# EFFECT OF SUPPLEMENTATION OF PRICKLY PEAR JUICE ON THE ANTIOXIDANT STATUS OF YOUNG ADULT FEMALES

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#### ABSTRACT

Prickly pear (Opuntia spp.) belongs to Cactaceae family and commonly known as cactus fruit. The fruit contains various phenolic compounds, antioxidant vitamins and hence possess good antioxidant activity. In the present study, the effect of prickly pear juice supplementation on the antioxidant status of young adult female was assessed. For this, 20 female subjects with the age group 20-25 years were enrolled in each control and experimental group. 100 ml of prickly pear juice was fed to the females in experimental group and 100 ml water was fed to the subjects in the control group for one month. Prickly pear juice contained 13.87mg% ascorbic acid, 237.75 mg% total phenols and 36.03 mg% flavonoids. The total antioxidant capacity of juice was 97.41 mg% while DPPH and ABTS radical scavenging activity of juice was 71.88% and 48.61% respectively. Thirty days supplementation of prickly pear juice significantly elevated blood glutathione level by 17.75%, blood vitamin C by 54.44% and plasma total antioxidant activity by 33.30%. The present study concludes that consumption of prickly pear juice positively affect the antioxidant status of an individual.

Keywords: prickly pear, antioxidants capacity, vitamin-C, glutathione

#### **INTRODUCTION**

Antioxidants are crucial for animal and plant life as they are involved in complex metabolic and signalling mechanisms [1, 2]. In animals, free radicals are steadily produced by oxidation reactions that can start multiple chain reactions and finally, it causes damage or death to the cell [3]. Antioxidants are the substances that delay, prevent or remove oxidative damage to a target molecule by their free radical scavenging activity and thus preventing the harmful chain reactions [3, 4, 5]. Antioxidants are capable of stabilizing, or deactivating, free radicals before they attack cells. Antioxidants are absolutely critical for maintaining optimal cellular and systemic health and well-being [6]. Antioxidants like vitamin-C, vitamin-E and  $\beta$ -carotene play a vital role in inactivating reactive oxygen species that are responsible for initiation as well as progression of various chronic diseases. Antioxidants are reported to decrease oxidative stress induced carcinogenesis [7, 8].

Prickly pears (*Opuntia spp.*) are underutilized and widely growing fruits belong to the family *Cactaceae* [9]. The other common names for prickly pears are cactus fruit, cactus

pear, Indian fig, and Barbary fig. In India, it is known as *Nagphani* and in Gujarat especially in Saurashtra region, it is popular as findla or hathla [10]. Prickly pears are fleshy berry, elongated, edible, and varying in colour and size as per their species. The taste of fruit is sweet due to presence appreciable amount of sugar [11]. These fruits contain vitamin-C, vitamin-E, β-carotene. minerals, free amino acids such as proline, phenylalanine, lysine, histindie as well as good amounts of total phenols, flavonoids, betalains and carotenoids [12] and hence, prickly pears are reported to possess good antioxidant activity. These nutritional compounds and nutraceuticals are associated mainly with better health of an individual [13]. A diet rich in prickly pear cactus is positively correlated with reduced risk of chronic diseases associated with oxidative stress, such as diabetes, cancer, cardiovascular and neurodegenerative diseases [14]. Hence, in this context, the present study was aimed to check the effect of supplementation of prickly pear juice on the antioxidant status of young adult females.

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#### METHODOLOGY

Development and analysis of prickly pear juice: Prickly pear fruits were procured from Jasdan taluka of Rajkot district of Gujarat. The fruits were cleaned, washed and peeled. The seeds were separated and the pulp was used for juice preparation. The pulp was mixed with water and sugar. The fruit pulp, water and sugar mixed in varying proportion to obtain different samples of prickly pear juices. All the juice samples were subjected to sensory evaluation for finalizing the composition of prickly pear juice for the feeding trial. Also as per the suggestions of the panellist, to increase the acceptability of juice, natural flavours like lemon juice and ginger juice were also added. The final composition and appearance of optimized prickly pear juice is presented in figure 1 and plate 1 respectively. The juice was analysed for ascorbic acid, total phenolic content, flavonoid content and total antioxidant capacity.

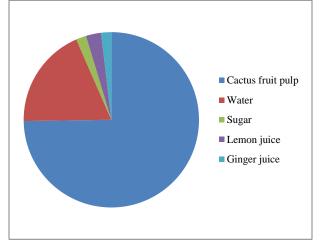


Figure 1: composition of prickly pear juice



Plate 1: Appearance of prickly pear juice

Ascorbic acid estimation: It was done by titration method with dye (2,6 dichlorophenol indophenols) [15]. For this, 5 ml of juice was mixed with 3% meta phosphoric acid and titrated against dye . The results are expressed in mg/100ml of juice.

**Sample extraction:** For this, 5 ml of prickly pear juice was extracted with 80% methanol and samples were stored at -20°C and used for total phenol flavonoids and antioxidant activity analysis.

**Determination of total phenol and flavonoids:** Total phenol estimation was done by folin ciocaltue method [16].The value of total phenol was expressed as % gallic acid equivalent per 100ml of juice. Flavonoid estimation was done by colorimetric method [17]. The result was expressed as % rutin equivalent per 100ml of juice.

**Determination of total antioxidant activity:** Ferric Reducing Antioxidant Power Assay (FRAP) was determined by method given by Benzie and strain [18] and the results were depicted as % trolox equivalent per 100 ml of juice. DPPH (1, 1- diphenyl, 2-picrylhydrazyl) scavenging activity was measured by the spectrophotometric method [19] and 2, 2, Azinobis, 3 ethyl benzo-thiazolin 6-sulphonic acid (ABTS) radical scavenging activity of juice was determined using the modified ABTS radical depolarization assay [20]. The results of DPPH and ABTS radical scavenging activity are expressed in % inhibition.

Clinical trial: Total 40 young adult females in the age group 20-25 years were enrolled on the basis of their willingness to participate in the study. The subjects were divided into control (N=20) and experimental group (N=20). All the subjects were explained the objectives of the study and written consent was taken. 100 ml of freshly prepared prickly pear juice was fed to the subjects in the experimental group and water was fed to each of the subject in the control group for 30 days. Antioxidant profile of the selected subject from both the groups was studied prior to and at the end of the study period. For this, 5 ml fasting venous blood sample was collected from each subject. Whole blood was checked for glutathione and ascorbic acid levels while from serum was analysed for total antioxidant activity. All the samples were analysed on the same day of the collection.

**Glutathione estimation:** Blood glutathione was evaluated by the method given by Ellman [21]. For this, 0.5 ml. of blood sample was mixed with 1 ml of 5% tricholoro acetic acid (TCA). The mixture was mixed and centrifuged. From the supernatant, 0.1 ml aliquote was taken. Sample was treated with 3.9 ml of phosphate buffer and 0.2 ml of DTNB solution.The samples were incubated at room temperature for 10 minutes. The absorbance was read at 412 nm.

**Vitamin-C estimation:** Blood vitamin C was analyzed by the method given by Roe and Kuether [22] and Bessy et al. [23]. For this, 0.5 ml of blood sample was mixed with 1 ml of chilled 5% TCA. The samples were centrifuged for ten minutes. The samples were prepared with 0.4 ml of supernatant and 0.6 ml of 5% TCA to make the volume 1.0 ml. The samples were treated with 0.2 ml of 2,4-Dinitrophenylhydrazine / Thiourea / Copper sulphate solution and were incubated for 3 hours at 37°C .After incubation, 1.5 ml of 65% sulphuric acid was added and the samples were further incubated for 30 minutes at room temperature. The colour developed in the samples were read at 520 nm.

Total Antioxidant Capacity using FRAP method: Serum total antioxidant activity was evaluated by the method of Benzie and Strain [18]. About 0.02 ml of serum was mixed with distilled water to make the volume upto  $300 \ \mu$ l. To this, 1.8 ml of FRAP reagent was added and incubated at 37°C for 10 minutes. The samples were read at 593 nm.

Statistical analysis: The data were analysed by SPSS (version 15.00). Results are expressed in mean  $\pm$  S.D. For studying the effect of supplementation, paired t test was done. A p value less than 0.05 was considered as spastically significant.

#### **RESULTS AND DISCUSSION**

Prickly pear juice was developed using its fruit pulp, sugar and water. Lemon juice and ginger juice were added to increase the flavour of juice. Overall acceptability of freshly prepared prickly pear juice was 88% as scored by the panellist.

Table 1 depicts the average value of ascorbic acid, total phenol, flavonoid content and antioxidant activity of prickly pear juice. Mean ascorbic acid content of prickly pear juice was 13.87 mg/100 ml. Kuti et al. [24] reported ascorbic acid content of different cactus fruit ranged from 12.1 mg% to 81.5 mg% . Diaz Medina et al. [25] and Fernández-López [26] have reported little higher ascorbic acid content of *opuntia ficus indica*. In the present study, the pulp was mixed with water, hence ascorbic acid content was found to be lower.

Ascorbic acid	$13.87 \pm 0.80$		
(mg/100ml)	10107 - 0100		
Total phenol	$237.75 \pm 19.09$		
(mgGAE/100ml)			
Flavonoids	$36.03 \pm 3.75$		
(mgRE/100ml)			
Antioxidant capacity	$97.41 \pm 4.95$		
(FRAP)			
(mgTE/100ml)			
DPPH radical	$71.88 \pm 0.76$		
scavenging activity			
(% inhibition)			
ABTS radical	$48.61 \pm 1.32$		
scavenging activity			
(% inhibition)			

Table 1: Ascorbic acid, total phenol,flavonoid content and antioxidant activity ofprickly pear juice.

Values are Mean ±S.D.

Polyphenols are an important group of natural compounds, recently considered to be of high scientific and therapeutic interest [27]. Polyphenols are also considered as a class of free radical terminator. The products of the metal oxide reduction have a blue colour that exhibits a broad light absorption with a maximum at 765 nm. The intensity of light absorption at that wavelength is proportional to the concentration of phenols [9]. Total phenol content of prickly pear juice was 237.75 mg GAE/100 ml of juice. The phenol content was found to be in the line with that reported by Fernández-López [26] and Yeddes et al. [27]. Albano et al. [13] reported lower value of total phenol of hydrophilic extract of purple cactus fruit. The mean value of flavonoid content of prickly pear juice was 36.03 mgRE/100 ml which was higher than the flavonoid content of prickly pears reported by Kuti et al.[24] and Fernández-López [26].

FRAP assay is a method to determine the total antioxidant power interpreted as the reducing

capacity of the sample. In the present study, antioxidant capacity by FRAP was 97.41 mg TE/ 100 ml of juice. As noted by Albano et al. [13], pulp of purple cactus pear fruit showed significant higher trolox equivalent antioxidant capcacity as compared to pulp of orange cactus pear fruits. Zenteno-Ramirez et al. [28] noted a positive correlation between betalain content and antioxidant capacity by FRAP method.

DPPH and ABTS radicals are commonly used to test the free radical scavenging ability of the sample. ABTS measure the activity of compounds of both lipophilic and hydrophilic nature, while DPPH can only be dissolved in organic media [29]. Higher reduction in colour indicates higher ability to scavenge these radical. In the present study, DPPH radical scavenging activity of prickly pear juice was 71.88 % that is higher than DPPH radical scavenging activity of pomegranate juice, orange juice, apple juice and cranberry juice as reported by Sreeram et al.[30]. While, ABTS radical scavenging activity was 48.61%. Hassan and Hassan [31] have reported 19.34% to 92.59% for 50 µl/ml to 600 µl/ml of cactus pear juice respectively. Madrigal-Santillán et al. [29] reported the best inhibition corresponded to the red-purple juice variety, reaching an inhibition of 65%

Butera et al. [32] have reported that antioxidant activity of cactus fruits is mainly attributed to its chief pigment betalian. The other contributory factors are ascorbic acid, tocopherols, phenolic compounds as well as various flavonoids [24, 33, 34]. Chavez et al. [33] reported higher antioxidant capacity of cactus fruit as compared to apples, pears, tomatoes, bananas and white grapes. Various factors affect phenolic content of cactus fruits such as cultivar, colour, geographical location [24, 35, 36,]. Also, the type of solvent used for extraction and processing of food also affect the antioxidant potential of the food [37].

Effect of supplementation of prickly pear juice on the antioxidant status of young adult females: Prickly pear juice was prepared freshly and supplemented to young adult females. Table 2 shows the mean values of blood glutathione and ascorbic acid level of the subjects prior to and at the end of the experimental period.

Glutathione is a powerful and major tissue antioxidant that prevents damage to cellular component by reactive oxygen species. Increased glutathione level decreases muscle damage, reduce recovery time as well as increases strength and endurance [38]. In the present study, initial level blood glutathione of control subjects was 8.07 mg/dl which increased to 8.59 mg/dl after 30 days, however no significant increase was noticed. In the experimental group, the initial level of blood glutathione was 8.73 mg/dl. Supplementation of prickly pear juice for 30 days significantly (p<0.01) increased it by 17.75%. Tesoriere et al., (2004)[38] reported that GSH :GSSG level in red blood cells was elevated by the supplementation of cactus pear which indicates reduction in oxidative stress. Glutathione is a tripeptide comprising of cystine, glycine and glutamic acid. Ali et al. [39] reported that red prickly pear fruits contains good amount of these three amino acids. In present study, increase in glutathione level in experimental subjects due to prickly pear supplementation may be attributed to presence of reduced glutathione and cysteine in prickly pear fruits [40] which are important constituents of reduced glutathione.

The mean level of whole blood vitamin-C of control subjects at initial level was 1.80 mg/dl. After 30 days, no significant change was noticed in average vitamin-C level. While, in experimental group, the initial level of whole blood vitamin C (1.80 mg/dl) significantly (p<0.01) increased up to 2.78 mg/dl. Supplementation of prickly pear juices for thirty days raised the level of vitamin C by 54.44%. This is due to presence of appreciable amount of vitamin C in prickly pear juice (13.87 mg%) analysed in the present study. According to Tesoriere et al [38], vitamin C is well characterized antioxidant in cactus pear fruit and hence, significant increase in vitamin C was noted after two weeks of cactus pear fruit supplementation to healthy human subjects.

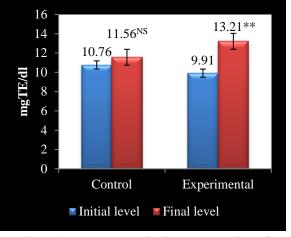


Figure 2: Plasma antioxidant capacity of control and experimental subjects

The difference in mean value of plasma antioxidant capacity of the subjects is presented in figure 2. The average value of plasma antioxidant capacity was 10.75 mg TE/dl at the initial level in control group. No significant change was observed in control group after 30 days pertaining to plasma antioxidant capacity. Prickly pear juice supplementation for one month to the experimental subjects showed a significant (p<0.01) elevation in plasma antioxidant capacity by 33.30%. Trolox equivalent antioxidant activity (TEAC) indicates body's overall antioxidant status. Betanin and indicaxanthin, two important pigments as well as other phenolic compounds, biothiols, water and lipid soluble antioxidant vitamins present in prickly pear are potent free radical scavengers [32, 40]. The rise in plasma antioxidant activity may be associated with the presence of phenolic compounds in the prickly pear fruits. Moreover, the increase in blood vitamin-C and glutathione levels might have contributed to rise in total antioxidant capacity among the experimental subjects in the present study.

### CONCLUSION

Supplementation of 100 ml of prickly pear fruit juice significantly increased blood vitamin-C and glutathione levels as well as plasma antioxidant capacity among young adult females. This reveals that prickly pear juice improves antioxidant status of an individual and may help to prevent many chronic diseases.

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Table 2: Blood glutathione and vitamin-C level of control and experimental subjects prior to and at
the end of experimental period

Parameter	Control			Experimental		
	Initial level	<b>Final level</b>	%	Initial level	<b>Final level</b>	% change
			change			
Glutathione	8.07	8.59 <sup>NS</sup>	6.44	8.73	10.28**	17.75
(mg/dl)	± 2.12	± 1.64		± 3.54	$\pm 2.83$	
Vitamin-C	2.10	2.24 <sup>NS</sup>	6.67	1.80	2.78**	54.44
(mg/dl)	$\pm 0.58$	± 0.59		± 0.65	±0.73	

Values are Mean  $\pm$  S.D., \*\* indicates significant difference at P<0.01 level and NS indicates no significant difference between initial and final level of a parameter