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Inhibition of Induced DNA Oxidative Damage by Beers: Correlation with the Content of Polyphenols and Melanoidins

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Beers are a source of dietary flavonoids; however, there exist differences in composition, alcohol concentration, and beneficial activities. To characterize these differences, three kinds of lager beer of habitual consumption in Spain, dark, blond, and alcohol-free, were assayed for total phenolic content, antioxidant activity, superoxide and hydroxyl radical scavenging activities, and in vitro inhibitory effect on DNA oxidative damage. Furthermore, their melanoidin content and correlation with antioxidant activity were evaluated. Dark beer contained the highest total phenolic (489 ± 52 mg/L) and melanoidin (1.49 ± 0.02 g/L) contents with a 2-fold difference observed when compared to the alcohol-free beer. For the three kinds of beer, the antioxidant activity measured as *N,N*-dimethyl-*p*-phenylenediamine dihydrochloride concentration was strongly correlated with the total polyphenol content ($R^2 = 0.91102$, $p < 0.005$) and with the melanoidin content ($R^2 = 0.7999$, $p < 0.05$). The results support a positive effect of beers on the protection of DNA oxidative damage, by decreasing the deoxyribose degradation, DNA scission (measured by electrophoresis), and inhibition of 8-hydroxydeoxyguanosine (8-OH-dG) formation. Furthermore, a correlation between the total melanoidin content ($R^2 = 0.7309$, $p < 0.01$) and inhibition of 8-OH-dG was observed.

KEYWORDS: Beer; antioxidant; phenolics; melanoidins, deoxyribose, 8-hydroxydeoxyguanosine (8-OHdG)

INTRODUCTION

Reactive oxygen species can attack some cellular structure and molecules such as lipid, protein, or DNA. DNA oxidative damage can lead to base mutation, single- and double-strand breaks, DNA cross-linking, and chromosomal breakage and rearrangement (*1*, *2*). In humans, this damage is associated with the aging process, and with chronic diseases including cancer and coronary heart disease (*3*). Nutrition strategies to improve the in vivo antioxidant status may be effective in lowering the risk of the toxic effect of reactive oxygen species (*4*, *5*). Nutritional antioxidants are usually found in vegetables, fruits, and beverages (*6*, *7*), and they can be investigated as pure compounds; however, it is more relevant to test them in the complex mixtures in which they occur in real foods.

Beer is a low-alcohol beverage brewed from natural ingredients which may be involved in the prevention of cardiovascular and carcinogenesis diseases (*8*−*11*). One mechanism through which it may contribute to positive health effects is related to its antioxidant properties (*12*−*16*). In a human study, an increase in plasma antioxidant capacity has been reported to occur following the consumption of certain types of beer, and this increase can be associated with changes in the levels of plasma flavonoids (*14*). Other studies showed beer to have antimutagenic and antitumor promotion effects (*15*, *16*) and the capacity to inhibit induced colonic carcinogenesis in rats (*17*, *18*). Among the compounds of beer that are involved in the health benefit effects are the melanoidins and polyphenols, which are the most significant sources of natural antioxidants in beer (*19*). The content of melanoidins and polyphenols in beer is largely influenced by the genetic factors of its raw materials and therefore by the environmental conditions in which they grow, and it is also influenced by technological brewing factors (*20*, *21*).

It is well-known that the polyphenols, especially flavonoids, are very effective scavengers of free radicals (*22*−*24*). These compounds are excellent candidates to explain the health benefits of diets rich in fruits, vegetables, and beverages such as wine or beer (*25*−*27*), and the polyphenol antioxidant activity depends on structural features, such as the number and positions of the hydroxyl moieties on the ring systems (*28*). Beer is supposed to be a good source of polyphenols because significant amounts of them are present in barley, malt, and hops. Common beer phenols are flavonols, isoflavonoids, phenolic acids, catechins, procyanidins, tannins, and chalcones (*29*−*32*).

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On the other hand, different Maillard reaction products are largely formed during the malting and brewing process. Among them, the melanoidins have an important role on different levels. They have an influence on color, aroma, and flavor needed for making some special styles of beers. They also contribute to the stabilization of the foam and to the antioxidant capacity of the beers (*19*, *33*–*36*). Melanoidins are colored compounds that are provided mainly from the malt, although they are also formed during mashing and wort boiling. The main difference between dark and blond beers is that they are elaborated from malt with diverse grades of toasting (*37*). Several studies suggest that melanoidins are responsible for the strong antioxidant properties and metal chelating ability of some foods (*38*) and may improve the defense system of organisms demonstrated in several in vitro and ex vivo models (*39*, *40*). This fact suggests that these compounds, together with polyphenols, could be responsible for the antioxidative activity of the beers. However, it is important to keep in mind that, under certain circumstances, the phenolic compounds and melanoidins have exhibited a double effect, showing both antioxidant and pro-oxidant properties (*41*, *42*).

The aim of this work was to study the antioxidant capacity of Spanish beers, especially their protective effect on induced DNA oxidative damage. Three kinds of beers were studied, the most consumed in Spain, blond, dark, and alcohol-free, all of them lager beers.

Levels of different phenolic compounds and total melanoidins and antioxidant and scavenger activity were evaluated and correlated with the protective effect against DNA oxidative damage induced by the system copper(II)–ascorbic acid. So the degradation of deoxyribose test, DNA scission (measured by electrophoresis), and 8-hydroxydeoxyguanosine (8-OH-dG) levels, used as a biomarker of DNA oxidation, implicated in carcinogenesis and associated with chronic infections and inflammation, were evaluated.

MATERIALS AND METHODS

Chemicals and Reagents. Calf thymus DNA, 2-deoxyguanosine (dG), 8-OH-dG, L-ascorbic acid, gallic acid, and catechin 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were purchased from Sigma Chemical Co. (St Louis, MO). *N*,*N*-Dimethyl-*p*-phenylenediamine dihydrochloride (DMPD) was from Fluka (Seelze, Germany). Agarose was from Bio-Rad (Richmond, CA), Tris, NaOH, phenazin methosulfate β-nicotinamide adenine dinucleotide (NADH), nitroblue tetrazolium (NBT), 1-butanol, ethanol, trichloroacetic (TCA), $CuSO_4$, and thiobarbituric acid (TBA) were obtained from Merck (Darmstadt, Germany), and Folin–Ciocalteu reagent was from Panreac (Barcelona, Spain).

Preparation of Beer Samples. Three kinds of beers were studied, all of them lager beers. The beers were divided according to commercial and technical characteristics into three groups: blond (clear beer with a normal content of alcohol), dark (high-roasting beer), and alcohol-free (blond beer but with alcohol levels lower than 0.5% Alc). Beers were transported to the laboratory and analyzed as soon as possible. Bottles, from four different lots or brewing processes, of each beer were analyzed. The analyses were carried out immediately upon opening the bottles to prevent loss of phenols by oxidation. Beer samples were used after 10-fold concentration under vacuum at *T*×bb < 35 °C.

Measurement of Phenolic Compound Contents. Three different families of phenolic compounds were measured: the total polyphenol (TPP) content was evaluated by the Folin–Ciocalteus reaction, and quantified as a GAE (gallic acid equivalent expressed in milligrams per liter) (*43*), the procyanidin (PRO) content was measured after their transformation by acid digestion, and quantified as an equivalent of cyanidin chloride expressed in milligrams per liter, and the catechin (CAT) content was measured by vanillin reaction, and quantified as an equivalent of D-catechin expressed in milligrams per liter (*44*).

Measurement of Melanoidin Content. Isolation of melanoidins was carried out according to the Cost Action 919 protocol (*45*). Beers were dialyzed using cellulose dialysis tubing (MW 5000). A known volume of beer was put into the dialysis tubing, which was placed in a glass vessel with 1 L of water. This system was maintained at 4 °C with stirring for 24 h. This procedure was repeated two times. This solution was freeze-dried and then used for the estimation of the melanoidin content of different beers. The total melanoidin levels were determined by weighing the yellow-brown powders obtained.

Determination of Total Antioxidative Capacity. The antioxidant activity was evaluated using DMPD, the radical cation of which gives a stable color solution, the inhibition of color formation observed spectrophotometrically being directly correlated with the antioxidant activity of the added product. The antioxidant ability of beer samples was expressed as TEAC (Trolox equivalent antioxidant capacity) in milligrams per liter using the calibration curve plotted with different amounts of Trolox (*46*).

Superoxide Radical Assay. Superoxide radicals are generated in a phenazin methosulfate β-nicotinamide adenine dinucleotide system by oxidation of NADH and assayed by the reduction of NBT. In this experiment, the superoxide radicals were generated in 3 mL of Tris–HCl buffer (16 mM, pH 8.0) which contained 78 μM NADH, 50 μM NBT, and 10 μM PMS, and the beers were tested at different volumes (0.5, 1, 5, and 10 μL). The color reaction between superoxide radicals and NBT was detected at OD = 560 nm (*47*).

Deoxyribose Test. The samples were incubated at 37 °C for 1 h in 50 mM phosphate buffer, pH 7.4, with deoxyribose (10 mM), ascorbic acid (1 mM), and copper(II) ions (100 μM), which were used to initiate the reaction. A 1 mL sample of the incubation mixture was mixed with 0.5 mL of 28% (w/v) trichloroacetic acid and 0.5 mL of TBA reagent (1% w/v, in 0.05 M NaOH), followed by heating at 100 °C for 15 min and subsequent cooling to room temperature. Degradation of deoxyribose in vitro produces malondialdehyde, which can be evaluated by reaction with TBA. The release of the thiobarbituric acid reactive products was measured by spectrophotometry at 532 nm against appropriate blanks following the methodology previously described (*48*).

Incubation of Calf Thymus DNA. The reaction mixture contained calf thymus DNA (200 μg), ascorbic acid (10 mM), Cu(II) (100 μM), and beer samples to be tested at different volumes (10, 50, and 100 μL). The mixture was incubated in a shaking water bath at 37 °C for 1 h.

Electrophoresis of DNA. Electrophoresis was performed after incubation of DNA samples in 0.7% agarose gels prepared in 100 mM sodium phosphate buffer, pH 7.8. A 50 μL sample of each DNA test solution and molecular weight markers were premixed with 10 μL of a buffered solution containing glycerol (20%) and 0.1% bromophenol blue as loading buffer. The resulting mixtures were run in the above buffer at 400 mA and stained in a solution of ethidium bromide (1 μL/mL running buffer). The DNA bands were viewed under UV light and photographed with a digital camera.

Enzymatic DNA Hydrolysis and 8-OH-dG Determination. DNA digestion was performed as previously described (*49*) with slight modifications (*50*). In brief, 200 μg/mL DNA after the pH was lowered to 5.1 with sodium acetate (0.54 M) was mixed with 5 units of nuclease P1. The mixtures were incubated for 1 h. The pH was then readjusted with 0.4 M Tris–HCl (pH 7.8) followed by the addition of 10 μL of alkaline phosphate (3 units), and the samples were incubated for 30 min. The DNA hydrolysates were dissolved in HPLC-grade water and filtered through a 0.22 μm syringe filter before their analysis by HPLC. The amount of 8-OH-dG in the DNA digest was measured by electrochemical detection. Elution conditions were those described by Ritcher et al. (*51*) using filtered and vacuum-degassed 50 mM phosphate buffer solution, pH 5.5, containing 10% acetonitrite and a Waters ODS HPLC column (25 cm × 0.46 cm i.d., 5 μm particle size). The amount of dG was quantified by a UV absorbance detector placed on line between the HPLC columns and the electrochemical detector (*49*). Standard samples of dG and 8-OH-dG were analyzed, and a calibration curve was constructed to ensure their good separation and to allow identification of those derived from calf thymus DNA.

Statistical Analysis. Statistical analysis of the data was carried out using one-way analysis of variance (ANOVA) to detect the factor effect

Table 1. Antioxidant Activity ([DMPD]), [TPP], [PRO], [CAT], and Melanoidin Content in the Three Groups of Studied Beers[a]

	[DMPD] (TEAC, mg/L)	[TPP] (GAE, mg/L)	[PRO] (mg/L Cyn)	[CAT] (mg/L Cat)	melanoidin content (g/L)
dark	99 ± 9^c	489 ± 52^c	168 ± 10^b	60 ± 5^b	1.49 ± 0.02^b
blond	71 ± 4^b	380 ± 6^b	138 ± 2^a	26 ± 2^a	0.61 ± 0.04^a
alcohol-free	36 ± 12^a	230 ± 37^a	127 ± 0.9^a	22 ± 2^a	0.58 ± 0.01^a

[a] Results are the mean ± SD of six experiments. Values with different superscript alphabetical letters are significantly different ($p < 0.05$).

of the “kind of beer”. Furthermore, the least significant difference (LSD) test was applied to determine the statistical difference value. A minimum significance level of 95% ($p < 0.05$) was considered. Linear regression was used to study the possible correlations between studied parameters. Statistical analyses were performed using the Statgraphics Plus 4.0 statistical package for Windows (*Statgraphics Plus for Windows 4.0. User's guide*; Manugistics, Inc.: Rockville, MD, 1999).

RESULTS

Table 1 shows the average values, by beer groups, of the results obtained in the quantitative study of the different parameters evaluated in the study: antioxidant activity and total polyphenol, procyanidin, catechin, and melanoidin contents. The ANOVA showed the factor effect of the “kind of beer” for all the parameters studied. Results of the LSD test noted that dark beers were the richest in phenol (GAE, mg/L) and melanoidin (g/L) levels. The content of melanoidins, expressed as milligrams per gram of dry weight of beer, were also highest in dark beer with values of 25.5 ± 2.9, with respect to 10.4 ± 0.67 in blond beer and 10.5 ± 0.08 for alcohol-free beer.

The total polyphenol content was statistically different ($p < 0.05$) among all the kinds of beers. Higher levels of procyanidins, catechins, and melanoidins were observed in the dark beer, while the blond and alcohol-free samples showed no significant difference ($p < 0.05$) between them. In relation to antioxidant activity measured by [DMPD], a statistically significant difference ($p < 0.05$) was observed among the beers studied.

A positive correlation between antioxidant activity and total polyphenol ($R^2 = 0.9110$, $p < 0.005$), procyanidin ($R^2 = 0.8533$, $p < 0.005$), and catechin ($R^2 = 0.8333$, $p < 0.005$) levels was observed. Similarly, a weak correlation existed between the antioxidant activity and melanoidin content ($R^2 = 0.7999$, $p < 0.05$).

Superoxide radical scavenging activity was measured at different beer concentrations. The scavenging activity was concentration dependent (**Figure 1**), and the superoxide radical inhibition increased between 65% and 75% when the beer concentration was increased 10-fold. No statistical significant differences were observed among beers. The superoxide radical scavenging activity of these products was correlated neither with their phenolic families studied nor with the total melanoidin content.

The results of the deoxyribose degradation, which is used as a measure of hydroxyl radical scavenger capacities, showed a positive effect of beers (**Figure 2**). Beer samples decreased significantly the deoxyribose degradation and a concentration-dependent effect was detected. For a volume of 10 μL inhibition percentages of 83%, 66%, and 62% for dark, blond, and alcohol-free beer, respectively, were shown, while 50 μL of beer showed an inhibition percentage higher than 90% for all of them. No pro-oxidant activity was observed at high concentrations (100 μL) of beer. The values of the inhibition of deoxyribose degradation found were well correlated with the total polyphenol content of the beers ($R^2 = 0.8267$, $p < 0.001$) and total melanoidin levels ($R^2 = 0.7609$, $p < 0.001$), showing the dark beers had a higher protective effect at the lower studied concentration. However, no difference was observed between blond and nonalcoholic beers.

Ascorbic acid and copper also induced damage to DNA due to the fact that they produce highly reactive oxygen species such •OH, responsible for the degradation of DNA. This mechanism has been proposed as a simple model of DNA scission produced in vivo by several substances that require metal ion for their action. **Figure 3** shows the concentration course experiment of the effect of the three different beer groups on DNA visualized by the electrophoretic separation of its damaged fragments. The obtained results indicated that the protective effect took place at 50 μL of volume, showing a maximal effect at 100 μL. A 10 μL volume of beer was not capable of protecting the DNA, which was degraded, and the fragments were clearly separated and were similar in size to those obtained by copper(II) and ascorbic acid.

Another method used to test the capacity of the beers to protect DNA from oxidation consisted in quantifying their capacity to inhibit the level of modified base 8-OH-dG formed during attack of the •OH on the DNA. So DNA was treated in solution with ascorbic acid and copper in the absence and

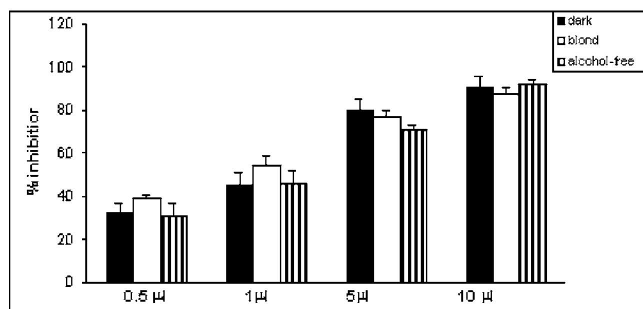

Figure 1. Influence of beers (dark, blond, and alcohol-free) on reduction of NBT by $O_2^{\bullet -}$ generated by PMS in the presence of NADH. Values are expressed as the mean ± SD ($n = 4$). No statistically significant differences were observed among the beers.

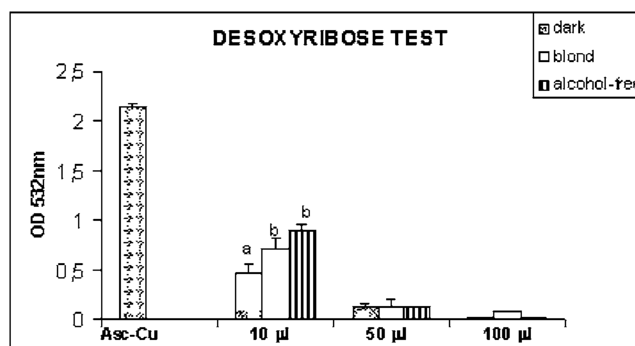

Figure 2. Degradation of deoxyribose induced by ascorbic acid–Cu(II) ions and the effect of beers. Results are the mean ± SD of four experiments. Values with different alphabetical letters are significantly different ($p < 0.05$).

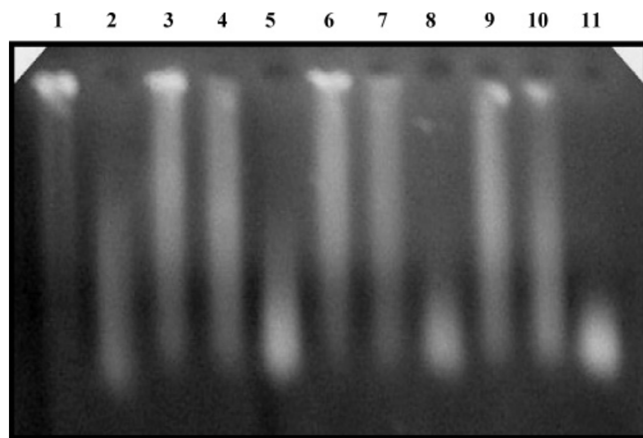

Figure 3. Agarose gel electrophoresis separation of damaged DNA induced by Cu(II)–ascorbic acid and the effect of beers: DNA alone (1); DNA plus Cu(II)–ascorbic acid (2); DNA plus Cu(II)–ascorbic acid plus 100 μL (3), plus 50 μL (4), and plus 10 μL (5) of blond beer; DNA plus Cu(II)–ascorbic acid plus 100 μL (6), plus 50 μL (7), and plus 10 μL (8) of dark beer; DNA plus Cu(II)–ascorbic acid plus 100 μL (9), plus 50 μL (10), and plus 10 μL (11) of alcohol-free beer.

Table 2. Effect of Beers on the Formation of 8-OH-dG/10^5dG in Calf Thymus DNA Induced by Ascorbic Acid and Copper(II)[a]

	8OH-dG/10^5 dG	inhibition (%)
DNA alone	nd	
DNA + dark beer	nd	
DNA + blond beer	nd	
DNA + alcohol-free beer	nd	
DNA + Cu–Asc	59.9 ± 1.8	
DNA + Cu–Asc + dark beer	15.2 ± 1.7[b]	74.5 ± 2.9[b]
DNA + Cu–Asc + blond beer	29.3 ± 6.9[b]	51.1 ± 11.6[a]
DNA + Cu–Asc + alcohol-free beer	22.7 ± 2.3[b]	62.0 ± 3.8[a]

[a] Results are the mean ± SD of four experiments. All the reactions contained 200 μg of calf thymus DNA. Other experimental conditions were as described in the Materials and Methods. Values of percent inhibition with different superscript nonitalic alphabetical letters are significantly different ($p < 0.05$). nd = not detected. [a] $p < 0.001$ for the effect of antioxidants compared with that of the DNA treated with ascorbic acid and copper.

presence of 100 μL of beer (**Table 2**). 8-OH-dG was not detected after incubation of DNA alone or in the presence of 100 μL of beer, so the beers did not induce any DNA damage. The Cu(II)−ascorbic acid system produced an important generation of free radical that induced a significant increase in 8-OH-dG/10^5dG levels in coincubated DNA. The presence of beers in the coincubated DNA system significantly inhibited oxidative DNA damage induced by copper(II)−ascorbic acid, and the stronger inhibition effect was shown by the dark beer. Additionally, a negative correlation between 8-OH-dG levels and total melanoidin contents ($R^2 = -0.7309$, $p < 0.01$) was observed. However, no significant correlations between 8-OH-dG and antioxidant activity and total polyphenol content were found.

DISCUSSION

In the past few years a major emphasis has been made on the positive influence of nutritional antioxidants on health (*5*). The content of compounds with antioxidant capacity in foods or beverages is one of the major factors in the determination of their antioxidant activity and their protective effect against oxidative stress in vitro and in biological systems (*12*, *52*).

In this paper, an in vitro study on the antioxidant activity and protective effect against DNA oxidative damage of three kinds of beers, dark, blond, and alcohol-free, is described. The correlation between these parameters and the content of different families of phenolic compounds and the total levels of melanoidins in the beers was studied.

It is well-known that polyphenols and melanoidins of beers are involved in their antioxidant activity (*19*, *42*, *53*, *54*). The concentration of polyphenols and melanoidins in the beers could be influenced by several conditions, which include the raw material and brewing process, which will also determine the characteristics of the different varieties of beer (dark, blond, alcohol-free) (*29*, *31*).

As was expected, the dark beers were the richest in phenolic compounds and melanoidins. On the other hand, the three kinds of beers showed a significant antioxidant activity measured by the DMPD method. This method is an index of the hydrogen-donating ability and indicates that a compound may act as a primary antioxidant protecting against the oxidation of molecules. Thus, the beer compounds can act as chain-breaking antioxidants in retardation of lipid peroxidation, because they are able to break the chain reaction as a result of their hydrogen-donating property to lipid hydroperoxides or other free radicals. The correlation study showed that the hydrogen-donating ability of beers is dependent on the total polyphenol levels but not on the total melanoidin contents. The relation between antioxidant capacity and total polyphenol contents has been previously reported by other authors (*36*, *54*).

In regard to the absence of correlation between DMPD and total melanoidin levels, it could be assumed that, in this case, the antioxidative activity of these compounds is based on other antioxidant abilities, such as reducing power or chelating ability. Several studies suggest that melanoidins are responsible for the strong antioxidant properties and metal chelating ability shown by different foods and beverages (*38*, *55*, *56*).

Superoxide or hydroxyl radical participates in the chain reaction of free radicals; for this, the study of the ability of a food or beverage to scavenger these radicals may be relevant in the evaluation of their antioxidant capacity. In relation to the capacity to scavenger the superoxide radical, no difference was observed among the three kinds of beers. However, we observed that the hydroxyl radical scavenger activity, measured as the inhibition of oxidation deoxyribose (*48*), was well correlated with the total polyphenol contents and with the total melanoidin levels. According to these results, beers could act as good scavengers of •OH radical.

Major attention has recently been placed on the delineation of the mechanisms that prevent oxidative damage to the base of DNA. The suggestion that beer plays a protective effect against DNA damage under the reported experimental conditions is consistent with a number of previous observations in which its antioxidant and free radical scavenger activity has been shown (*14*, *20*, *57*). However, a relationship between the levels of beer total polyphenols and total melanoidins and the protective effect against DNA oxidative damage is still under consideration.

Numerous studies have shown the usefulness of using DNA base scission as an effective in vitro method for evaluating antioxidant activity against active oxygen species (*58*). It is known that hydroxyl radical reacts with DNA and produces DNA strand breaks that can be observed by electrophoresis. The different beers were able to protect DNA from damage in a manner similar to that of other hydroxyl radical scavengers such as thiourea, GSH, etc. In fact, 100 μL of the three types of beers tested clearly preserves the normal electrophoretic pattern of DNA. The inhibition of DNA damage achieved by

beers is a consequence of their potential free radical scavenging properties or quelant activity by removing free copper(II) ions from solution before they can complex with ascorbic acid. Therefore, in addition to their antioxidant capability a chelating effect by beers cannot be ruled out and could also be important in the protection achieved by them, since in the absence of copper(II) they do not damage DNA.

The copper(II)−ascorbic acid system also produces marked sequence-dependent damage of DNA with preferential oxidation of guanine bases, which indicates a localized production of reactive oxygen species as a result of metal binding to specific regions on the DNA molecule (*59*). It is known that the oxygen free radical attacks DNA, resulting in production of oxidized bases such as 8-OH-dG which contribute to mutations and tumor promotion. The obtained correlations showed that the studied beers protected the DNA from oxidation by •OH, significantly reducing the 8-OH-dG levels, and this effect is dependent on the total content of melanoidins but not on the content of polyphenols of the beers.

The difference in DNA protection exerted by the three studied beers is probably related to a distinct capacity of them to compete with DNA for trapping oxidants or to adsorb onto the DNA molecule. Dark beers were more effective in the protection of DNA damage than blond and alcohol-free ones. This difference is probably due to the higher levels of polyphenols and melanoidins in dark beers, and it is supposed that they could have acted synergistically. Harper et al. (*60*) suggested that the DNA-binding capacity of molecules with similar structure provides one mechanism by which they can protect DNA against oxidation. Furthermore, it was reported that the antioxidant activities of malts, which affect the efficacy of cancer chemoprevention, were produced at least partly by the Maillard reaction (*18*, *33*). The inhibitory effect on induced colonic carcinogenesis formation of malt and of different beers has also been reported. These authors observed that there were considerable variations among brands of beer and suggested that this variation may be due to the contents of components responsible for the preventive function. They also proposed that components in dark beer generated from the brewing process, other than those rich in dark-colored malts, are responsible for the protective effect of this beer.

The obtained results are satisfactory and positive, so actually "in vivo" analyses are being carried out. The real importance of beer in the prevention of oxidative damage to DNA occurring in vivo will soon be tested.

In conclusion, the protective effects of beers were observed using an in vitro model. A significant decrease in the deoxyribose damage and 8-OH-dG levels in DNA treated with ascorbic acid and copper may suggest that the components of beer inhibit DNA damage. Furthermore, comparison among the different beers suggests the strongest protective effect is by dark beer, in which the compounds formed during malting and brewing, such as melanoidins, are involved. Further studies are needed to clarify the components responsible and the underlying mechanisms.

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