr>
<td>Protein*</td>
<td>0.25</td>
<td>0.53</td>
</tr>
</tbody>
</table>

*Values shown in grams per 6oz serving

2.2 Blood Samples

Blood samples from subjects were obtained by phlebotomy technicians at Lab Corp, Inc. (Scottsdale AZ), and were transported directly to Lab Corp facilities for testing. Test results were submitted from Lab Corp to NIS Labs, an independent contract research organization, where data analysis was performed.

2.3 Statistical Analysis

Statistical significance of the raw data and the percent changes from baseline to later assessments was evaluated by ‘between-groups’ analysis using the 2-tailed independent Student’s t-test. ‘Within-subject’ analysis was performed using the 2-tailed, paired t-test. Statistical significance was indicated if \( P < .05 \) (*), a high level of significance was indicated if \( P < .01 \) (**), and a very high level of significance was indicated if \( P < .001 \) (***)

3. RESULTS AND DISCUSSION

3.1 Participation

The numbers of participants that were screened, enrolled, and analyzed are shown in the consort flow chart in Fig. 1. Of the 395 subjects screened, 287 had CRP levels within the range of 3.0 mg/L or lower. Of these, 145 participants were assigned to the Nopal fruit juice (NFJ) group and 142 participants assigned to the control (apricot) juice group. Out of these qualifying participants, 54 people dropped out for various reasons, 32 from the apricot juice group and 22 from the NFJ group. An additional 37 people presented with elevated CRP levels at either the 8-week or 12-week visit, 19 from the apricot juice group and 18 from the Nopal fruit juice group, and their data were removed from the per-protocol data analysis. The age and...
gender distribution was similar between the two groups (Table 2).

3.2 Reduction in Serum C-reactive Protein Levels

The serum C-reactive protein (CRP) level in participants ingesting Nopal fruit juice was compared to CRP levels in participants consuming apricot juice using ‘between-groups’ statistical analysis. The CRP levels at baseline were not significantly different between the two groups, however, the CRP levels were significantly lower in the Nopal fruit juice group than the apricot juice group at 8 weeks ($P < .05$) (Fig. 2). The CRP levels were reduced in both groups over the course of the study, with a more robust reduction seen in the group consuming Nopal fruit juice. After 8 weeks consumption, the percent change in the Nopal fruit juice group was significantly higher than the percent changes seen in the apricot juice group ($P < .05$).

Table 2. Gender and age distribution of the study population

<table>
<thead>
<tr>
<th></th>
<th>Nopal fruit juice</th>
<th>Control (apricot) juice</th>
<th>$P^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Females</td>
<td>61</td>
<td>54</td>
<td></td>
</tr>
<tr>
<td>Age average $^b$</td>
<td>57.8±9.1</td>
<td>58.5±9.7</td>
<td>.47</td>
</tr>
<tr>
<td>Age range</td>
<td>38.9–74.4</td>
<td>34.7–75.2</td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>44</td>
<td>37</td>
<td></td>
</tr>
<tr>
<td>Age average $^b$</td>
<td>60.0±9.1</td>
<td>61.9±9.9</td>
<td>.11</td>
</tr>
<tr>
<td>Age range</td>
<td>33.5–75.5</td>
<td>36.5–82.0</td>
<td></td>
</tr>
<tr>
<td>Female: Male ratio</td>
<td>6:4</td>
<td>6:4</td>
<td></td>
</tr>
</tbody>
</table>

$^a$There was no significant age difference between the two groups

$^b$The average ± standard deviation is shown

Consort flow chart

Fig. 1. Consort flow chart of the study participants, showing the number of people screened, randomized, and analyzed. A total of 196 people completed the study participation
Serum levels of CRP in participants ingesting Nopal fruit juice were reduced by an average of 21.0% over a period of eight weeks ($P < .001$). This effect diminished with time in that CRP levels were reduced by only 10.6% compared to baseline at 12 weeks ($P < .065$).

Serum CRP levels in participants ingesting apricot juice were reduced by an average of 6.6% over a period of 8 weeks. By 12 weeks, this effect diminished and average CRP level was only 2.6% less than at baseline. Neither at 8 or at 12 week assessments were these reductions statistically significant.

### 3.3 Changes in Serum Uric Acid

Small, but statistically significant elevations of serum uric acid were observed in both the treatment and control groups of participants at the eight week assessment. This increase was not significant between the NFJ and the control group (Fig. 3). When analyzing changes within each group, differences were seen. Serum concentrations of uric acid in participants ingesting Nopal fruit juice increased by 3.6% over baseline at the 8-week follow-up ($P < .02$), however, this returned towards baseline values at the 12-week follow-up and was no longer significantly different from baseline. In contrast, the control group consuming apricot juice showed a mild but highly significant increase in serum concentration of uric acid at 8 weeks ($P < .001$), and this increase remained statistically significant at 12 weeks when compared to baseline ($P < .04$).

### 3.4 Discussion

The goal for the controlled pilot study presented here was to perform a comparison of two fruit juices on selected biomarkers pertaining to antioxidant and inflammatory status. Fruit juice consumption is one of many ways to get dietary vitamins, antioxidants, including phenolic compounds with the ability to help reduce oxidative stress [17]. However, different fruit juices may have diverse effects on overall health, depending on the balance between sugars and anti-inflammatory compounds. A high intake of fructose has been associated with metabolic syndrome, as characterized by insulin resistance, dyslipidemia, and hypertension, as it can cause an increase in uric acid levels, thus directly affecting nitric oxide availability. The direct negative effects of high fructose intake on oxidative stress and vascular health are well-documented in animal studies [18-20].
Uric acid

![Graph showing serum uric acid levels](image)

**Fig. 3. Serum levels of uric acid.**

Data are shown as the group averages ± standard error of the mean (SEM) for the group consuming Nopal fruit juice (solid line) and for the group consuming apricot juice (dashed line). There was no statistically significant difference between the average uric acid levels for the two groups at baseline, 8, or 12 weeks. Within-subject analysis of the Nopal fruit juice group showed a mild but significant increase in uric acid levels at 8 weeks ($P<.05$, *); however, significance was lost at 12 weeks. Similar analysis of the apricot juice (control) group showed a highly significant increase in uric acid levels at 8 weeks ($P<.001$, ***)**, remaining significantly higher than baseline at 12 weeks ($P<.05$, *)

This pilot study demonstrated that 8 weeks daily consumption of Nopal fruit juice (NFJ) resulted in a statistically significant decrease in C-reactive protein in healthy subjects with levels of C-reactive protein under 3 mg/L. C-reactive protein levels remained lower at the 12 week mark when compared to baseline starting measurements, but were higher than levels recorded at the eight week time point. This is not unusual in studies of this nature, as the interest in participating may decline over the final weeks of the study, and compliance may begin to decline. It is also possible that people became more physically active. While similar trends were observed in the control group consuming 6 oz of apricot juice per day, there was no statistically significant decrease in C-reactive protein levels recorded in the control group. It is noteworthy that 8 weeks consumption of Nopal fruit juice was associated with an average CRP level below 1mg/L, which has been suggested as the “lowest-risk” range. [21].

The daily consumption of both fruit juices were associated with a mild increase in uric acid. The average increase in uric acid levels was higher in the control group consuming apricot juice, and within this group the change was highly significant. In contrast, the group consuming NFJ only showed a mild and transient increase in uric acid levels, suggesting that phenolic and betalain compounds in the juice may help ameliorate effects of fructose.

This study has value as a pilot study on Nopal fruit juice’s effects, and suggests that the effects are linked (either directly or indirectly) to modulation of inflammation. The implications of this study was limited in that the potential anti-inflammatory effects of Nopalea were only observed in healthy subjects whose levels of C-reactive protein were maintained below 3 mg/L throughout the duration of the study. Further clinical studies on Nopal fruit juice are warranted, and should include the specific study of effects pertaining to metabolic markers, cardiovascular risk factors, sports recovery, and general wellbeing. A future placebo-controlled clinical trial should incorporate a wash-out from other juices before study start, and track information on diet and nutritional supplements. Future clinical trials should include data collection to allow for data analysis of subgroups, for example pertaining to BMI, diet, smoker status, history of cardiovascular disease, stress level, and levels of physical activity/exercise.

Future studies should also include measurements at time points earlier than 8 weeks, as this would help evaluate whether other biological changes preceded the reduction in C-reactive protein at 8 and 12 weeks. At this time we can
only speculate as to whether a direct modulation of inflammation was seen, or whether the reduction in C-reactive protein was an after-effect of other, earlier changes to a person's antioxidant protection, immune status, cardiovascular and metabolic function, or other aspects of health. Previous studies on natural product-based consumables have shown changes in antioxidant status within 7 days, [22] and other studies have shown measurable changes after ingestion of a single dose [23-26]. It is possible that changes in antioxidant status may happen rapidly after ingesting Nopal fruit juice, followed by changes within a few weeks in metabolic markers. If so, this would suggest that the effect of daily consumption of NFJ on C-reactive protein is an indirect effect happening as a consequence to direct effects on antioxidant status and metabolic function.

Future studies should also aim at further characterization of active compounds, both betalains and non-betalain compounds. The prickly pear fruit puree is uniquely different from other Opuntia-based nutritional products, such as those based on the leaves, [27] and those associated specifically with extracts of the skin, [4,28] while still containing fibers common to all parts of the Opuntia cactus. Such further work could potentially lead to novel food applications of the Opuntia fruit.

4. CONCLUSION

In conclusion, the comparison of two fruit-based juice products showed better reduction of C-reactive protein levels with consumption of Nopal fruit juice than with apricot juice. Furthermore, consumption of apricot juice led to higher and more prolonged increase in uric acid than consumption of Nopal fruit juice. The results support the potential use of Nopal fruit juice as a modulator of inflammation in healthy individuals across a wide age range.

ETHICAL APPROVAL

The study protocol and consent form was approved by University of Bridgeport's Institutional Review Board.

ACKNOWLEDGEMENTS

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COMPETING INTERESTS

The author is an employee of NIS Labs, an independent contract research organization, and has no financial interest in the subject matter.

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