

EFFECT OF BLACK TEA PREPARATION ON VITAMIN C ABSORPTION IN ALBINO RAT ILEUM USING EVERTED GUT SAC TECHNIQUE

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ABSTRACT

Introduction: Vitamin C is a vital antioxidant molecule. It plays a critical role in the normal functioning of the body and optimum health. Black Tea is the most popular flavoured and functional drink worldwide. Aim of the work was to study the effect of black tea on vitamin C absorption in albino rat ileum. **Methodology:** Ten adult male albino rats, weighing 300-350 g, were divided into equal two groups. In group one, rats were starved for 24 hours, killed by cervical dislocation and the entire small intestine quickly excised and flushed with tyrode solution. Everted gut sac technique was prepared according to Barthe et al. (1998). Each sac was placed in a beaker containing 20 ml of tyrode solution with 20 mg Vitamin C. The beakers were placed in a shaking water bath for homogeneity for (5, 15, 30, and 60 minutes). Sacs were removed, washed in tyrode, and blotted dry. 0.1 ml of each sac contents was taken into beaker containing 9.9 ml buffer (PH 5.4). In the second group, 1 ml of black tea extract (50 mg dried black tea/ml) was added to 19 ml tyrode solution containing 20 mg vitamin C. The final tyrode mixture contained 1 mg/ml vitamin C and 2.5 mg dried black tea/ml tyrode. The absorbed vitamin C concentration was measured using spectrophotometer. **Results and conclusion:** Vitamin C absorption was increased by time, either alone or in the presence of black tea extract. Black tea extract increased the absorption of vitamin C compared to the absorption of vitamin C alone.

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Introduction

Vitamin C is a water-soluble vitamin and an essential dietary component. [1] It is required for the biosynthesis of collagen, L-carnitine, and certain neurotransmitters, and involved in protein metabolism, [1, 2] with important physiological antioxidants. [3] Vitamin C regenerates other antioxidants within the body, including alpha-tocopherol. [4] In addition, it plays an important role in immune function and improves the absorption of nonheme iron. [5]

Black tea is one of the most consumed beverages in the world. It comes from *Camellia sinensis* plant. It offers a variety of health benefits because it contains antioxidants that helps to decrease the risk of chronic diseases [6, 7] and can reduce body inflammation. [7] Consumption of black tea beverages may reduce many risk factors for heart diseases, [6, 8, 9] and may lower the risk of stroke. [10] Several compounds in black tea were shown to improve insulin levels [11] and sugar metabolism [12] and may play a role in regulating cancer cell growth and reducing new cell development. [13]

Aim of the work

The aim of this work was to study the effect of black tea preparation on the absorption of vitamin C using "Everted gut sac" method.

Materials and Methods

Materials

Sodium chloride, magnesium chloride, and potassium chloride were purchased from Carlo Erba, SPA. Calcium chloride 2-hydrate, sodium hydrogen carbonate, and glucose were purchased from Riedel-De-Haen AG, Franc. Sodium dihydrogen orthophosphate dihydrate was purchased from Fisher Scientific, UK. Potassium dihydrogen orthophosphate was purchased from BDH Analar, BDH Limited Poole, England. Sodium monohydrogen phosphate was purchased from Park Scientific Limited; Northampton, UK. Sodium oxalate was purchased from BDH Limited Poole England. Orthophosphoric acid was

purchased from T-Baker Lab chemicals, INDIA. Vitamin C was purchased from pharmaceutical industries society (PIS), Ben Arous, Tunisia.

Instrument

Spectrophotometer designed and manufactured in the UK by Bibby Scientific Ltd. Dunmow Essex CM6 3LB at the wavelength of 270 nm and quartz cell was used. Shaking water bath from Gesellschaft fur Labortechnik mbH, Germany, Ultrasonics Telsonic TPC-15 Frauenfeld, Switzerland, and PH meter from HANNA instruments, Romania, were used.

Black tea preparation

For the preparation of the black tea solution, 5 g of black tea was soaked in 100 ml of boiling water for 2 min and filtered. [14] The filtrate of black tea solution had a concentration of 50 mg dried black tea/ml. 1 ml of black tea preparation was added to 19 ml tyrode solution contain 20 mg vitamin C. The final tyrode mixture contained 1 mg/ml vitamin C and 2.5 mg dried black tea/ml tyrode.

Methodology

An everted gut sac technique was used. 10 adult male albino rats, weighing 300-350 g, were divided into two groups; each group consisted of 5 adult male albino rats. Rats were starved for 24 hours, killed by cervical dislocation and the entire small intestine quickly excised and flushed several times with physiological salt solution of tyrode (pH 7) [15] at room temperature. Ileum was immediately placed in Petri dish containing Tyrode solution with oxygen aeration. 5 cm of the ileum was slid onto a glass rod (2.5 mm diameter). Then, the ileum gently everted over the rod and the everted ileum slid into fresh oxygenated Tyrode solution. One end of the ileum fastened with silk suture, and the whole length of the ileum was filled with 2 ml fresh oxygenated Tyrode physiological solution. The second end of the ileum was fastened with second silk suture (sac). Each sac was then placed in beaker containing 20 ml of Tyrode solution with 20 mg Vitamin C (1 mg/ml). Then the beakers were placed in shaking water bath for homogeneity for 5, 15, 30, and 60 minutes. Sacs were removed, washed four times in Tyrode and blotted dry. The sacs were cut open and the fluid content drained into small tubes. [16] 0.1 ml of each sac contents were taken into beaker of 20 ml containing 9.9 ml buffer (PH 5.4). [17] The solution then was thoroughly mixed and then the absorbed vitamin C concentration was measured using spectrophotometer. [18]

Different concentrations of vitamin C (0, 0.25, 0.5, and 1 mg/ml) were prepared for dose-response curve in buffer (PH 5.7). Calibration of spectrophotometer was carried out using 0 mg/ml vitamin C. The absorbed vitamin C concentration was measured at wavelength of 270 nm (figure 1). [18]



Figure 1. Steps of ileum everted sac preparation.

Statistical Analysis

Descriptive statistical analysis was applied on parameters of different samples using Statistical Package for Social Sciences version 17 (SPSS). Kolmogorov-Smirnov maximum deviation test, for goodness of fit to find out whether the observed samples were parametric. If the parameters normally distributed, the comparison between groups was analyzed using one-way ANOVA, followed by a post-hoc test (LSD tests). If the parameters of samples were non-parametric, treatments were compared by applying Mann-Whitney two samples (none matched) test. The difference was considered significant at $p \leq 0.05$.

Results

The dose-response curve for different concentrations of vitamin C ranged between 0.25-1 mg/ml was carried out; the correlation coefficient of the curve was 0.969. The absorption of vitamin C in the group treated with vitamin C alone showed insignificant increase in vitamin C absorption during the first 15 minutes ($p > 0.05$); after 30 minutes and 60 minutes, the absorption of vitamin C was increased significantly ($p \leq 0.05$). Absorption of vitamin C in the presence of black tea preparation was increased significantly by time during the 60 minutes ($p \leq 0.05$). Vitamin C absorption in the presence of black tea preparation was increased insignificantly during the first 15 minutes ($p \leq 0.05$) and significantly increase after 30 and 60 minutes compared to the amount of vitamin C absorbed compared with vitamin C alone-treated group ($p \leq 0.05$) (table 1, figure 2).

Table 1. Absorption of vitamin C in the presence of black tea using everted gut sac technique.

Treatments	5 min	15 min	30 min	60 min
Vitamin C (1mg/ml) (n=5)	0.239±0.032	0.444±0.097	0.651±0.095 a,	0.860±0.089 a, b
Vitamin C in presence of black tea (2.5mg/ml) (n=5)	0.352±0.046	0.644±0.02 a,	0.841±0.007 *,a, b	1.104±0.011 *, a, b, c

* Significantly different from absorbed vitamin C of the same time at $p \leq 0.05$. a, Significantly different from absorbed vitamin C after 5 min of the same treated group at $p \leq 0.05$. b, Significantly different from absorbed vitamin C after 15 min of the same treated group at $p \leq 0.05$. c, Significantly different from absorbed vitamin C after 30 min of the same treated group at $p \leq 0.05$.

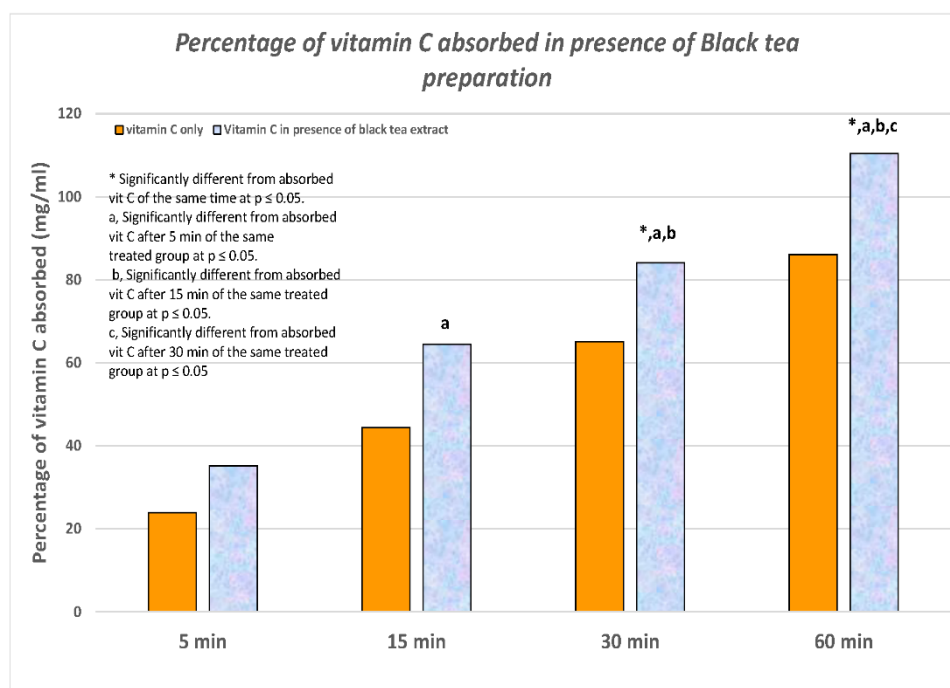


Figure 2. Percentage of vitamin C absorbed in the presence of black tea.

Discussion

Vitamin C absorption was increased by time. The absorption of vitamin C in the presence of black tea was increased compared to the amount of vitamin C absorbed when present alone.

One of the black tea constituents is caffeine, [19] which is a CNS stimulant. It may increase energy production in the body. The increase of energy production may stimulate the active transport of vitamin C through SVCT1 and SVCT2, and lead to increase the absorption of vitamin C.

Black tea was found to possess antidiarrheal activity, which is antagonized by Naloxone. [20] This indicates that black tea has the role of opioid system in its antidiarrheal activity. Stimulation of the opioid receptor by black tea may reduce the motility of the intestine [14] leading to an increase in the chance for the absorption of vitamin C. The antihyperglycaemic activity of black tea [21] decreased the levels of glucose leading to an increase in the transportation of vitamin C through glucose transporter (GLUT).

Black tea has low acid composition, [20] with a pH of 4.9. [22] The absorption of Vitamin C in the presence of black tea was increased, due to the presence of vitamin C in unionized form in acidic media induced by black tea.

Conclusion

Vitamin C absorption was increased by time, either when it is administered alone or in the presence of black tea. Vitamin C absorption was potentiated in the presence of black tea when administered together, compared to the absorption of vitamin C when administered alone.

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