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A comparative study on the effect of feeding an Antioxidant rich drink and Dark chocolate on blood antioxidant status of Young Females

ABSTRACT

The investigation was carried out to study the effect of feeding antioxidant rich drink and dark chocolate on the antioxidant status and lipid profile of young female. The respondents (young females, 20 to 22 yrs.) were divided into two groups – Group-I (n=15) and Group-II (n=15). Group-I was fed 30 ml of antioxidant rich drink while Group-II was fed 3 gm of dark chocolate. Both the groups were supplied with equal amounts of phenols through antioxidant rich drink and dark chocolate. Before and after feeding of juice fresh whole blood was analyzed for glutathione and vitamin-C while serum was estimated for total antioxidant capacity (using Ferric Reducing Antioxidant Power assay (FRAP) method) lipid profile and vitamin E. The result of Sensory Evaluation of four types of drinks indicated that the sample containing pomegranate, black grapes, lemon grass and lemon juice (70+15+10+5) had highest overall acceptability and therefore was selected for feeding trial for one month. The total phenolic content of the dark chocolate was observed higher than the antioxidant rich drink. The flavonoid content of dark chocolate was found to be significantly higher ($P \leq 0.01$) than the antioxidant rich drink. The total antioxidant capacity (FRAP, DPPH, ABTS) content of dark chocolate was found to be higher than the antioxidant rich drink. FRAP and ABTS values of dark chocolate was significantly higher ($P \leq 0.01$) than the antioxidant rich drink. The blood glutathione levels in group-I were non-significantly increased after feeding antioxidant rich drink. Feeding of dark chocolate significantly ($P \leq 0.01$) increased blood glutathione. The level of ascorbic acid in group-I was non-significantly increased after one month feeding of antioxidant rich drink. Feeding of dark chocolate significantly increased ($P \leq 0.01$) blood ascorbic acid. The percentage increase in the serum vitamin E levels was found to be 184.94 and 231.61 after feeding the antioxidant rich drink and dark chocolate. In conclusion dark chocolate appears to be more effective in increasing the antioxidant markers.

Keywords: - Antioxidant rich drink, dark chocolate, blood, glutathione, vitamin-C, total antioxidant capacity (FRAP, DPPH, ABTS)

INTRODUCTION

Damage to cells caused by free radicals is believed to play a central role in the aging process and in disease progression. Antioxidants are our first line of defense against free radical damage, and are critical for maintaining optimum health and wellbeing. The need for antioxidants becomes even more critical with increased exposure to free radicals. Pollution, cigarette smoke, drugs, illness, stress, and even exercise can increase free radical exposure. Because so many factors can contribute to oxidative stress, individual assessment of susceptibility becomes important. As part of a healthy lifestyle and a well-balanced, wholesome diet, antioxidant supplementation is now being recognized as an important means of improving free radical protection. The ability to utilize oxygen has provided humans with the benefit of metabolizing fats, proteins, and carbohydrates for energy (Percival, 1996).

Oxygen is a highly reactive atom that is capable of becoming part of potentially damaging molecules commonly called “free radicals.” Free radicals are capable of attacking the healthy cells of the body, causing them to lose their structure and function. Cell damage caused by free radicals appears to be a major contributor to aging and to degenerative diseases of aging such as cancer, cardiovascular disease, cataracts, immune system decline, and brain dysfunction (Sies et al, 1992). Free radicals are a chemical species that possess unpaired electrons. These electrons account for their reduced chemical stability and high reactivity. They are produced continuously within the cells (Chithra, 2010).
Reactive oxygen species (ROS) is a term which encompasses all highly reactive, oxygen-containing molecules, including free radicals (Percival, 1996). Reactive Oxygen Species (ROS), eg. Superoxide radical, hydroxyl radical, peroxy radical, hydrogen peroxide, singlet oxygen, nitric oxide radical, peroxynitrite, hypochlorous acid, etc (Halliwell, 1994).

Fruits are produced from flowers and the ripened ovary or ovaries of a plant together with adjacent tissues. Fruits are fleshy or pulpy in character often juicy and usually sweet with fragrant, aromatic flavors. Fruits are divided into groups depending upon the shape, cell structure and type of seed or natural habitat (Srilakshmi, 2007).

The pomegranate, Punica granatum L., is an ancient, mystical and unique fruit borne on a small, long-living tree cultivated throughout the Mediterranean region, the Himalayas, in Southeast Asia, and in California and Arizona in the United States. Pomegranate in addition to its ancient historical uses, is used in several systems of medicine for a variety of ailments. The synergistic action of the pomegranate constituents appears to be superior to that of single constituents. In the past ten years, numerous studies on the antioxidant, ant carcinogenic, and anti-inflammatory properties of pomegranate constituents have been performed and published, focusing on treatment and prevention of cancer, cardiovascular disease, diabetes, dental conditions, erectile dysfunction, bacterial infections and antibiotic resistance, and ultraviolet radiation-induced skin damage. Some other potential applications of pomegranate include infant brain ischemia, male infertility, Alzheimer’s disease, arthritis, and obesity (Jurenka et al, 2008). Pomegranates help in preventing or treating various disease risk factors like high blood pressure, high cholesterol, oxidative stress, hyperglycemia, and inflammatory activities. It is demonstrated that certain components of pomegranate such as polyphenols have potential antioxidant, anti-inflammatory, and anti carcinogenic effects. The antioxidant potential of pomegranate juice is more than that of red wine and green tea, which is induced through ellagitannins and hydrosable tannins. Pomegranate juice reduces macrophage oxidative stress, free radicals, and lipid peroxidation. Moreover, pomegranate fruit extract prevents cell growth and induces apoptosis, which may lead to its anti carcinogenic effects.
In addition, promoter inhibition of some inflammatory markers and their production are blocked via ellagitannins (Zarfeshany et al, 2014).

The varying colors of grapes bring a spectrum of antioxidant protective power to this versatile fruit. The components in grape juice are reported to reduce the risk of cancer, stroke, heart disease and memory loss. Purple grapes contain resveratrol, the potent cancer-fighting antioxidant found in red wine and other grape products that have been making headlines around the world. Grape juice has also been shown to provide benefits in areas of anti-aging, anti-bacterial/viral, anti-inflammatory, antioxidant arterial flexibility, brain, skin, eye health, cardiovascular health, inhibiting prostate cancer

(www.javierfuller.com/docs/Monavie). Cymbopogon citratus (DC.) Stapf. (Gramineae), also known as lemongrass, is a plant cultured in almost all tropical and subtropical countries as a source of essential oil.

Lemongrass is used in Peru for preparing soft drinks and is used as an aromatic, pleasant-tasting herbal tea all around its distribution area. The infusion or decoction of its aerial parts has widespread use in folk medicine (Cheel et al, 2005). The plant is recommended to treat digestive disorders, inflammation, diabetes, nervous disorders, and fever as well as other health problems. It is a great interest due to its commercially valuable essential oils and widely used in food technology as well as in traditional medicine (Mirghani, 2012).

Lemon (Citrus limon L) is the third most important species of citrus fruit after orange and mandarin. The presence of bioactive compounds, such as hydrocinnamic acid, ferulic acid, cyaniding glucoside, flavonoid, vitamin C, carotenoid, hesperidin and naringin content contribute to the value of lemon in terms of it being associated with promoting good health.

Therefore, the study aimed at investigating the effect of feeding an antioxidant rich drink and dark chocolate in improving the blood antioxidant status of young females.

MATERIALS AND METHODOLOGY

The present investigation was planned to develop an antioxidant rich drink using pomegranate, black grapes, lemongrass, and lemon juice. The effect of feeding the antioxidant rich juice and dark chocolate on the blood antioxidant status and lipid profile was also studied.

Locale of study:

The research work was conducted at the laboratory of Foods and Nutrition, Jashbhai Khodabhai Patel, Department of Home Science, Sardar Patel University, Vallabh Vidyanagar, Anand.

The entire experiment was divided into three phases.

Phase 1:

It deals with procuring raw materials, development of an antioxidant rich drink having pomegranate, black grapes, lemongrass, and lemon juice mixed in different proportions and studying the organoleptic qualities of the juice.

(a) Procurement of raw material:

Pomegranate, black grapes, lemongrass, and lemon were purchased from local market of Anand.

Selection of dark chocolate:

Dark chocolate was selected on the basis of content of cocoa. Dark chocolate (Amul brand) was procured from the local market of Anand.

(b) Development of Antioxidant rich drink:

The drink was successfully prepared in the laboratory condition. For this repeated trials of the juice preparation was carried out. For this the juice was extracted from pomegranate, black grapes, and lemon

(Maharaja). Lemongrass was boiled with known quantity of water and strained through a strainer. These juices were mixed in different proportions for the preparation of antioxidant drink. Different samples of the antioxidant rich drink were subjected to sensory evaluation. The ingredients were mixed in different proportions based on the sensory trials. The composition of the antioxidant rich drink is presented in the Table - 1.

Table: 1 Composition of antioxidant rich drink

Ingredients	Sample A	Sample B	Sample C	Sample D
Pomegranate juice (ml)	100	50	60	70
Black grapes juice (ml)	_	20	10	15
Lemongrass extract (ml)	_	25	25	10
Lemon juice (ml)	_	5	5	5
Water (ml)	25	25	25	25

(c) Studying the organoleptic qualities of the drink:

Drinks containing different proportion of each ingredient were subjected to sensory evaluation by a team of six panel members using composite scoring test (Srilakshmi, 2003) for consecutive three days. The organoleptic qualities studied were color, smell, flavor and overall acceptability. The drink having highest overall acceptability was used for feeding trial.

Phase 2:

It deals with chemical analysis of drink and dark chocolate.

- **Parameters studied**:
  - Total Antioxidant Capacity (TAC)
    a) Ferric Reducing Antioxidant Power assay (FRAP; Benzie et al, 1996)
    b) DPPH Radical Scavenging Activity Assay (DPPH-RSA; Bran-Williams et al, 1995)
    c)ABTS Radical Scavenging Activity Assay (ABTS-RSA; Re et al, 1999)
  - Total phenols (Folin-Ciocalteu method by Singleton and Rossi, 1965)
  - Flavonoids (Singleton and Rossi, 1999)

Phase 3:

It deals with studying the effect of feeding the antioxidant rich juice as well as dark chocolate for one month on the blood antioxidant status of young females (20 to 22 yrs).

(a) Feeding Trial:-

- At the onset, the total phenolic content of 30 ml of drink was analyzed. The weight of the dark chocolate providing equal amount of phenolic content as the drink was used for feeding trial.
- Thirty young females in the age group of 20 to 22 yrs. were recruited from P.G. Department of Home Science; Sardar Patel University. The selection was done on the basis of
  (a) Absence of any apparent diseases/illness
  (b) Willingness to participate
  The subjects were divided into two groups Group-I (n=15) was fed the antioxidant drink (30 ml/day) while Group-II (n=15) was fed dark chocolate (3 gm/day) for a period of 30 days. Prior to feeding and at the end of the experimental period the blood and serum were collected and analyzed, for antioxidant profile.

(b) Collection of blood sample:-

The subjects were requested to report to the laboratory of department of Foods and Nutrition, after an overnight fast (12 hours). Approximately 5 ml of venous fasting blood drawn from each subject and collected in a clean dry centrifuge tube. The tubes were centrifuged to separate the serum for analysis of antioxidants.

(c) Final data:-

At the end of experimental period all the initial parameters were again checked from blood as well as serum.

- **Initial and final parameters**

  - Antioxidant profile.
    a) Whole blood analysis
    - Glutathione (Ellman, 1959)
    - Vitamin-C (Roe and Kuether, 1943 and Bessey et al, 1947)

    b) Serum analysis
    - Vitamin-E (Emmerie and Engel, 1938 modified by Desai,1986)
    - Total antioxidant capacity (TAC; Benzie and Strain, 1996)

Statistical Analysis

All the assays were carried out in triplicates and results were standardized and expressed as Means ± SEM. The difference between sensory variables was tested for significance by ANOVA using SPSS version 15. The observations obtained were compared satisfactorily using descriptive test, t-test: two sample Assuring equal variance, regression and scattered chart y M. S. Excel (2007).

RESULT AND DISCUSSION

The results obtained are discussed here under the following heads:

1) Sensory Evaluation

2) Chemical analysis of antioxidant rich drink and dark chocolate
3) Feeding Trial

Sensory evaluation

Sensory score of different antioxidant rich drinks are presented in Table-2

When the quality of a food product is assessed by means of human sensory organs, the evaluation is said to be sensory. The composite scoring test was helpful in grading products and comparison of quality attributes by indicating which characteristic is at fault in a poor product (Srilakshmi, 2007).

The color scores of different antioxidant rich drinks ranged from 6.78 to 7.94. The highest score was observed in sample D (7.94). Sample B and C had a score of 7.06 and 7.44, respectively. The flavor scores of different antioxidant rich drinks ranged from 6.72 to 7.94. The highest score was observed in sample D (7.94). Sample D containing pomegranate, black grapes,

Table:2 Sensory scores of different Antioxidant rich drink

Sample	Color	Flavor	Taste	Overall acceptability	Total
A	6.78^{a} ± 0.29	6.72^{ab} ± 0.29	6.78^{a} ± 0.36	6.78^{a} ± 0.26	27.11^{ab} ± 1.09
B	7.06^{a} ± 0.25	5.94^{a} ± 0.34	6.44^{a} ± 0.22	6.67^{a} ± 0.19	26.28^{a} ± 0.84
C	7.44^{ab} ± 0.25	7.56^{bc} ± 0.28	7.11^{a} ± 0.23	7.61^{b} ± 0.20	29.72^{bc} ± 0.87
D	7.94^{b} ± 0.37	7.94^{c} ± 0.33	7.89^{b} ± 0.25	8.11^{b} ± 0.28	31.88^{c} ± 1.15
F-value	3.031*	8.250*	5.257*	8.425*	6.581*

Values are Mean ± SEM of three observations

* indicates significant difference ($P \leq 0.01$)

Values with different superscripts within the column differ significantly ($P \leq 0.05$)

Lemongrass and lemon juice (70+15+10+5 ml) had significantly higher ($P \leq 0.05$) flavor score as compared to sample A and sample B. The taste scores of different antioxidant rich drinks ranged from 6.78 to 7.89. The highest score was observed in sample D (7.89). Sample D containing pomegranate, black grapes, lemongrass and lemon juice (70+15+10+5 ml) had significantly higher $P \leq 0.05$) score as compared to sample A, B and C. The overall acceptability scores of different antioxidant rich drinks ranged from 6.78 to 8.11. The highest score was observed in sample D (8.11). Sample B and C had a score of 6.67 and 7.61, respectively. The scores of all the sensory attributes of sample D was found to be highest which may be due to incorporation of pomegranate, black grapes, lemongrass and lemon juice (70+15+10+5). Based on the results of sensory evaluation, Sample D was used for feeding trial. The cost of the formulated drink is Rs. 4.13 per 30 ml.

Chemical analysis of Antioxidant rich drink and Dark Chocolate:-

Total Phenol:

The total phenolic content of the dark chocolate was 1309.89 mg GAE/100gm while that of antioxidant rich drink was found to be 114.58 mg GAE/100gm. A high significant difference ($P \leq 0.01$) was observed between the two values. Tzanakis et al (2006) concluded that pomegranate shows the highest values of total phenols followed by wild pear and in other fruits especially to apple. Pomegranate contains more than seven to eleven times higher phenol values than green apple. Vinson et al (2006) reported that chocolate products contain a great deal of polyphenol antioxidants. Thus, chocolate contributes over 20% of the antioxidants provided by fruits and vegetables. Pimentel et al. (2010), represented that 34% of polyphenols in dark chocolate are from flavonoids group. The best results were found using 71% cocoa dark chocolate (polyphenols = 62.9 ± 0.1 lmol of catechin equivalents/g).

Flavonoid:

The flavonoid content of dark chocolate (1308.77 mg RE/100gm) was found to be significantly higher ($P \leq 0.01$) than the antioxidant rich drink (47.45 mg RE/100gm). Zvaigzne et al (2009) concluded that the total flavonoid content was usually higher in peels than in the tissues in citrus fruits. Allen et al (2008) reported that cocoa and cocoa containing products, including chocolate can be rich sources of a subclass of flavonoids known as flavanols. A negative and non-significant correlationship existed between total phenols and flavonoid content of dark chocolate ($R^2 = 0.685$, $p \leq 0.05$). Pimentel et al. (2010), also depicted that the cocoa has also been described as being a good source of flavonoids, such as catechins. In this context, dark chocolate with high content of cocoa has been recognised as an important alternative antioxidant in the diet. The best results were found using 71% cocoa dark chocolate (flavonoids = 21.6 ± 2.4 lmol of catechin equivalents/g).

Total antioxidant capacity:

FRAP:

The FRAP value of dark chocolate was 1317.71 mg TE/100gm while that of antioxidant rich drink was found to be 542.98 mg TE/100gm. Dark chocolate had significantly higher ($P \leq 0.01$) FRAP value than the antioxidant rich drink. Serum et al (2008) reported that pomegranate had the greatest antioxidant potency composite index among the beverages tested and was at least

Table: 3 represents the mean values of total phenols, flavonoid and total antioxidant capacity (FRAP, DPPH, ABTS) of Antioxidant rich drink and Dark Chocolate

Parameters	Antioxidant rich drink	Dark chocolate	T - value
Phenol (mg GAE/100gm)	114.58 ± 2.19	1309.89 ± 45.28	-26.37**
Flavonoid (mg RE/100gm)	47.45 ± 3.40	1308.77 ± 63.58	-19.80**
FRAP (mg TE/100gm)	542.98 ± 13.80	1317.71 ± 23.96	-28.02**
DPPH RSA (mg TE/100gm)	519.89 ± 35.63	2298.56 ± 14.93	-46.04**
ABTS RSA (mg TE/100gm)	291.00 ± 4.28	4663.00 ± 65.49	-66.61**

- Values are Mean ± SEM

** indicates high significant difference (P ≤ 0.01)

20% greater than any of the other beverages tested. A negative and significant correlationship existed between total phenols and FRAP value of antioxidant rich drink (R^2 = 0.859, p ≤ 0.05). A negative and non-significant correlationship existed between total phenols and FRAP value of dark chocolate (R^2 = 0.598, p ≤ 0.05).

DPPH RSA:

The DPPH RSA of dark chocolate was 2298.56 mg TE/100gm while that of antioxidant rich drink was found to be 519.89 mg TE/100gm. A high significant difference (P ≤ 0.01) was observed between the two values. Zvaigzne et al (2009) reported that the DPPH RSA of freshly squeezed juice (except lemon) was higher than that of the reconstituted juice since it is dependent on the content of vitamin C, total phenolics and carotenoids in raw material.There was a close correlation between the RSA and total phenolic content and vitamin C. The higher the content of total phenolics, vitamin C and carotenoids in juice, the higher is RSA percentage. A negative and non-significant correlationship existed between total phenols and DPPH RSA of antioxidant rich drink (R^2 = 0.249, p ≤ 0.05). A positive and non-significant correlationship existed between total phenols and DPPH RSA of dark chocolate (R^2 = 0.137, p ≤ 0.05).

ABTSRSA:

The ABTS RSA of dark chocolate (4663.00 mg TE/100gm) was found to be significantly higher (P ≤ 0.01) than the antioxidant rich drink (291.00 mg TE/100gm). A negative and non-significant correlationship existed between total phenols and ABTS RSA of antioxidant rich drink (R^2 = 0.312, p ≤ 0.05). A negative and non-significant correlationship existed between total phenols and ABTS RSA of dark chocolate (R^2 = 0.157, p ≤ 0.05).

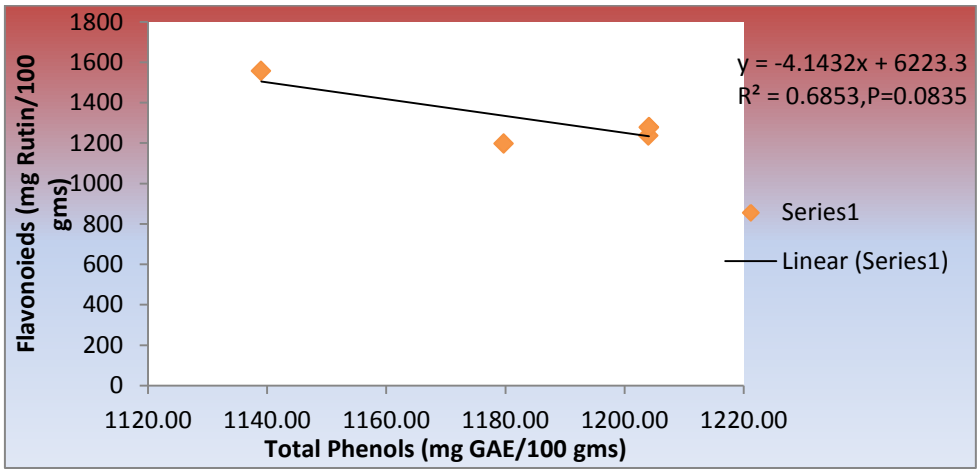

Figure:1 Regression analysis between total phenols and flavonoids content of Dark Chocolate

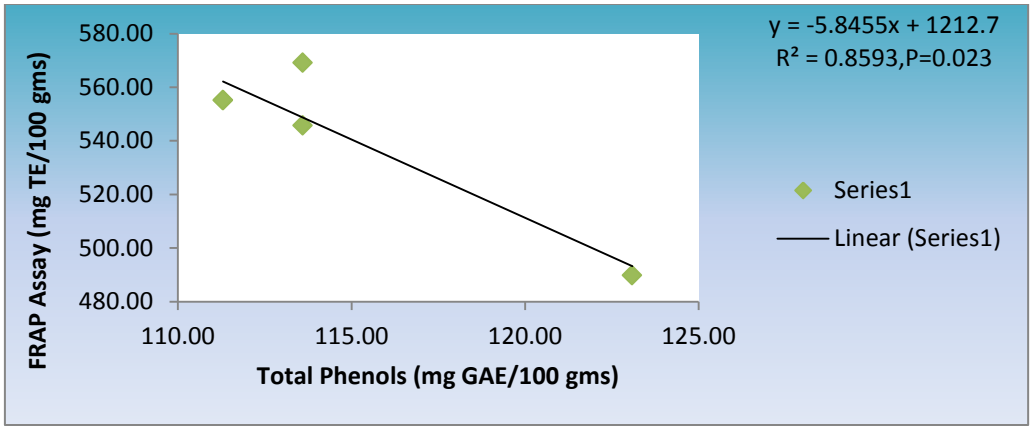

Figure: 2 Regression analysis between total phenols and FRAP values of Antioxidant rich drink

Feeding Trial:

The purpose of this study was to compare the efficacy of antioxidant rich drink and dark chocolate on improving the antioxidant status and lipid profile in young females.

Blood Glutathione:

The initial level of blood glutathione in group-I was found to be 20.73 mg% which non-significantly increased to 23.67 mg% after one month feeding of antioxidant rich drink. The initial level of blood glutathione in group-II was found to be 16.75 mg% which significantly increased ($P \leq 0.05$) to 26.92 mg% after one month feeding of dark chocolate. The percentage increase in the whole blood glutathione levels was 14.13 and 60.81 after feeding the antioxidant rich drink and dark chocolate, respectively. Feeding of dark chocolate showed a higher increase of blood glutathione levels as compared to antioxidant rich drink. Reactive oxygen species (ROS) cause oxidant stress in tissues of living organisms. The main ROS to be considered is superoxide anion (O2-), which is predominantly generated by the mitochondria. Hydrogen peroxide (H2O2) is produced from O2 –by the action of superoxide dismutase (SOD), and peroxynitrite (ONOO-) is generated by the reaction of O2 - with nitric oxide (NO). These ROS are scavenged by antioxidant enzymes namely SOD, GSH-Px, and CAT. Under certain circumstances, these endogenous antioxidative defenses are likely to be disturbed as a result of overproduction of oxygen radicals and failure to regenerate antioxidants in tissues adequately. Besides the antioxidant enzymes and vitamins, some foods that have antioxidant power may ameliorate oxidant stress (Aslihan, 2010). The result of the present study is consistent with the few scientists. Nagai et al, (2002) demonstrated that supplementing a meal with grape seed proanthocyanidins can minimize the postprandial oxidative stress by decreasing the oxidants and increasing the antioxidant levels in plasma, and, as a consequence, enhance the resistance to oxidative modification of LDL.

Blood Ascorbic Acid:

The initial level of ascorbic acid in group-I was found to be 4.21 mg% which non-significantly increased to 8.77 mg% after one month feeding of antioxidant rich drink. The initial level of ascorbic acid in group-II was found to be 4.72 mg% which highly significantly increased ($P \leq 0.01$) to 8.55 mg% after one month feeding of dark chocolate. The percentage increase in the whole blood ascorbic acid levels was found to be 108.31 and 81.14 after feeding the antioxidant rich drink and dark chocolate, respectively.

Feeding of antioxidant rich drink showed a higher increase of blood ascorbic acid levels as compared to dark chocolate. This may be attributed to presence of vitamin C rich ingredients in the antioxidant rich drink. Nalsen et al (2006) reported an increase in ascorbic acid levels after consumption of dietary antioxidant. Franch et al (2008) also reported an increase in plasma concentration of ascorbic acid by 68.2%. Chen et al, (2003) reported that after having fruits and vegetable concentrates, ascorbic acid levels increased significantly compared to the placebo treatment.

Serum total antioxidant capacity using FRAP assay:

The initial level of serum TAC in group-I was found to be 67.62 mg% which non significantly decreased to 64.30 mg% after one month feeding of antioxidant rich drink. The initial level of serum TAC in group-II was found to be 71.35 mg% which non-significantly decreased to 60.76 mg% after one month feeding of dark chocolate. The percentage decrease in the serum TAC levels was found to be 4.91 and 14.81 after feeding the antioxidant rich drink and dark chocolate, respectively. Feeding of antioxidant rich drink showed a lower decrease of serum TAC levels as compared to dark chocolate.

In the present study a non-significantly decrease was observed in the TAC levels after feeding antioxidant rich drink as well as dark chocolate as compared to their initial levels which may be attributed to oxidative stress among the various respondents of both the groups which might have caused a decrease in the antioxidant defence system (Aslihan et al, 2010).

Vitamin E:

The initial level of vitamin E in group-I was found to be 4.25 mg% which highly significantly ($P \leq 0.01$) increased to 12.11 mg% after one month feeding of antioxidant rich drink. The initial level of vitamin E in group-II was found to be 3.66 mg% which highly significantly ($P \leq 0.01$) increased to 12.14 mg% after one month feeding of dark chocolate. The percentage increase in the serum vitamin E levels was found to be 184.94 and 231.61 after feeding the antioxidant rich drink and dark chocolate, respectively. Feeding of dark chocolate showed a higher increase of serum vitamin E levels as compared to antioxidant rich drink. Luepker et al (1993) reported that levels of α-tocopherol increased significantly in the fruit and vegetable juice concentrate group vs placebo. Elderly subjects supplemented with fruit and vegetable juice concentrates for 80 days experienced significant increases in serum α-tocopherol (from 22.56 to 28.75 µg/ml; p=0.003) and also reported improvement in several markers of immune function, including increased natural killer cell activity and improved IL-2 levels, regardless of smoking status.

SUMMARY AND CONCLUSION

In conclusion the present study showed favorable effects of antioxidant rich drink and dark chocolate on plasma antioxidant markers such as glutathione, ascorbic acid and vitamin E in young healthy females. Dark chocolate appears to be more effective than the antioxidant rich drink in increasing the antioxidant markers.

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