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Original Article

Melatonin present in beer contributes to increase the levels of melatonin and antioxidant capacity of the human serum

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SUMMARY

Background & aim: Melatonin is a molecule with antioxidative properties including direct free radical scavenging and indirect stimulatory actions on a variety of antioxidative enzymes which further promote its ability to reduce the toxicity of radicals and their associated reactants. Beer is an integral element of the diet of numerous people and is rich in antioxidants. We analyzed if melatonin is present in beer and if so, at what concentration. It further determines whether the moderate consumption of beer has an effect on the total antioxidant status (TAS) of human serum.

Methods: We analyzed 18 brands of beer with different percentage of alcohol content in order to determine the concentration of melatonin. Serum samples were collected from 7 healthy volunteers. These samples were used to measure melatonin and TAS on basal conditions and after drinking beer.

Results: Showed that all the beer analyzed did indeed contain melatonin and the more they have got, the greater was its degree of alcohol. Both melatonin and TAS in human serum increased after drinking beer.

Conclusions: Melatonin present in the beer does contribute to the total antioxidative capability of human serum and moderate beer consumption can protect organism from overall oxidative stress.

1. Introduction

Beer is a beverage associated with the diet of numerous people, containing a great array of bioactive phytochemicals (polyphenols and antioxidants) and nutraceutical compounds.[1] The moderate consumption of alcohol, like wine or beer, seems to have a beneficial impact on the organism compared to alcohol abuse or abstinence.[2,3] The beneficial effects on the health may be due not only to the small amount of alcohol but also to the antioxidants (B vitamins complex, citric acid, ascorbic acid, silicic acid, etc.,)[4] and other existing components in beer (barley, hop, malt, etc.,).[4] Melatonin is a molecule with a wide range of antioxidative properties including direct free radical scavenging and indirect stimulatory actions on a variety of antioxidative enzymes (e.g., superoxide dismutase, glutathione peroxidase, glutathione reductase, etc.) which further promote its ability to reduce the toxicity of radicals and their associated reactants.[5] Furthermore, several melatonin metabolites that are generated when melatonin interacts with toxic reactants are themselves direct free radical scavengers, e.g., N1-acetyl-N2-formyl-5-methoxykynuramine (AFMK) and N1-acetyl-5-methoxykynuramine (AMK). The process by which melatonin and its metabolites successively scavenge reactive oxygen species (ROS)/reactive nitrogen species (RNS) is referred as the free radical scavenging cascade. This cascade reaction makes melatonin highly effective and explains how it differs from other conventional antioxidants.[6] As results, it has been demonstrated that melatonin in vivo and in vitro, at physiological or pharmacological concentrations, may provide protection against diseases that involve degenerative or proliferative changes by shielding macromolecules (DNA, lipids, and proteins) from free radical damage.[7] Also, melatonin has been functionally linked to the regulation of circadian and seasonal rhythms,[8] immune function,[9] lipids metabolism[10] and retinal physiology[11] among the others. The goal of present study was to analyze if the beer contained melatonin and if the moderate consumption of beer would have an influence on the total antioxidant status of the human serum.

2. Material and methods

2.1. Beer samples

Different brands of beer with varying percentages of alcohol content were selected from among the brands most highly consumed in our geographical area and they were evaluated. For each beer tested, samples were analyzed in triplicate.

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2.2. Serum samples

Serum samples were collected between 09:00 and 09:30 a.m. (basal conditions), from 7 healthy volunteers (4 men and 3 women) ranging from 20 to 30 years of age. All volunteers were informed and consented to the present study. None of the subjects were taking any medication known to influence melatonin concentration or TAS. Participants fasted overnight prior to the collection of the samples and alcoholic drinks were prohibited during the study. When blood samples were collected on basal conditions, the subjects consumed a moderate intake of Volt-Damm beer (330 ml for women and 660 ml for men) for 10 min and a new sample of blood was obtained 45 min later. Samples were frozen at −20 °C until assayed. TAS of the serum was measured within 48 h of sample collection. In addition to TAS, melatonin levels were estimated in all serum samples. A basic biochemical profile was also run to determine the general health of the individuals.

2.2.1. Alcohol and gender

Females show greater sensitivity to alcohol than males. The combination of those different behaviours related to pharmacokinetics may increase women's vulnerability to the effects of ethanol. The mechanisms that may cause these different reactions could underlie in their different physiological processing and metabolic clearance of alcohol and also in the different reactions of their nervous systems to alcohol. Some researchers[2] have suggested that such differences are mainly due to a lower alcohol-dehydrogenase activity in women. Also, alcohol intolerance is caused by rapid acetaldehyde formation in conjunction with low acetaldehyde removal (low aldehyde dehydrogenase activity). For this reason, this experimental design was carried out having in mind gender distinctions and in accordance with those researches[12] that recommend the consumption of 330 ml of beer for women and 660 ml for men.

2.2.2. Exogenous melatonin on bioavailability and ingestion

Melatonin itself has a very short average life in the blood (a range of 20–40 min, depending on the conditions), so that a high amount of melatonin in blood cannot persist there after many hours. According with the concentration–time curve of our laboratory and other researches,[13] 45 min is the necessary time for the oral melatonin to be absorbed in the gastrointestinal tract (GIT) and also to be detected in blood before its metabolism and elimination take place.

2.3. Total antioxidant status determination

Total antioxidant status (TAS) was measured using a kit purchased from Randox Laboratories. In this assay, Met-myoglobin reacts with H_2O_2 to form the radical species Ferryl-myoglobin. A chromogen (2,2′-azinodi-[ethylbenzthiazoline sulfonate]; ABTS) is incubated with the Ferryl-myoglobin to produce the radical cation species $ABTS^+$. This has a relatively stable blue–green color which is measured at 600 nm. Antioxidants in the added sample cause suppression of this color production to a degree which is proportional to their concentration. The assay is calibrated using 6-hydroxy-2, 5, 7, 8-te-tramethylchroman-2-carboxylic acid and results are expressed as mmol/l. The assay range is 0–2.5 mmol/l. Intra and inter assay precision studies showed coefficients of variation lower than 2 and 3% respectively.

2.4. Melatonin determinations

Beer and serum melatonin concentrations were measured by a competitive enzyme immunoassay [Melatonin ELISA, Immuno-Biological Laboratories (IBL) Hamburg Company]. This assay is based on the competition principle and the microtiter plate separation. An unknown amount of antigen present in the sample and a fixed amount of enzyme labelled antigen compete for the binding sites of the antibodies coated onto the wells. After incubation the wells are washed to stop the competition reaction. Having added the p-nitro-phenyl phosphate (PNPP) substrate solution the concentration of antigen is inversely proportional to the optical density measured. The measured ODs of the standards are used to construct a calibration curve against which the unknown samples are calculated. The lower limit of the assay was 2.6 pg/ml, and the intra-essay and inter-essay coefficients of variance were less than 10%. Melatonin content in beer and in the human serum was also assayed by High-Pressure Liquid Chromatography (HPLC).

2.5. Data analysis

All data are presented as the mean ± standard error (S.E.M.). All statistical procedures were performed using GraphPad InStat statistical software package program 3.0. The linear Pearson correlation analysis and Student's paired t-test were used as appropriate. A value of $p < 0.05$ was considered as statistically significant.

3. Results

Table 1 shows a total of 18 samples of beer obtained from the Spanish market and including different brands of beer with varying percentage of alcohol content. All beers analyzed had melatonin the more they have got, the greater was its alcohol degree. In this way, we analyze how the concentration of melatonin varies according to the percentage of alcohol content with a correlation coefficient $(r) = 0.8752$, a coefficient of determination $(r^2) = 0.7659$ and p value was 0.0001 considered highly significant. Also, we could differentiate two groups of beer: a) beers with alcohol content between 4% and 7% and b) alcohol free beers (0–1%). When the group of beers with alcohol content was analyzed, this showed a correlation coefficient $(r) = 0.5642$, a coefficient of determination $(r^2) = 0.3183$ and p value was 0.0446 considered significant.

Volt-Damm beer showed the greater concentration of melatonin with 169.7 pg/ml and 7.2 alcohol content and was selected for the study.

Table 1
Melatonin concentration in beer, analyzed by a competitive enzyme immunoassay (ELISA). SEM: standard error of mean.

Brands of beer	Average concentration of melatonin [pg/ml] (±SEM)	Alcoholic degrees
Volt-Damm	**169.7** (±8.7)	7.2
Murphy's	**142.7**(±3.9)	5.0
Mahou Negra	**138.6** (±2.1)	5.5
Amstel	**128.1** (±4.7)	5.0
Coronita	**127.6** 13.6)	4.6
Budweisser	**119.8** (±9.8)	5.0
Guiness	**118.1** (±0.3)	4.2
Cruzcampo	**111.9** (±9.9)	4.8
Carlsberg	**104.4** (±0.2)	5.0
Mahou 5 estrellas	**101.9** (±4.9)	5.5
Heineken	**98.1** (±2.3)	5.0
San Miguel Sp	**97.6** (±2.8)	5.4
Mahou Clásica	**84.6** (±7.2)	4.8
Laiker Sin	**68.6** (±3)	1.0
San Miguel 0.0	**61.6** (±1.7)	0.0
Buckler Sin	**55.6** (±0.1)	0.0
Kaliber Sin	**52.7** (±0.2)	1.0
Buckler 0.0	**51.8** (±2.2)	0.0

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The second time, we analyzed the concentration of melatonin in the blood before (basal conditions) and after drinking beer (post-ingestion conditions). Fig. 1 shows that both groups of samples, basal and post-ingestion conditions, had melatonin, being greater the concentrations in the group post-ingestion with differences considered significant ($p = 0.0124$). Similar results were obtained when a HPLC was used to measure melatonin (data not shown).

To determine whether the melatonin present in the beer modified the levels of antioxidant status of the blood, TAS levels before (basal conditions) and after drinking beer (post-ingestion conditions) were compared by paired *t*-test. Fig. 2 shows that both groups of samples, basal and post-ingestion conditions, had different levels of TAS, being greater in the group that drank beer, with differences considered highly significant ($p = 0.0008$).

4. Discussion

The results of the present study show that beer does contain melatonin and that the brands of beer with alcohol content are those that presented greater concentrations of melatonin and vice versa. Melatonin is a soluble molecule in alcohol, with properties of amphiphilicity. We think that in the processes of industrialization, when they extract the alcohol of the beer, they could also be dragging the melatonin. This is important to take into account because thus, the industries breweries would be able to seek new methods to extract the alcohol of the beer without extract also the melatonin, or perhaps the alcohol free beers could be enriched with the melatonin that initially they lost. On the other hand, the properties of amphiphilicity of the melatonin would explain why melatonin is still present in the de-alcoholised beer.

In quantitative terms, we do not know the possible sources of melatonin in the processes of beer fermentation, neither hop nor malt may suffice for explaining the values measured, but they may be the yeasts that are decisive. Thus, *Saccharomyces cerevisiae* showed capacity to produce high amounts of melatonin.[14,15] On the other hand, we cannot forget that melatonin can be released from the GIT to the circulation in remarkable amounts, upon chemical stimuli, in particular, tryptophan.[16] Whether or not, or to what extent, melatonin-releasing compounds are present in the beer remains to be identified. The presence of tryptamine in beer, e.g., is well-known. Since rises in melatonin after intake of nutrients rich in this indole are inconclusive, corresponding experiments with de-melatonized material are required.[17]

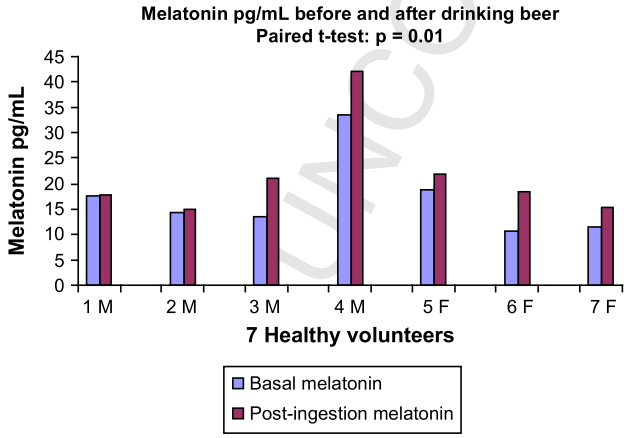

Fig. 1. Melatonin concentration in serum samples from 7 (1–4 M: male and 5–7 F: female) healthy volunteers before (basal conditions) and after drinking beer (post-ingestion), with *p* value = 0.0124 considered significant. Each column is the mean ± SEM of 3 determinations. Both groups of samples, basal and post-ingestion conditions, had melatonin, being greater the concentrations in the group post-ingestion.

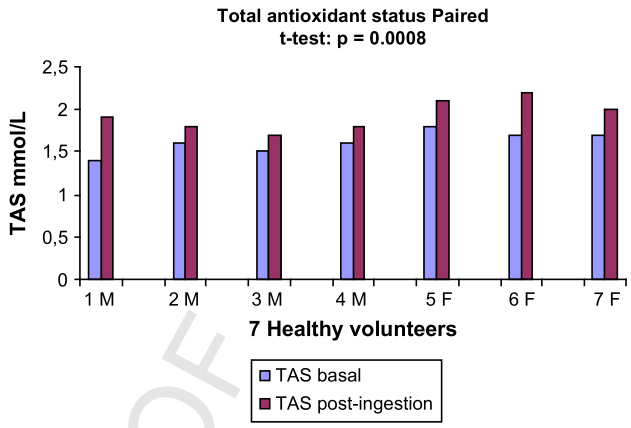

Fig. 2. Values of TAS in serum samples from 7 (1–4 M: male and 5–7 F: female) healthy volunteers before (TAS basal) and after drinking beer (TAS post-ingestion), with *p* value = 0.0008 considered highly significant. Each column is the mean ± SEM of 3 determinations. Both groups of samples, basal and post-ingestion conditions, showed levels of TAS, being greater the level in the group post-ingestion.

Beer is an integral element of the diet of numerous people and accumulating evidence suggests that it may have health benefits that include reduction of free radical generations, decrease of risk factors of coronary heart disease, prevention of several varieties of cancers, and modification of immune and inflammatory responses.[18,19] Beer appears to be an example of a functional drink, with varied components that may contribute to its overall therapeutic characteristics. Melatonin is an ingredient in beer with similar properties that this (antioxidant, oncostatic, immunoenhancing effects, etc.),[20,21] for this reason, part of the beneficial effects of beer they are produced by melatonin.

The persistent oxidative stress in the organism has been associated with various diseases that are free radical-based, such as neurodegenerative diseases (Alzheimer's and Parkinson diseases),[22,23] retinopathy degenerative (glaucoma and macular degeneration),[11] cardiovascular diseases[24] and brain ischemia/reperfusion injury (cardiac attack and stroke)[25,26] among the others; melatonin is a molecule produced by pineal gland and extra-pineal sites, in vertebrates, in yeast[14,15] and in plants.[27,28] Based on the data available, melatonin seems to have protective properties via its direct free radical scavenger and its indirect antioxidant activity. Melatonin efficiently interacts with various reactive oxygen and reactive nitrogen species and it also upregulates antioxidant enzymes and downregulates pro-oxidant enzymes.[29] These mechanisms of action help to clarify the beneficial effects of the beer, due to the fact that it contains melatonin, which helps to prevent the conditions induced by oxidative stress.[30,31]

The second step of the results show that increments of melatonin in human serum, after drinking beer, may well be relevant in terms of total antioxidant capacity of the serum. Herein we show basal and post-ingestion variations in melatonin and TAS. Both parameters exhibited a pattern, with basal values smaller that post-ingestion values (Figs. 1 and 2). The volunteer number 4 presented a significantly higher melatonin level than the others (Fig. 1) but not a higher expected TAS (Fig. 2). Methodological factors such as judge him as an outlier or high inter-individual variability to the melatonin, can explain the levels noted in this case. While the sample size was small the universal response of the healthy volunteers lent us support to suggest that the melatonin in beer contributes, in addition to other compound antioxidants, to the total antioxidative capacity of human serum,[31] especially in beer moderates consumers.

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A number of studies have shown that other existing antioxidants in beer such as B vitamins complex, citric acid, silicic acid and resveratrol may also contribute to increase the TAS of the human serum or, at least, have prevailed[32–34] and thus, contribute to avoid the free radical damage. Examples of situations in which beer (silicic acid) has been found to lower induced oxidative damage include cerebral oxidation caused by aluminium toxicity,[35] or as the resveratrol of the beer has very beneficial antioxidant and anti-inflammatory effects on pancreatitis.[36]

In conclusion, these results suggest that melatonin contained in beer contributes, in addition to other compounds, to increase the antioxidant properties of human serum. Consequently, the moderate consumption of beer can improve diet quality in regard to melatonin in healthy adults, although further research needs to be done.

Conflict of interest

None of the authors of this manuscript have any financial interest that has influenced the results or interpretations of this manuscript.

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