# Cell Membranes and Free Radical Research

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Areas of particular interest are four topics. They are;

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**B- Oxidative Stress** (Antioxidant vitamins, antioxidant enzymes, metabolism of nitric oxide, oxidative stress, biophysics, biochemistry and physiology of free oxygen radicals

#### C- Interaction Between Oxidative Stress and Ion Channels

(Effects of the oxidative stress on the activation of the voltage sensitive cation channels, effect of ADP-Ribose and NAD<sup>+</sup> on activation of the cation channels which are sensitive to voltage, effect of the oxidative stress on activation of the TRP channels)

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#### **Keywords**

lon channels, cell biochemistry, biophysics, calcium signaling, cellular function, cellular physiology, metabolism, apoptosis, lipid peroxidation, nitric oxide synthase, ageing, antioxidants, neuropathy.

# Effects of beer, Hops (Humulus lupulus) on total antioxidant capacity in plasma of stressed subjects

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### List of abbreviations ?????

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#### Abstract

The fermented drinks of low graduation like beer has many healthy properties such as low-calories, protein compounds, minerals and trace elements, vitamins A, D and E, carbon dioxide and polyphenols. Beer is the only drink which contains Hops (*Humulus lupulus*) and this plant produces a greater and better antioxidant effect. The objective of our experiment was measured the *Antioxidant Capacity* after consumed a nonalcoholic beer. The trial was conducted in a stressed population of 17 female healthy volunteers who drank beer in dinner during two weeks. We analyzed the *Antioxidant Capacity* in urine. The results showed that the consumption of non-alcoholic beer at dinner increases the *Antioxidant Capacity*. In conclusion, our assayed population which ingested a non-alcoholic beer increased the antioxidant levels.

#### Keywords

Antioxidant, hops, beer, resveratrol and xanthohumol.

#### Introduction

The source for the majority of life is photosynthesis which turns sun energy into redox energy (Demmig-Adams, 2002). Plants have high concentration of active antioxidants which work like a secondary metabolite, for example, alcohols and polyphenols like xanthohumol and resveratrol, and also carotenoids, glutation, ascorbic acid, antioxidant enzymes, which help to prevent dangerous damage as oxidative stress to cell components (Demmig-Adams, 2002; Benzie, 2003; and Pinto, 2012).

Beer is a fermented drink has many nutritional properties, and contains polyphenols and low levels of graduation (0.1-5%) of alcohol, which generate in moderate consumption an effect healthy (Sánchez, 2010; Arranz, 2012). Beer is the only drink that contains Hops (Humulus lupulus) which give the typical bitter flavor. The polyphenols from the hops and the beer: xanthohumol and resveratrol generate in this beverage the antioxidant properties and may reach 0,148 mmol/100 g depending on the type of beer (Bente, 2011).

Flavonoids are a group of natural substances found in the plant kingdom. Have discovered more than 4000 different species of flavonoids, found in fruits, vegetables, seeds, stems, flowers, thus being constituents important in the human diet. His basic structure is: 2 rings benzenes bonded through a ring pyrone (Bravo, 1998). The chemical structure of the phenolic compounds, is conferred by their ability to act as free radical scavengers (Heim, 2002). Beer contains a significant amount of flavonoids. Researchers from King's College London have published an investigation into the presence of flavonoids in various types of beverages and potential role as an antioxidant ferulic acid is found in low alcohol beers (Bourne, 2000). In another study of Oregon State University has shown that hops flavonoids dependent monooxygenases can inhibit cytochrome P-450, suggesting a potential role in modulating the activation of carcinogens (Henderson, 2000). The same group of researchers has confirmed that chalcones and flavanones are inducers of guinone reductase and may have a preventive role in the progression of hepatomas (Miranda, 2000).

Xanthohumol is one of the major flavonoids present in beer from Hops. This biomolecule is used to preserve beer as well as to give the characteristic aroma and flavor to beer (Pinto, 2012). The mechanism of action of xanthohumol has not yet been fully elucidated. Although the evidence for the beneficial effect of xanthohumol is promising, most studies were carried out in vitro. Recently, it has been reported that supplementing the drinking water of rats with xanthohumol prevents agains heterocyclic aromatic mutagens which induce preneoplastic lesions and DNA damage in the liver and colon (Ferk, 2010).

Resveratrol (3, 4, 5-trihydroxy-trans-stilbene) has been found in some hop varieties (Chiva-Blanch, 2010). It is a natural nonflavonoid polyphenolic found in the skin of red grapes (Shakibaei, 2009). Its antioxidant capacity is based in changes in low-density lipoproteins (LDL) properties by oxidation of polyunsaturated fatty acids (PUFA) is believed to play a major role in atherosclerosis. The oxidation affects the protein moiety (Apo B) of LDL particles impairing their catabolism by the regulated apo B/E receptor system. Therefore, the protective role of foods rich in phenolic compounds has been attributed to their antioxidant properties (Rice-Evans, 1997) were the first to demonstrate that trans-resveratrol added to human LDL, reduced the copper-catalyzed oxidation (Frankel 1993). Other benefits of resveratrol are the prevention of the inflammation and cellular senescence (Chung, 2012).

For all theses scientific arguments the objective of our experiment was measured the *Antioxidant Capacity* after consumed a non-alcoholic beer, in a stressed population.

#### Materials and methods Subjects

The trial was conducted in a population of 17 female volunteers (auxiliary, nurses and doctors) belonging to the Extremadura Health Service (SES) Badajoz (Spain), which work in rotating shifts and / or night shift, and with an elevated level of work stress and / or mood (assessed by a questionnaire of stress). These women were healthy (40.9±10.5 years), with normal weight and not under any prescription that might influence the study objectives. This project was approved by the Ethics Committee of the University of Extremadura.

#### **Antioxidant Analysis**

We chose a longitudinal intervention design in which each subject was his own control. The experimental period was 3 weeks, being the first 7 days the (Week Control), in which the individuals did not ingests any beer at dinner, until the second week (Week 1), in which the individuals ingested 330 mL of non-alcoholic beer during dinner, and the third week (Week 2) is the second week that consumed beer. Urine samples were collected at the end of each of the three weeks of the study. The *Antioxidant Capacity* was analyzed by the improved spectrophotometric "TEAC" method (*Trolox Equivalent Antioxidant Capacity*) (Cubero, 2009). This analysis allows us to calculate the percent inhibition of ABTS-+ radical cation by Trolox, a water soluble analogue of alpha-tocopherol (vitamin E), which is used as a standard antioxidant. The analysis was carried out on a TECAN plate reader M200 (Männedorf / Switzerland). The wavelength for the study was 730 nm.

#### **Statistical Analysis**

After confirming that the population belonged to the Normal, the results were analyzed by *t-Student* test for comparisons between the two groups. We used software GraphPad Prism 5.

#### Results

Results obtained in a population with high levels of stress (Table I), showed that the consumption of nonalcoholic beer at dinner increases the antioxidant levels (0.02 mg Trolox) measured in urine.

We observed a growing of *Antioxidant Capacity* in Treatment Group after drinking a non-alcoholic beer for two weeks (0.15±0.03 mg Trolox), versus the Control group without drinking a beer at dinner (0.13±0.01 mg Trolox, Figure 1).

#### Discusion

It is demonstrated that of free radicals with drinking non-alcoholic beer at dinner is very important for people who work at night because in those hours of sleep, our system acts against free radicals produced in our diurnal life, in addition to fighting viruses and bacteria. Besides, the components of beer are very important of treated oxidative damage, for example, Hops have been used to treat a variety of pathological processes including rheumatism, inflammation and climacteric complaints (Zanoli, 2008). Thus the polyphenol xanthohumol prevents the acute hepatic injury induced by carbon tetrachloride (CCl4) in rats (Pinto, 2012); respecting to polyphenols many of the biological effects of them have been attributed to their antioxidant properties, either through their reducing capacities per se or through their possible influences on intracellular redox status (Angeloni, 2012).

 Table 1. Demographic values of the stressed population (n=17).

Parameters	Mean X±SD
Age (years)	40.90±10.50
Weight (kg)	62.40±11.50
Height (m)	1.61±0.07
CMI (kg/m <sup>2</sup> )	24.40±5.30

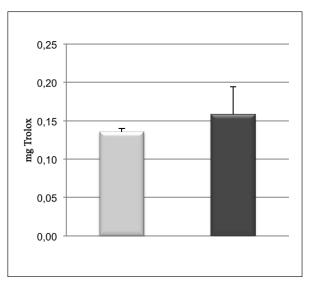
We report that treatment with non-alcoholic beer shows antioxidant effect of Hops. Our data suggest that the protective properties of non-alcoholic beer are based on the antioxidant effect of the components of Hops: resveratrol and xanthohumol. Our *in vivo* results agree with previous in vivo studies (Pinto 2012) and *in vitro* studies reporting the efficiency of polyphenols (Rodriguez 2001).

There were found similar results with the nonalcoholic beer and this *antioxidant ability* (Gonzalez, 2001), by Garrido, (2010) and Bravo, (2012) using cherries and cereals enriched in tryptophan respectively which were supplied to people in dinner.

Referent our trail in stressed population, this Antioxidant Capacity will be more higher with higher doses (660 ml) and more time (30 days) of ingest of non alcoholic beer showing the indications of the investigations by Codoñer-Franch, 2012 and Arnao, 2001. For this reason we recommend to increase the ingestion the nonalcoholic beer with this higher dose and more duration.

Apart from that polyphenols show antimicrobial activity, being primarily responsible for the presence of gallic acid. This acid is a natural defense mechanism in many fruits against the development of microbial and fungal diseases (Chung, 1993).

Finally, highlight that the bitter acids components of the Hops modulate on the amino acid GABA (main inhibitory neurotransmitter in the central nervous system), at the same time as myrcenol a terpenoid and xanthohumol in beer from Hops improve its sedative properties (Franco, 2012a and Franco, 2012b).



**Figure 1.** Comparison of *Antioxidant Capacity* (mg of Trolox) between the Control Group and Group of two weeks of Treatment with non-alcoholic beer.

#### Conclusion

Drinking a non-alcoholic beer could increases Antioxidant Capacity which was measured in the urine from stressed population.

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