Indian Journal of
Nutrition

Open Science Publications

Volume 3, Issue 1 - 2016

www.opensciencepublications.com

# *In vitro* Oxidative Damage of Protein by Carbonated Soft Drink and its Restoration by Vitamin C

## Research Article

**Pamela Banerjee*, Aniruddha Mukhopadhyay**

*Department of Environmental Science, University of Calcutta, 35 Ballygunge Circular Road, Kolkata-700019, India*

***Corresponding author:** Pamela Banerjee, Department of Environmental Science, University of Calcutta, 35 Ballygunge Circular Road, Kolkata-700019, India, Tel: +919830915163; E-mail: pbanerjee232@gmail.com

**Article Information:** **Submission:** 03/06/2016; **Accepted:** 20/07/2016; **Published:** 26/07/2016



**Abstract**

Carbonated soft drink causes oxidative damage of liver, kidney and serum proteins with apoptosis of these tissues in guinea pigs. Protein carbonyl and bityrosin formation and tryptophan loss took place under such condition indicating protein oxidation. In *in vivo* experiments these damages could be prevented by supplementation of Vitamin C to carbonated soft drink fed to guinea pigs. The present study was aimed at weather vitamin C can inhibit protein oxidation *in vitro* caused by carbonated soft drink when added in both dose and time dependent manner to pure protein solution.

**Keywords:** Oxidative damage; Carbonated soft drinks

## Introduction

Carbonated soft drink have their extreme influence in world's consumer market and are now almost a part of our daily food habit [1]. The main active ingredients in these drinks are carbonated water, high sugar or fructose corn syrup, phosphoric acid, caramel colour, natural flavour and caffeine [2]. Excess sugar consumption increases production of destructive free radicals and lower level of key antioxidants as reported by [3]. Type II diabetes resulting from high sugar consumption is also associated with an increase in free radical generation, leading to damage of proteins and DNA [4]. High fructose feeding in rats showed increased level of oxidative stress markers and increased reactive oxygen species in circulatory leukocytes [5].

This has been reported earlier that carbonated soft drink (obtained from Indian market) causes oxidative damage of liver, kidney and serum proteins with apoptosis of these tissues in guinea pigs [6]. Protein carbonyl and bityrosine formation and tryptophan loss took place under such condition indicating protein oxidation compared to control when using Bovine Serum Albumin (BSA) was incubated with carbonated soft drink [6].

These damages could be prevented by supplementation of Vitamin C to carbonated soft drink fed guinea pigs [6]. The present study was aimed at whether vitamin C can inhibit protein oxidation *in vitro* caused by carbonated soft drink when added in both dose and time dependent manner to pure protein bovine serum albumin (BSA).

In order to check whether the observed oxidative damage induced by carbonated soft drink in animal tissues in our studies is caused directly by oxidant(s) furnished by the carbonated soft drinks or indirectly, through generation of secondary cellular effects *in vivo,* we initially treated a pure protein i.e. bovine serum albumin or BSA directly with carbonated soft drinks and checked for any possible oxidation. Our results show (Figures 1-9) that carbonated soft drinks are capable of directly oxidizing such pure proteins *in vitro* indicating the apparent presence of oxidant(s) in the carbonated soft drinks. Such oxidative modification by significant extent was interestingly prevented with vitamin C, similar to that observed in our *in vivo*


ISSN: 2395-2326

studies [6]. This indicated that carbonated soft drinks contained potent oxidant(s), which may be partly or wholly responsible for the observed carbonated soft drinks- induced oxidative damage.

## Materials and Methods

All necessary details have been described earlier [2]. All the reagents used were of analytical grade and were purchased from the Sigma Chemical Company, E.Merck Ltd and SRL (India).

### *Oxidative tests*

For determination of such protein oxidation studies were made involving

(a) Carbonyl formation S

(b) Bityrosine formation

(c) Tryphtophan loss

(a) Carbonyl assay

To solution of pure bovine serum albumin (BSA) 1mg/100µl, a micro volume of carbonated soft drink was added both on a dose dependent and time dependent manner. While in the experiments by dose dependent, to a fixed volume of BSA solution (1mg/100µl) carbonated soft drink varying from 10µl to 100µl was added. The dose was chosen on an average consumption of 1 liter of carbonated soft drink by an adult individual of 60 kg body weight and converting the dose for a guinea pig of 350-500 gm body weight. On the other hand in time dependant experiments, both concentration and volume of BSA and volume of carbonated soft drink were kept fixed at 1 mg/100µl and 50µl respectively and the experiment time after addition of carbonated soft drink to pure protein were varied from 10-160 minutes under incubation (Figures 1,2).

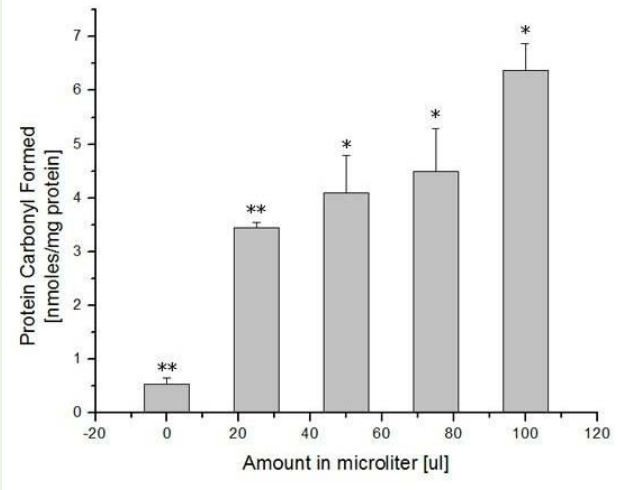


**Figure 1:** Dose Dependent *in vitro* assay of Protein Carbonyl Formation by carbonated soft drinks using Bovine Serum Albumin (BSA). Bovine serum albumin (BSA) was exposed to carbonated soft drink in a dose-dependent manner and possible protein carbonyl formation was assessed by standard protocols described under 'Materials and Methods'. represents p<0.001 and represents <0.01.

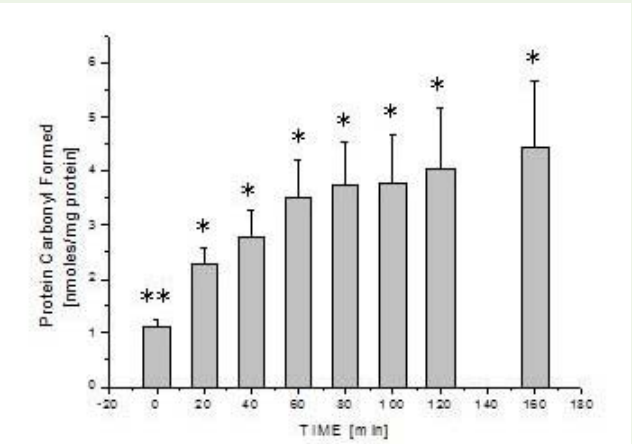


**Figure 2:** Time Dependent *in vitro* assay of Protein Carbonyl Formation by carbonated soft drink using Bovine Serum Albumin (BSA). Bovine serum albumin (BSA) was exposed to carbonated soft drink in a time-dependent manner and possible protein carbonyl formation was assessed by standard protocols described under 'Materials and Methods'. represents p<0.001and represents p<0.01.

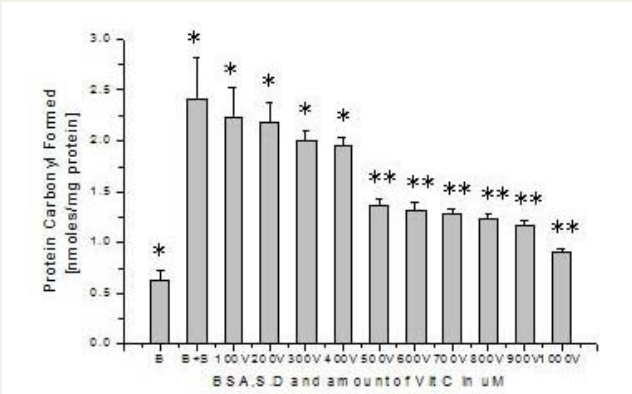


**Figure 3:** *In vitro* assay of Protein Carbonyl Formation by carbonated soft drink using Bovine Serum Albumin (BSA) and concentration dependent protection by vitamin C. To a fixed amount of BSA (1mg) was added varying amount of vitamin C (100µM-1000µM), following which a fixed amount of carbonated soft drink was added and incubated. Conditions of incubation and estimation of carbonyl groups are described under 'Materials and Methods'. represents p<0.001and represents p<0.01.

Formation of carbonyl was measured at 390 nm spectrometrically.

Again in *in vitro* studies, to a fixed volume and concentration of BSA (1mg/100ml) was added varying amount of vitamin C (100µM-1000µM), in presence of fixed amount of carbonated soft drink, for a varying time between 10-160 minutes under incubation in order to study the effect (Figure 3).

(b) Bityrosine formaion

Experiments have been carried out *in vitro* both on dose dependant and on time dependent manner to find out such damage due to carbonated soft drink supplementation. Experiments were


**Citation:** Banerjee P, Mukhopadhyay A. *In vitro* Oxidative Damage of Protein by Carbonated Soft Drink and its Restoration by Vitamin C. Indian J Nutri. 2016;3(1): 130.

done by selection of varying dose of carbonated soft drink between 0-100µl and by varying time between 1 to 140 hours as presented in the following figures (Figures 4,5).

Again, experiments were carried with vitamin C supplementation along with carbonated soft drink, in doses varying from100µl to 1ml as presented in the figure (Figure 6).

(c) Typtophan Loss

Both dose and time dependant studies, on varying amount of carbonated soft drink between 0-100 µl and 0-160 minutes was done alone with vitamin C supplementation in a dose level between 800-1000µm and the effect was observed (Figures 7,8 and 9).

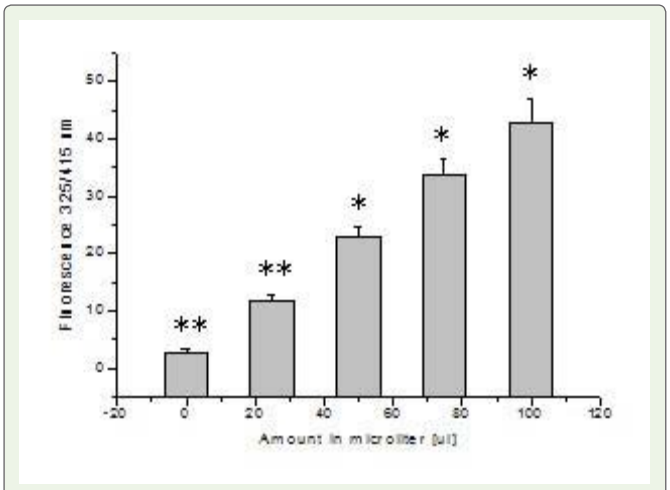


**Figure 4:** Dose Dependent *in vitro* assay of Protein Bityrosine Formation by carbonated soft drinks using Bovine Serum Albumin (BSA). Bovine serum albumin (BSA) was exposed to carbonated soft drink in a dose-dependent manner and possible protein bityrosine formation was assessed by standard protocols described under 'Materials and Methods'. represents p<0.001and represents p<0.01.

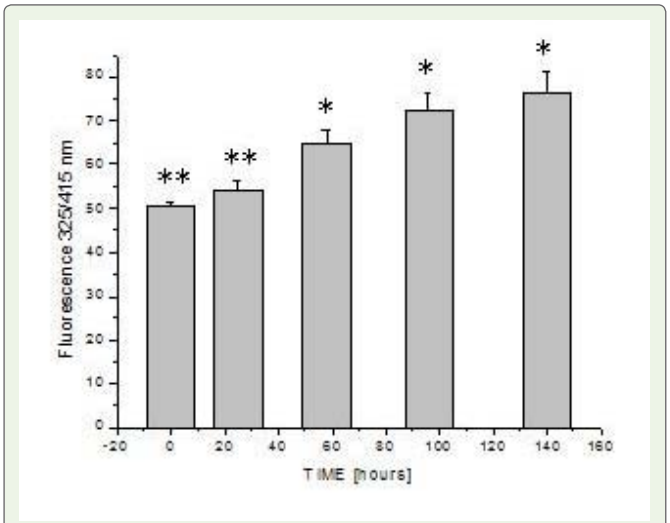


**Figure 5:** Time Dependent *in vitro* assay of Protein Bityrosine Formation by carbonated soft drink using Bovine Serum Albumin (BSA). Bovine serum albumin (BSA) was exposed to carbonated soft drink in a time-dependent manner and possible protein bityrosine formation was assessed by standard protocols described under 'Materials and Methods'. represents p<0.001 and represents p<0.01.

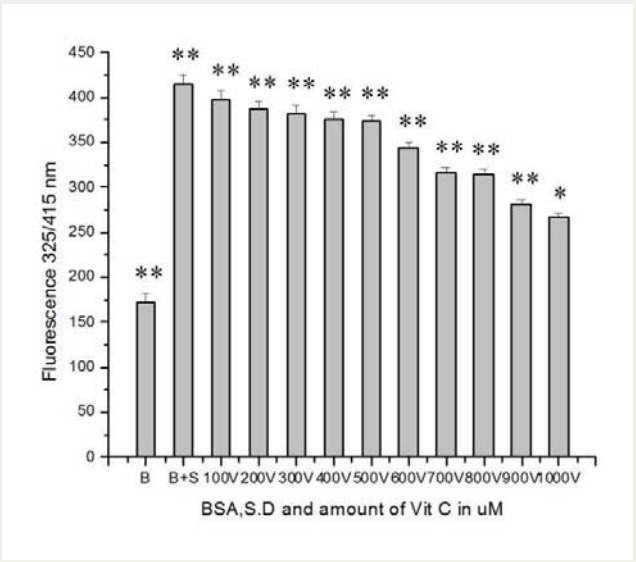


**Figure 6:** *In vitro* assay of Protein Bityrosine Formation by carbonated soft drink using Bovine Serum Albumin (BSA) and concentration dependent protection by vitamin C. A fixed volume and concentration of BSA (1mg/100ml) was added to varying amount of vitamin C (100 µM-1000µM), with addition of fixed amount of carbonated soft drink and it has been observed that addition of vitamin C could almost substantially neutralized bityrosine formation. Conditions of incubation and estimation of bityrosine groups are described under 'Materials and Methods'. Here soft drink is represented by 'S' and vit C as 'V'. represents p<0.001and represents p<0.01.

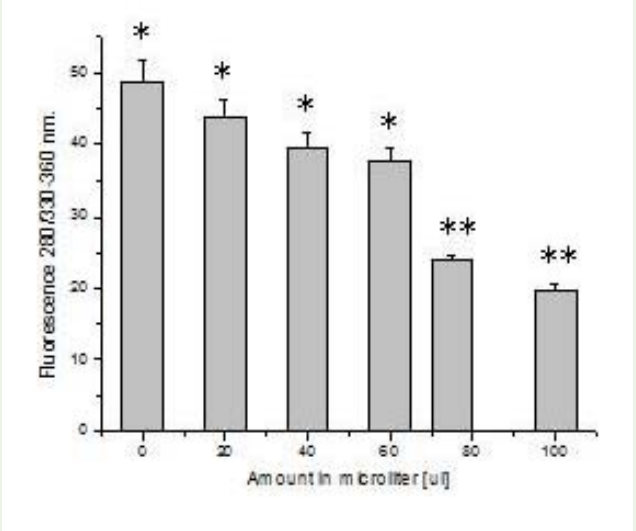


**Figure 7:** Dose Dependent *in vitro* assay of Protein Tryptophan Loss by carbonated soft drink using Bovine Serum Albumin (BSA). Bovine serum albumin (BSA) was exposed to carbonated soft drink in a dose-dependent manner and possible protein tryptophan loss was assessed by standard protocols described under 'Materials and Methods'. represents p<0.001 and represents p<0.01.



**Citation:** Banerjee P, Mukhopadhyay A. *In vitro* Oxidative Damage of Protein by Carbonated Soft Drink and its Restoration by Vitamin C. Indian J Nutri. 2016;3(1): 130.

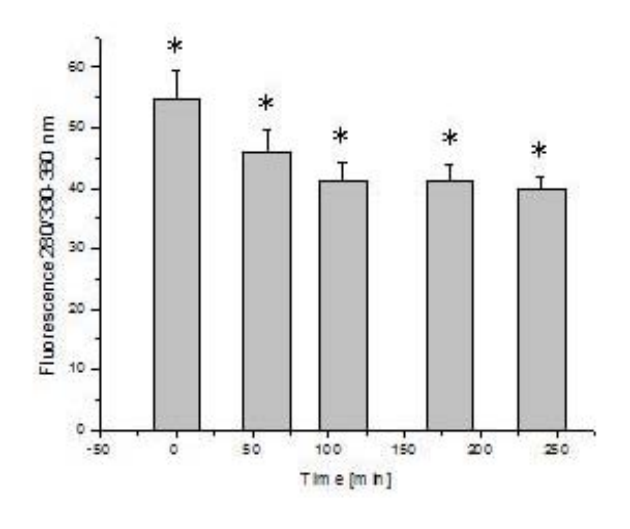


**Figure 8:** Time Dependent *in vitro* assay of Protein Tryptophan Loss by carbonated soft drink using Bovine Serum Albumin (BSA). Bovine serum albumin (BSA) was exposed to carbonated soft drink in a time-dependent manner and possible protein tryptophan loss was assessed by standard protocols described under 'Materials and Methods'. represents p<0.001.

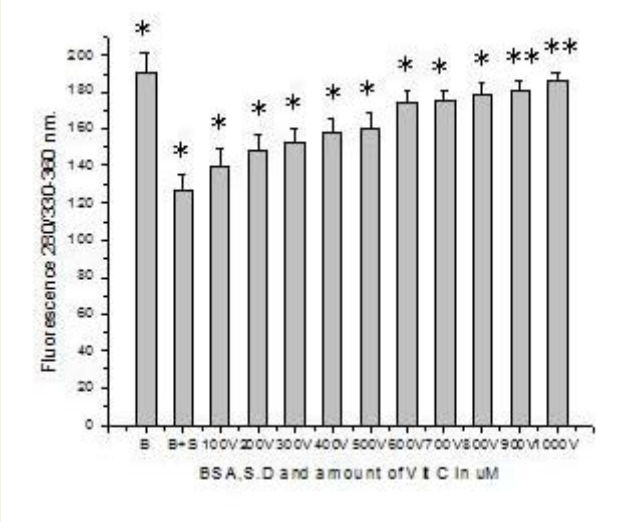


**Figure 9:** *In vitro* assay of Protein Tryptophan Loss by carbonated soft drink using Bovine Serum Albumin (BSA) and concentration dependent protection by ascorbate (AH2). A fixed volume and concentration of BSA (1mg/100ml) was added to varying amount of vitamin C (100 μM-1000 μM), with addition of fixed amount of carbonated soft drink and it has been observed that addition of vitamin C could almost substantially neutralized tryptophan loss. Conditions of incubation and estimation of tryptophan groups are described under 'Materials and Methods'. Here soft drink is represented by 'S' and vit C as 'V'. represents p<0.001and represents p<0.01.

### Statistical analysis

All values expressed as mean ± standard deviation (SD) of four different sets of experiments. Data analyse using Origin 61 data analysis and graphic software. All statements were significant based on probability ≤ 0.05.

## Results

For determination of protein oxidation *in vitro*, studies were made involving assay of:

(a) Carbonyl formation (b) Bityrosine formation (c) Tryphtophan loss

(a) Carbonyl Assay

It was observed that in *in vitro* experiment, carbonyl formation occurs immediately on addition of carbonated soft drink to pure BSA solution, indicating the fast onset of protein oxidation and it increases with increase in the dose of soft drink. Such oxidation was also observed to take place in a time-dependent manner (Figures 1,2).

Figure 3 shows the effect of different concentrations of vitamin C (100μM-1000μM) on the carbonated soft drink-induced oxidation of 1 mg BSA. 500 μM vitamin C and higher doses could substantially prevent such carbonyl formation. Similarly addition of vitamin C also reduces carbonated soft drink induced carbonyl formation in BSA in a time-dependent manner (Figure 2).

(b) Bityrosine formaion

Experiments have been carried out *in vitro* both in a dose-dependent and time-dependent manner. Both experiments show increasing level of bityrosine formation using varying doses (0-100 μl) of the carbonated soft drink as well as by varying time (1 to 140 hrs) of incubation with a fixed amount of the drink (Figures 4 ,5). Such carbonated soft drink-induced oxidative modification could be prevented significantly by vitamin C when supplemented along with the carbonated soft drink (Figure 6).

(c) Typtophan Loss

Both dose dependent and time dependent studies, using varying amounts of carbonated soft drink (0-100μl) and time of incubation (0-160 minutes) have resulted in significant tryptophan loss, which was prevented by vitamin C supplementation and almost prevented by using a dose of 800 μM or higher dose of vitamin C (Figures. 7, 8 and 9).

## Discussion

This paper proves that though carbonated soft drink have strong oxidant in it as causing direct oxidation in BSA in both dose and time dependent manner, vitamin C however cannot work markedly in *in vitro* system as it does in *in vivo* system, may be because of some physiological conditions which are required for vitamin C to act, obtained in *in vivo* system.

Here we see that carbonated soft drink can furnish potent oxidant(s) those are capable of directly oxidizing proteins even without the intervention of secondary cellular metabolite. This paves for future identification of harmful agents in this otherwise dispensable yet popular drink.

Carbonated soft drink contains oxidants which directly causes oxidation of protein *in vitro* as have been shown in the studies here. But vitamin C can protect such oxidation as have been found in these studies.


**Citation:** Banerjee P, Mukhopadhyay A. *In vitro* Oxidative Damage of Protein by Carbonated Soft Drink and its Restoration by Vitamin C. Indian J Nutri. 2016;3(1): 130.

Variety of population with different food habits and genetic variations in India consume carbonated soft drink. Many people may not have proper nutritional diets, which are more prone in rural areas, but are fond of carbonated soft drink particularly due to hot and humid weather in most calendar months. Such consumption of carbonated soft drink may oxidise body proteins but adequate intake of vitamin C may protect such oxidation.

## References

1. Teerasong S, Chan-Eam S, Sereenonchai K, Amornthammarong N, Ratanawimarnwong N (2010) A reagent-free SIA module for monitoring of sugar, color and dissolved CO2 content in soft drinks. Analytica Chimica Acta. 668: 47-53.
2. Encyclopaedia Britannica (2012) Soft drink. Encyclopaedia Britannica Online. December 2012.
3. Mohanty P, Hamouda W, Aljada R, Garg A, Ghanim A, et al. (2000) Glucose challenge stimulates reactive oxygen species (ROS) generation by leucocytes. J Clin Endocrinol Metab 85: 2970-2973.
4. Tappy L, Le KA (2010) Metabolic effects of fructose and the worldwide increase in obesity. Physiol Rev 90: 23-46.
5. Al-Awwadi NA, Araiz C, Bornet A, Delbosc S, Cristol JP, et al. (2005) Extracts enriched in different polyphenolic families normalize increased cardiac NADPH oxidase expression while having differential effects on insulin resistance, hypertension and cardiac hypertrophy in high-fructose-fed rats. J Agric Food Chem 53: 151-157.
6. Banerjee P, Panda K, Nandi P, Banerjee SK (2013) Oxidative damage of liver, kidney and serum proteins with apoptosis of above tissues in guinea pigs fed on carbonated soft drink. Asian Journal of Biochemistry 8: 1-13.



**Citation:** Banerjee P, Mukhopadhyay A. *In vitro* Oxidative Damage of Protein by Carbonated Soft Drink and its Restoration by Vitamin C. Indian J Nutri. 2016;3(1): 130.