



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

Gitte S. Jensen^{1*}

¹NIS Labs, Klamath Falls, Oregon, USA.

Author's contribution

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

Article Information

DOI: 10.9734/EJNFS/2016/18218

Original Research Article

Received 10th April 2015
Accepted 16th August 2015
Published 7th September 2015

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.

*Corresponding author: Email: gitte@nislabs.com;

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 hepato-protection [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/nitrite-induced oxidation [11]. Oral consumption of the betaxanthin Indicaxanthin from Nopal fruit was found to inhibit release of PGE₂, NO, IL-1 β 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

University of Bridgeport's Institutional Review Board. The trial was performed at the Trivita Wellness Center LLC (Scottsdale, Arizona USA). Participants were recruited via IRB-approved advertisement disseminated by radio, TV, flyers, and word of mouth. People were excluded from study participation if they were currently consuming Nopal fruit juice, had recent, major change in the last three weeks of prescription or non-prescription medication, were diagnosed with Type I Diabetes, were diagnosed with an acquired or congenital immune system deficiency, had surgery within 30 days prior to screening, had unstable angina or had a stroke within the last six months, had clinically overt peripheral vascular disease, or were diagnosed with or demonstrated symptoms consistent with congestive heart failure. Screening of 395 potential study participants identified a total of 287 men and women, between 33-82 years of age, who were recruited and randomly assigned to a treatment group (Nopal fruit juice: 145 subjects) and a control group (Apricot juice: 142 subjects). Subjects were randomized to receive one of two different kinds of juices with similar mouth feel, color, and calories (Table 1), bottled in similar containers with similar labels. The Nopal fruit juice (Nopalea™, Trivita Inc., 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, pH adjusted to 3.93 +/- 0.02, and Brix o 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 Beverages, Pleasanton CA), blending with 0.1% cherry red powder (GNT USA, Terrytown NY), 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

	Nopal fruit juice	Apricot juice
Calories	80	105
Sugars*	14	24
Fat*	0	0
Fiber*	0	0
Sodium*	0.001	0.005
Protein*	0.25	0.53

*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

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

^aThere was no significant age difference between the two groups

^bThe average±standard deviation is shown

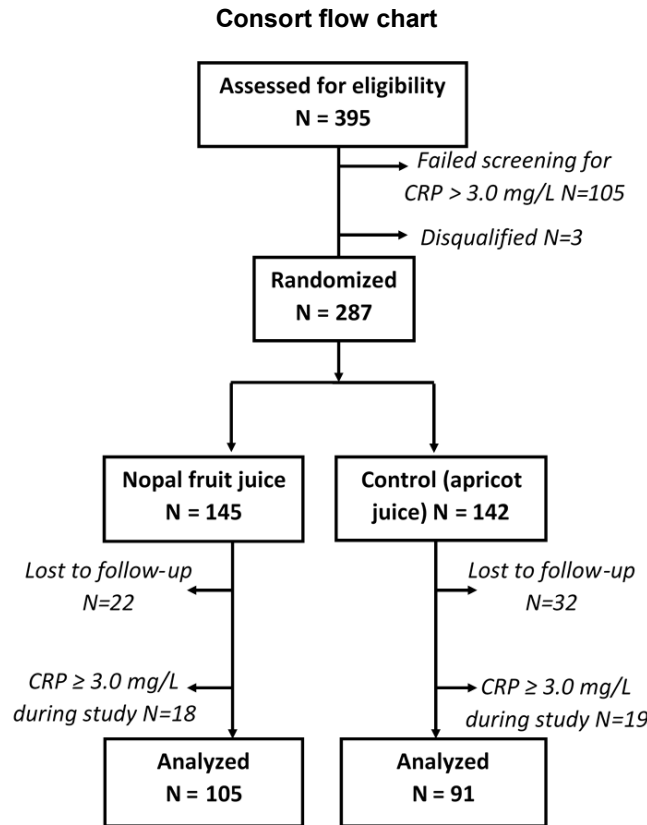


Fig. 1. Consort flow chart of the study participants, showing the number of people screened, randomized, and analyzed. A total of 196 people completed the study participation

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].

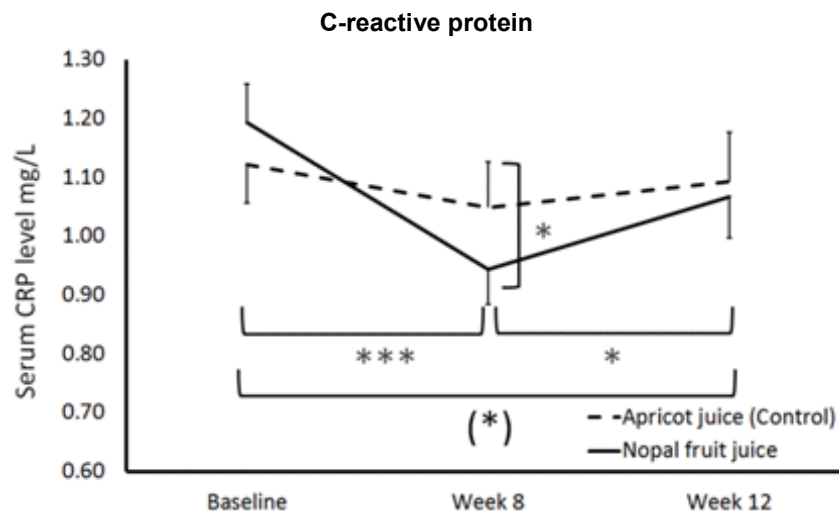


Fig. 2. Serum levels of C-reactive protein (CRP)

Data are shown as the group averages \pm standard error of the mean (SEM) for the group consuming Nopal fruit juice (solid line) and for the control group consuming apricot juice (dashed line). There was no statistically significant difference between the CRP levels for each group at baseline. There was a statistically significant difference in CRP levels between the two groups at 8 weeks ($P < .05$, * at vertical clamp). Within-subject analysis of the control group did not show significant changes. In contrast, within-subject analysis of the subjects in the Nopal fruit juice group showed a highly significant reduction in CRP levels at 8 weeks compared to baseline ($P < .001$, ***), remaining a trend at 12 weeks ($P < .065$, *)

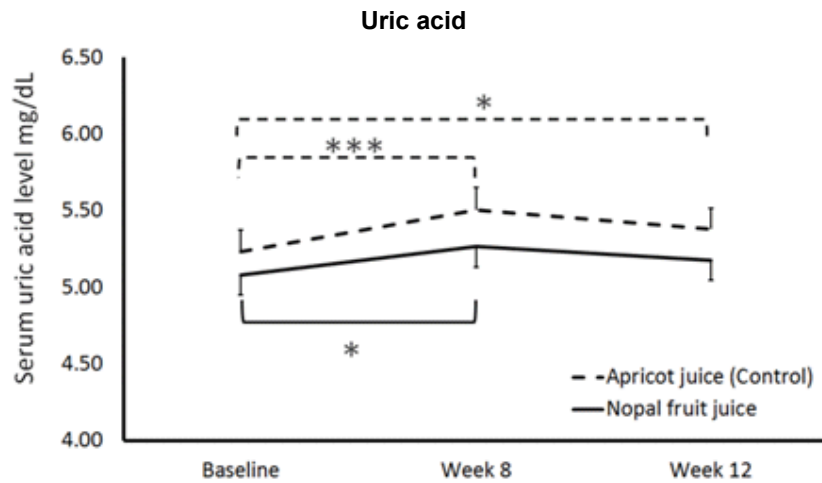


Fig. 3. Serum levels of uric acid.

Data are shown as the group averages \pm 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 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

This study was sponsored by Trivita, Inc., the manufacturer and distributor of the Nopal cactus fruit juice product.

COMPETING INTERESTS

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

REFERENCES

1. Heinrich M, Frei Haller B, Leonti M. A perspective on natural products research and ethnopharmacology in Mexico: The eagle and the serpent on the prickly pear cactus. *J Nat Prod*. 2014;77(3):678-89.
2. Nuñez-López MA, Paredes-López O, Reynoso-Camacho R. Functional and hypoglycemic properties of nopal cladodes (*O. ficus-indica*) at different maturity stages using in vitro and in vivo tests. *J Agric Food Chem*. 2013;61(46):10981-6.
3. Lee EB, Hyun JE, Li DW, Moon YI. Effects of *Opuntia ficus-indica* var. Saboten stem on gastric damages in rats. *Arch Pharm Res*. 2002;25(1):67-70.
4. Wiese J, Mc Pherson S, Odden MC, Shlipak MG. Effect of *Opuntia ficus-indica* on symptoms of the alcohol hangover. *Arch Intern Med*. 2004;164(12):1334-40.
5. Madrigal-Santillán E, Madrigal-Bujaidar E, Alvarez-González I, Sumaya-Martínez MT, Gutiérrez-Salinas J, Bautista M, Morales-González A, García-Luna Y, González-Rubio M, Aguilar-Faisal JL, Morales-González JA. Review of natural products with hepatoprotective effects. *World J Gastroenterol*. 2014;20(40):14787-14804.
6. Cejudo-Bastante MJ, Chaalal M, Louaileche H, Parrado J, Heredia FJ. Betalain profile, phenolic content, and color characterization of different parts and varieties of *Opuntia ficus-indica*. *J Agric Food Chem*. 2014;62(33):8491-9.
7. Francis FJ. *Colorants*. Egan Press;1999. ISBN 1-891127-00-4.
8. Stafford Helen A. Anthocyanins and betalains: Evolution of the mutually exclusive pathways. *Plant Science*. 1994; 101(2):91-98.
9. Madrigal-Santillán E, García-Melo F, Morales-González JA, Vázquez-Alvarado P, Muñoz-Juárez S, Zuñiga-Pérez C, Sumaya-Martínez MT, Madrigal-Bujaidar E, Hernández-Ceruelos A. Antioxidant and anticlastogenic capacity of prickly pear juice. *Nutrients*. 2013;5(10):4145-4158.

10. Gentile C, Tesoriere L, Allegra M, Livrea MA, D' Alessio P. Antioxidant betalains from cactus pear inhibit endothelial ICAM-1 expression. *Ann NY Acad Sci.* 2004;1028: 481-6.
11. Allegra M, Tesoriere L, Livrea MA. Betanin inhibits the myeloperoxidase/nitrite-induced oxidation of human low-density lipoproteins. *Free Radic Res.* 2007;41(3): 335-41.
12. Allegra M, Ianaro A, Tersigni M, Panza E, Tesoriere L, Livrea MA. Indicaxanthin from cactus pear fruit exerts anti-inflammatory effects in carrageenin-induced rat pleurisy. *J Nutr.* 2014;144(2):185-92. DOI:10.3945/jn.113.183657. Epub 2013 Dec 4.
13. Kim SH, Jeon BJ, Kim TI, Lee HK, Han DS, Lee JH, Kim TB, Kim JW, Sung SH. Prickly pear cactus (*Opuntia ficus indica* var. saboten) protects against stress-induced acute gastric lesions in rats. *J Med Food.* 2012;15(11):968-73.
14. Tesoriere L, Butera D, Pintaudi AM, Allegra M, Livrea MA. Supplementation with cactus pear (*Opuntia ficus-indica*) fruit decreases oxidative stress in healthy humans: A comparative study with vitamin C. *Am J Clin Nutr.* 2004;80(2):391-5.
15. Rho YH, Zhu Y, Choi HK. The epidemiology of uric acid and fructose. *Semin Nephrol.* 2011;31(5):410-419.
16. Wang DD, Sievenpiper JL, de Souza RJ, Chiavaroli L, Ha V, Cozma AI, Mirrahimi A, Yu ME, Carleton AJ, Di Buono M, Jenkins AL, Leiter LA, Wolever TMS, Beyene J, Kendall CWC, Jenkins DJA. The Effects of fructose intake on serum uric acid vary among controlled dietary trials. *J Nutr.* 2012;142(5):916-923.
17. Jensen GS, Wu X, Patterson KM, Barnes J, Carter SG, Scherwitz L, Beaman R, Endres JR, Schauss AG. In vitro and in vivo antioxidant and anti-inflammatory capacities of an antioxidant-rich fruit and berry juice blend. Results of a pilot and randomized, double-blinded, placebo-controlled, crossover study. *J Agric Food Chem.* 2008;56(18):8326-33.
18. Mahmoud AA, Elshazly SM. Ursodeoxycholic acid ameliorates fructose-induced metabolic syndrome in rats. *Plos One.* 2014;9(9):e106993.
19. Essawy SS, Abdel-Sater KA, Elbaz AA. Comparing the effects of inorganic nitrate and allopurinol in renovascular complications of metabolic syndrome in rats: role of nitric oxide and uric acid. *Arch Med Sci.* 2014;10(3):537-45.
20. El-Bassossy HM, Dsokey N, Fahmy A. Characterization of vascular complications in experimental model of fructose-induced metabolic syndrome. *Toxicol Mech Methods.* 2014;24(8):536-43.
21. Pepys MB, Hirschfield GM. C-reactive protein: A critical update. *J Clin Invest.* 2003;112(2):299.
22. Chang WH, Hu SP, Huang YF, Yeh TS, Liu JF. Effect of purple sweet potato leaves consumption on exercise-induced oxidative stress and IL-6 and HSP72 levels. *J Appl Physiol (1985).* 2010;109(6):1710-5. Pub Med.
23. Jensen GS, Wu X, Patterson KM, Barnes J, Carter SG, Scherwitz L, Beaman R, Endres JR, Schauss AG. In vitro and in vivo antioxidant and anti-inflammatory capacities of an antioxidant-rich fruit and berry juice blend. Results of a pilot and randomized, double-blinded, placebo-controlled, crossover study. *J Agric Food Chem.* 2008;56(18):8326-33. PubMed
24. Jensen GS, Redman KA, Benson KF, Carter SG, Mitzner MA, Reeves S, Robinson L. Antioxidant bioavailability and rapid immune-modulating effects after consumption of a single acute dose of a high-metabolite yeast immunogen: Results of a placebo-controlled double-blinded crossover pilot study. *J Med Food.* 2011; 14(9):1002-10. PubMed.
25. Davison G, Callister R, Williamson G, Cooper KA, Gleeson M. The effect of acute pre-exercise dark chocolate consumption on plasma antioxidant status, oxidative stress and immunoendocrine responses to prolonged exercise. *Eur J Nutr.* 2012;51(1): 69-79. PubMed.
26. Wylie LJ, Kelly J, Bailey SJ, Blackwell JR, Skiba PF, Winyard PG, Jeukendrup AE, Vanhatalo A, Jones AM. Beetroot juice and exercise: Pharmacodynamic and dose-response relationships. *J Appl Physiol (1985).* 2013;115(3):325-36. Pubmed.
27. Scientific opinion of the panel on dietetic products, nutrition and allergies on a

request from bio Seræ on Neopuntia® and improvement of blood lipid parameters associated with cardiovascular risks, especially HDL cholesterol. The EFSA Journal. 2008;788:1-10.

and “rapid recovery of cellular activity post stress” pursuant to Article 13(5) of Regulation (EC) No 1924/2006. EFSA Journal. 2013;11(7):3330-3337.

28. Scientific Opinion on the substantiation of a health claim related to Preservation®

© 2016 Jensen; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.