JOURNAL OF PHYSIOLOGY AND PHARMACOLOGY 2015, 66, 3, 441-447
www.jpp.krakow.pl

A. FEDERICO[1], M. DALLIO[1], A.G. GRAVINA[1], C. IANNOTTA[2], M. ROMANO[1], G. ROSSETTI[1], F. SOMALVICO[3], C. TUCCILLO[1], C. LOGUERCIO[1]

A PILOT STUDY ON THE ABILITY OF CLINOPTILOLITE TO ABSORB ETHANOL *IN VIVO* IN HEALTHY DRINKERS: EFFECT OF GENDER

[1]Department of Clinical and Experimental Medicine, Second University of Naples, Naples, Italy; [2]Department of Anaesthesiological, Surgical and Emergency Science, Second University of Naples, Naples, Italy; [3]Society for Statistics, Milan, Italy

Zeolites are microscopic minerals of volcanic origin, and the zeolite most commonly used in medicine is clinoptilolite. Over the years, clinoptilolite has been tested in several ways: as an antioxidant, as an adjuvant in anticancer therapy due to its ability to capture chemotoxins, as an antidiarrhoeal agent and as a chelating agent for heavy metals. The aim of this study was to evaluate the ability of clinoptilolite to absorb ethanol *in vivo* in healthy drinkers. We enrolled 12 healthy drinkers in this study. The study was conducted as follows: phase 1: consumption of a hydroalcoholic solution containing 25 g of ethanol; phase 2: use of a 16.25 mL medical device containing clinoptilolite (2.5 g of clinoptilolite within a single-dose sachet) + consumption of a hydroalcoholic solution containing 25 g of ethanol; phase 3: use of a 32.5 mL medical device (5 g of clinoptilolite within a single-dose sachet) + consumption of a hydroalcoholic solution containing 25 g of ethanol. At the time of blood sampling, alcohol ingestion was also measured using an Alcolmeter instrument, and the results showed that the two methods overlapped. Reductions of 43%, 35%, 41% and 34% in blood ethanol at 30, 60, 90 and 120 minutes, respectively, were observed after the consumption of 5 g of clinoptilolite + 25 g of ethanol in both males and females, whereas the consumption of 2.5 g of clinoptilolite did not result in a statistically significant reduction in blood ethanol. In particular, the blood ethanol reduction was more significant in males. Our study highlights and confirms the ability of clinoptilolite to decrease the absorption of ingested ethanol by reducing blood alcohol levels. This effect was statistically significant at a dose of 5 g.

Key words: *clinoptilolite, zeolite, ethanol, gender, healthy drinkers, blood alcohol levels*

INTRODUCTION

Zeolite is a microscopic mineral of volcanic origin that is composed of SiO_4 (silicon tetraoxide) and AlO_4 (aluminium tetraoxide), or crystals of silicon and aluminium joined by oxygen bridges (1). The mineral's chemical composition gives it a three-dimensional configuration that allows it to absorb a number of chemical elements (particularly cations), free radicals, ammonia, heavy metals, aflatoxins, and especially low-molecular-weight polar compounds, such as ethanol (2-6), into its crystal lattice.

In nature, over 100 types of zeolites are known to exist, and among these, the zeolite most commonly used in medicine is clinoptilolite. This mineral has been in use since 1986, and is mainly used in the preparation of cosmetics and as a dietary supplement, as well as for its ability to "filter" bacterial toxins (7, 8).

Clinoptilolite is not toxic to the body and passes through the entire gastrointestinal tract without being absorbed systemically. Clinoptilolite is stable under various environmental conditions and can withstand temperatures of up to 450°C. It does not polymerise, but it resists alkaline and acidic environments without decomposition. In addition, this mineral is not costly and is readily available in nature (3, 9).

Over the years, clinoptilolite has been tested in several ways: as an antioxidant, an adjuvant in anticancer therapy due to its ability to capture chemotoxins, an antidiarrhoeal agent and as a chelation agent for heavy metals (9-14).

The best-known beneficial biological activity of natural clinoptilolite is its action as an antidiarrhoeal drug (8). Clinoptilolite has been shown to reduce morbidity and mortality (diarrhoeal syndrome) due to intestinal diseases in pigs, rats and cattle (8). Based on these results and on natural clinoptilolite's status as an active material, a comprehensive study of antidiarrhoeal drugs was performed for the treatment of acute diarrhoeal diseases in humans (8). This research led to the approval of the antidiarrhoeal drug Enterex for use in humans. In addition, many studies have shown that zeolites play an important role in regulating the immune system (10, 11).

When given orally in mice and dogs suffering from a variety of types of tumours, clinoptilolite led to significant shrinkage of certain tumours and to an improvement in the overall health status of certain animals (9). The observed effects were diverse, ranging from a negative antitumour response to a normalisation of biochemical parameters, a prolongation of lifespan and a decrease in tumour size (15). The best results in animal models have been observed in the treatment of skin cancer in dogs, suggesting that the

adsorption of certain active components, i.e., direct contact with clinoptilolite, may be responsible for this compound's activity (9).

To our knowledge, no data on the effects of zeolite or clinoptilolite on ethanol absorption in humans have been published in the literature. Therefore, this study was performed to evaluate *in vivo* the ability of clinoptilolite to reduce the absorption of ethanol in healthy drinkers. Also, as a secondary aim, we assessed if there was any gender difference in the ability of clinoptilolite to reduce ethanol absorption.

MATERIALS AND METHODS

The study protocol was approved by the ethics committee of the Company University Hospital of the Second University of Naples on September 13, 2010 (protocol number 194), and written, informed consent was obtained from each study participant.

Twelve healthy subjects, between 18 and 50 years of age (median age 39 years of age) were enrolled in the study; there were equal numbers of males and females. Participants were not obese, had good nutrition and had not used drugs for at least two weeks. The participants were chronic alcohol users, with a mean alcohol intake of 25 g/day, and all were subjected to a basal evaluation of alcohol use history (AUDIT-C test) (16).

Before the study began, each subject underwent blood chemistry and instrumental investigations to rule out active or past conditions that would affect the research: haemochrome measurements with differential leukocyte counts and platelet counts; measurements of the levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), blood urea nitrogen, creatinine, gamma-glutamyltranspeptidase and alkaline phosphatase, iron, ferritin, transferrin, uric acid, cholesterol, triglycerides, total bilirubin, HBsAg and anti-HCV antibodies; serum protein electrophoresis; and electrocardiography.

We also evaluated the body mass index (BMI) and body composition of each participant using the impedance method (Akern BIA 101S instrument). We paid particular attention to the amount of body water, which can influence the body distribution of ethanol and, therefore, the blood ethanol concentration (17).

Clinoptilolite: mineralogical and chemical analysis

Clinoptilolite is a natural zeolite belonging to the heulandite family of minerals, which was formed from molten amorphous glassy material of volcanic origin; this molten material took crystalline form when it came in contact with sea water (1-6). Clinoptilolite deposits are located in different parts of the world. Noteworthy deposits are in Italy and Anatolia (Turkey).

Mineralogical analysis by X-ray diffraction showed that the composition of the clinoptilolite used in this study was as follows: clinoptilolite 88 – 95%, 3 – 5% feldspar, montmorillonite 2 – 5%, 0 – 2% cristobalite, muscovite 0 – 3%.

Chemical analysis showed that the composition of the clinoptilolite used in this study was as follows: SiO_2 65 – 72%; Al_2O_3 10 – 12%; CaO 2.5 – 3.7%; K_2O 2.3 – 3.5%; Fe_2O_3 0.8 – 1.9%; 0.9 – 1.2% MgO; Na_2O 0.3 – 0.65%; TiO_2 0 – 0.1; MnO 0 – 0.08%; SiO_2/Al_2O_3 5.4 – 6.0; loss of ignition 9 – 12%.

After extraction, the mineral is subjected to the process of disintegration and tribomechanical activation (*via* special disintegrators that lead to impact between them).

This method of micronisation greatly increases the surface area of the particles, and therefore, also increases the reactivity. The average diameter of the resulting particles is 20 µm, and the total surface area is over 1200 m^2/g.

Clinoptilolite samples are then subjected to a process of purification and chemical activation using a standardised protocol (HF Europe Srl, Bassano del Grappa-VI, Italy).

The study was conducted as follows:

Phase 1: Consumption of a hydroalcoholic solution containing 25 g of ethanol.

Phase 2: Use of a 16.25-mL medical device containing clinoptilolite (2.5 g of clinoptilolite within a single-dose sachet) + consumption of a hydroalcoholic solution containing 25 g of ethanol.

Phase 3: Use of a 32.5-mL medical device containing clinoptilolite (5 g of clinoptilolite within a single-dose sachet) + consumption of a hydroalcoholic solution containing 25 g of ethanol.

In particular, at the same time in the morning, the subjects received two drinks of a 12% water-alcohol solution (together containing a total of 25 g of ethanol) while in the fasting state. In phases 2 and 3, the two drinks were preceded by the use of a medical device (with volumes of 16.25 mL and 32.5 mL, respectively). The time between alcohol consumption and ingestion of the product was 2 – 3 minutes.

Venous blood samples were taken from the antecubital vein in all phases at 0, 30, 60, 90 and 120 minutes.

At the same time points (0, 30, 60, 90 and 120 minutes), alcohol ingestion was also measured using an Alcolmeter SD-400 instrument (Lion Laboratories Limited, UK; series 075963 D; December 2008) to analyse exhalation from alveolar regions. The instrument displayed values expressed in g/L. The Alcolmeter SD-400 was supplied by the Italian State Police; this instrument has recognised validity and is calibrated appropriately (18).

Plasma samples frozen at –80°C were successively evaluated for ethanolaemia (by the spectrophotometric method; Roche Diagnostics, Milan, Italy; sensitivity, 0.1 mg/L). Additionally, at 0 and 120 minutes, testing was performed to monitor the levels of AST/ALT, gamma-glutamyltranspeptidase, iron, uric acid and triglycerides (by a colorimetric/enzymatic test).

Statistical analysis

All study hypotheses were tested using the statistical package SPSS version 18.

We calculated the sample size of this two-treatment crossover study on the assumption that the within-patient standard deviation of the response variable is 1. Thus, a total of at least eight patients would need to enter this study. The significance level of the statistical tests used for the evaluation of the endpoints was determined considering a two-tailed probabilistic level of significance of 5% ($P < 0.05$). The statistical tests used were chosen after verification of the normality of the distribution of the sample data using the Kolmogorov-Smirnov test. Every variation in the concentrations (25 g of ethanol, 2.5 g of clinoptilolite + 25 mL ethanol and 5 g of clinoptilolite + 25 g of ethanol) over time was examined by analysis of variance (ANOVA), and comparisons among time points were performed using the Scheffe test for multiple comparisons. Comparisons among concentrations as a function of time (AUC; area under the concentration-versus-time curve, determined after logarithmic transformation of the AUC values for individual patients, performed to make the variances independent of the average value and to make the distribution more symmetric; $AUC_{25\ g\ of\ ethanol}$, $AUC_{2.5\ g\ of\ clinoptilolite\ +\ 25\ g\ of\ ethanol}$, $AUC_{5\ g\ of\ clinoptilolite\ +\ 25\ g\ of\ ethanol}$) and maximum concentrations (Cmax; $Cmax_{25\ g\ of\ ethanol}$, $Cmax_{2.5\ g\ of\ clinoptilolite\ +\ 25\ g\ of\ ethanol}$, $Cmax_{5\ g\ of\ clinoptilolite\ +\ 25\ g\ of\ ethanol}$) were verified by ANOVA, and comparisons between doses were performed using Tukey's test for multiple comparisons.

The times required to achieve the maximum concentrations (Tmax; $Tmax_{25\ g\ of\ ethanol}$, $Tmax_{2.5\ g\ of\ clinoptilolite\ +\ 25\ g\ of\ ethanol}$, $Tmax_{5\ g\ of\ clinoptilolite\ +\ 25\ g\ of\ ethanol}$) were determined using Friedman's test for

pairs, and a comparison between doses was performed using the Wilcoxon test for matched pairs.

An evaluation of concentrations as a function of time (AUC) or gender was performed *via* a two-way ANOVA and using Tukey's test for multiple comparisons.

To investigate the possible difference between sexes, the maximum concentration (Cmax) and the time required to reach the maximum concentration (Tmax) were used as follows:

- For each sex: The Friedman test was used to determine whether a difference among doses exists and the Wilcoxon test was used to highlight the difference among doses.

- For each dose: the Mann-Whitney U test was used to evaluate differences between the sexes.

RESULTS

This clinical research was conducted as scheduled, and there were no adverse events.

The median BMIs of males and females were 24.9 (range 23.5 – 26.9) and 21.6 (range 19.5 – 24.2), respectively. Total body water content was significantly different between males and females (31.8 ± 1.4 L and 46.2 ± 3.6 L, respectively; $P < 0.05$), with extracellular water content higher in females than in males (27.4 ± 3.6 L versus 14.3 ± 0.5 L, respectively; $P < 0.05$), whereas intracellular water volume was similar in males and females (17.5 ± 1.1 L and 18.8 ± 4.8 L, respectively).

No significant differences in any plasma biochemistry data were observed between males and females or between 0 and 120 minutes either under basal conditions or at the end of the three experiments (data not shown).

We found an overlap in the measurements of ethanolaemia obtained by spectroscopy and using the Alcolmeter instrument (*Table 1*). These values were also similar between males and females.

In the first phase (intake of two drinks of 12% water-alcohol solution, containing a total of 25 g of ethanol), there was a statistically significant difference ($P < 0.001$) between T0 and each time point after T0, with changes greater than 100%. Furthermore, a statistically significant difference was evident between T120 and both T30 and T60 ($P < 0.0001$ versus T30 and $P = 0.002$ versus T60). In particular, we demonstrated a change of 42.8% between T120 and T30 and of 36.5% between T120 and T60. Both methods indicated a different distribution of ethanol levels between males and females; *Table 1* shows greater increases in ethanol plasma levels and Alcolmeter readings in females compared with males at each time point.

Fig. 1 shows the mean effects of the use of a 16.25 mL medical device containing 2.5 g of clinoptilolite (phase 2) and of a 32.5 mL medical device containing 5 g of clinoptilolite (phase 3) on plasma

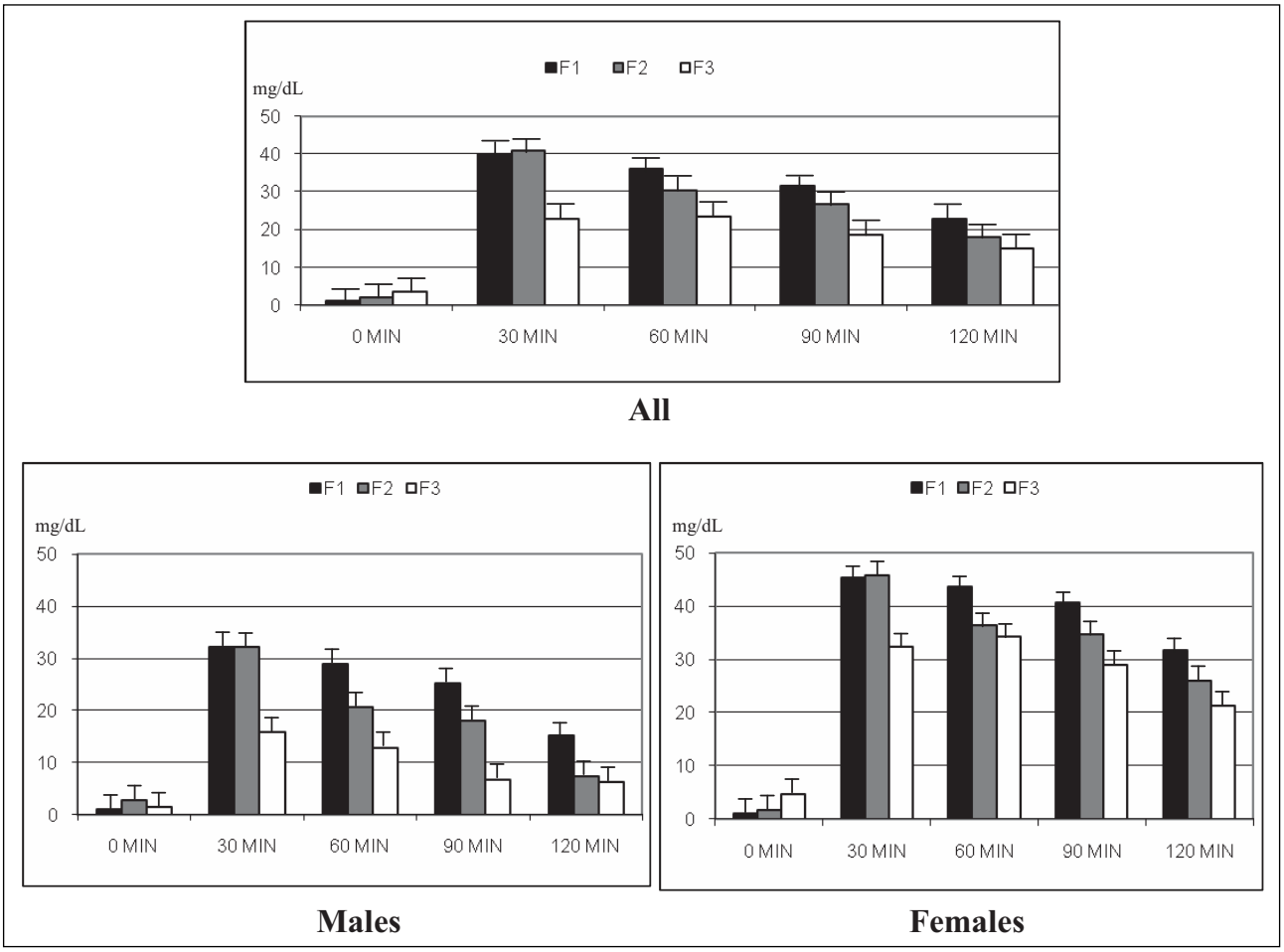

Fig. 1. Mean effects of the use of a 16.25 mL medical device containing clinoptilolite (2.5 g of clinoptilolite within a single-dose sachet (phase 2) and of a 32.5 mL medical device containing clinoptilolite (5 g of clinoptilolite within a single-dose sachet (phase 3) after the intake of two drinks of a 12% water-alcohol solution, together containing a total of 25 g of ethanol, on blood ethanol levels (spectrophotometric method; mg/dL) in relation to gender (F1 = 25 g of ethanol; F2 = 2.5 g of clinoptilolite + 25 g of ethanol; F3 = 5 g of clinoptilolite + 25 g of ethanol).

Table 1. Comparison between ethanol plasma levels and Alcolmeter in relation to gender at phase 1 (assumption of two drinks of 12% water-alcohol solution, containing 25 g of ethanol) (mg/dL).

	M + F		**M**		**F**	
	Blood levels	*Alcolmeter*	*Blood levels*	*Alcolmeter*	*Blood levels*	*Alcolmeter*
T0	1.13±0.9	0.00±0.00	0.95±0.94	0.00±0.00	1.06±0.95	0.00±0.00
T30	39.97±15.16*	43.2±11.0*	32.25±12.91*	34.7±5.9*	45.3±16.47*	51.7±7.3*
T60	36.06±10.69*	32.9±9.5*	28.85±3.73*	24.8±4.1*	43.56±10.12*	41±5*
T90	31.55±11.99*	24.5±9.9*	25.15±3.28*	16.2±2.9*	40.68±10.28*	32.8±6.4*
T120	22.85±11.28*^°	19.4±9.3*^°	15.2±2.02*^°	11.5±2.1*^°	31.7±9.37*^°	27.3±6.0*^°

*P < 0.001 versus T0; ^P < 0.0001 versus T30; °P = 0.002 versus T60.
T = time (min), M = males, F = females

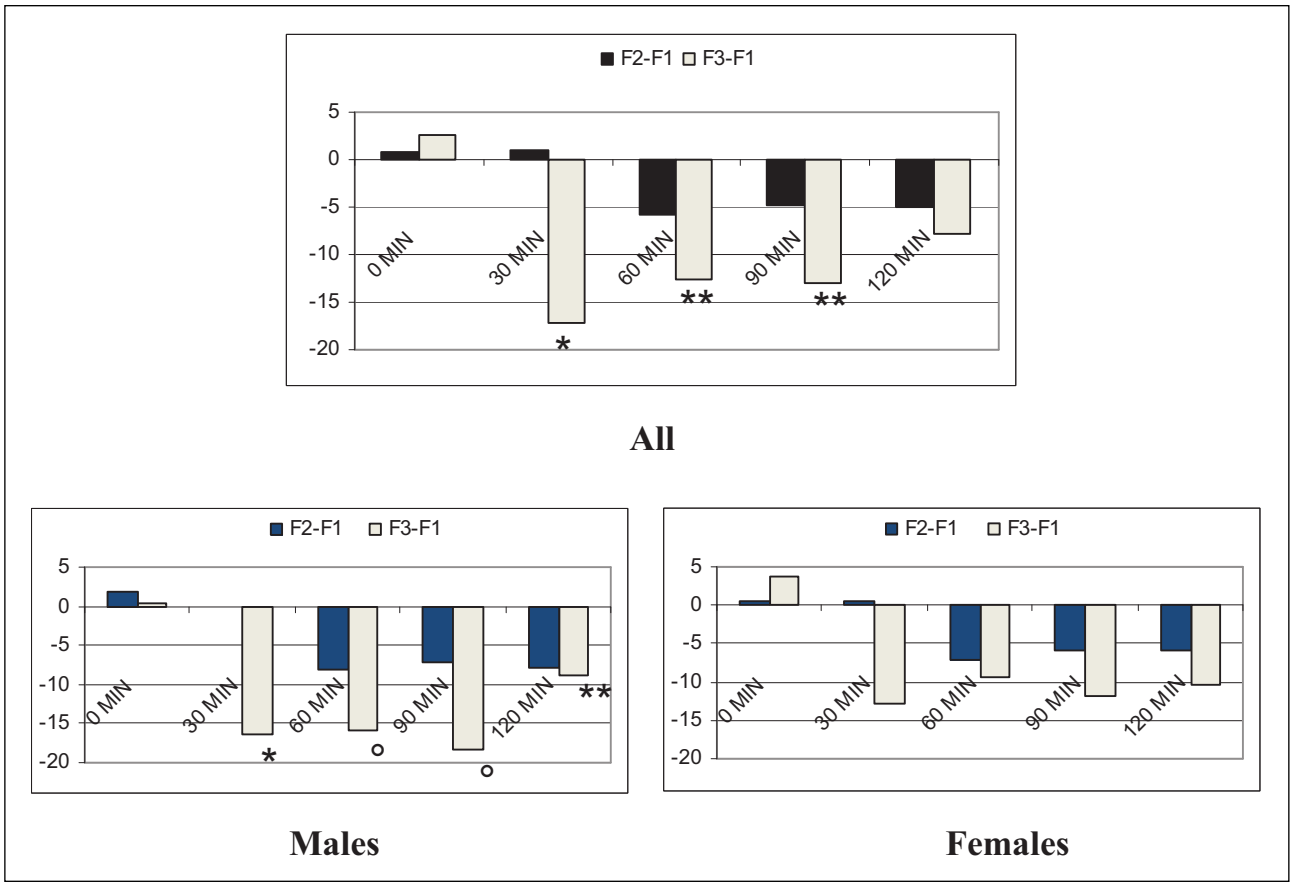

Fig. 2. Mean differences in blood ethanol levels (mg/dL) between F2-F1 and F3-F1 (*P < 0.01; **P < 0.05; °P < 0.001) in relation to gender (F1 = 25 g of ethanol; F2 = 2.5 g of clinoptilolite + 25 g of ethanol; F3 = 5 g of clinoptilolite + 25 g of ethanol).

Table 2. Area Under the Curve (AUC) of plasma concentration of ethanol (mg/dL) with respect to time at various phases of the study.

Area Under the Curve (AUC)	**Mean ± S.D.**	**95% IC**	
$AUC_{25\ g\ of\ ethanol}$	59.80 ± 19.97	47.11	72.49
$AUC_{2.5\ g\ of\ clinoptilolite\ +\ 25\ g\ of\ ethanol}$	54.01 ± 16.22	43.70	64.31
$AUC_{5\ g\ of\ clinoptilolite\ +\ 25\ g\ of\ ethanol}$	37.19 ± 21.44*	23.56	50.81

*P = 0.0002 versus $AUC_{25\ g\ of\ ethanol}$ and $AUC_{2.5\ g\ of\ clinoptilolite\ +\ 25\ g\ of\ ethanol}$

levels of ethanol (mg/dL) in relation to gender (F1 = 25 g of ethanol; F2 = 2.5 g of clinoptilolite + 25 g of ethanol; F3 = 5 g of clinoptilolite + 25 g of ethanol). As shown in *Fig. 2*, the intake of 2.5 g of clinoptilolite did not significantly affect ethanolaemia in either sex, while reductions of 43%, 35%, 41% and 34% in blood ethanol levels at 30, 60, 90 and 120 minutes, respectively, were observed after the consumption of 5 g of clinoptilolite in both males and females.

The mean differences in blood ethanol levels between F2-F1 and F3-F1 in relation to gender are shown in *Fig. 2* (*P < 0.01; **P < 0.05; °P < 0.001). In particular, a statistically significant reduction in blood ethanol was observed in males (*Fig. 2*).

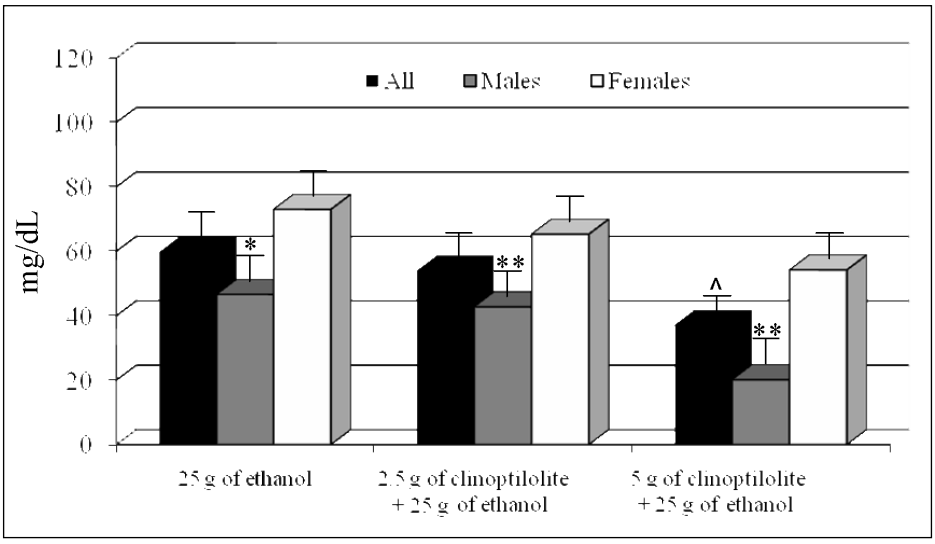

Fig. 3. AUC values for the serum concentration of ethanol in relation to gender (F1 = 25 g of ethanol; F2 = 2.5 g of clinoptilolite + 25 g of ethanol; F3 = 5 g of clinoptilolite + 25 g of ethanol). (*P = 0.0002 and **P = 0.0004 versus females; ^P = 0.0002 versus 25 g of ethanol and 16.25 mL of BBSlow® + 25 g of ethanol; °P = 0.002 versus F1 in females).

Table 3. Cmax of ethanol at various phases of the study after logarithmic transformation of the data.

Variable	Mean ± S.D.	95% IC
$Cmax_{25\ g\ of\ ethanol}$	42.817 ± 13.547	34.209 51.424
$Cmax_{2.5\ g\ of\ clinoptilolite\ +\ 25\ g\ of\ ethanol}$	40.983 ± 10.305	34.436 47.531
$Cmax_{5\ g\ of\ clinoptilolite\ +\ 25\ g\ of\ ethanol}$	25.358 ± 13.217*	16.961 33.756

Cmax= maximum concentration of ethanol.
*P = 0.0002 versus $Cmax_{25\ g\ of\ ethanol}$ and $Cmax_{2.5\ g\ of\ clinoptilolite\ +\ 25\ g\ of\ ethanol}$

Table 4. Tmax of ethanol at various phases of the study.

Variable	M ± S.D.	MIN	MAX
$Tmax_{25\ g\ of\ ethanol}$	0.667 ± 0.246	0.5	1.0
$Tmax_{2.5\ g\ of\ clinoptilolite\ +\ 25\ g\ of\ ethanol}$	0.500 ± 0.000*	0.5	0.5
$Tmax_{5\ g\ of\ clinoptilolite\ +\ 25\ g\ of\ ethanol}$	0.833 ± 0.325°	0.5	1.5

Tmax = time required for the achievement of maximum concentration.
*P = 0.046 verus $Tmax_{25\ g\ of\ ethanol}$ and $Tmax_{5\ g\ of\ clinoptilolite\ +\ 25\ g\ of\ ethanol}$; °P = 0.011 versus $Tmax_{2.5\ g\ of\ clinoptilolite\ +\ 25\ g\ of\ ethanol}$;

Table 2 summarises AUC values in various phases of the study. There were statistically significant differences between the mean $AUC_{5\ g\ of\ clinoptilolite\ +\ 25\ g\ of\ ethanol}$ and $AUC_{25\ g\ of\ ethanol}$ values (P = 0.0002), resulting in a percentage difference of 37.8% and between the mean $AUC_{5\ g\ of\ clinoptilolite\ +\ 25\ g\ of\ ethanol}$ and $AUC_{2.5\ g\ of\ clinoptilolite\ +\ 25\ g\ of\ ethanol}$ (P = 0.0002), resulting in a percentage difference of 31.2%.

After ingestion of 25 g of ethanol, a difference in the AUC for the plasma concentration of ethanol was observed in relation to gender, with values of 46.63 ± 9.12 mg/dL in males and 72.97 ± 19.4 mg/dL in females (P = 0.0002), for a difference of 56.5%. After clinoptilolite intake, the same difference between males and females was confirmed, resulting in a percentage difference in $AUC_{2.5\ g\ of\ clinoptilolite\ +\ 25\ g\ of\ ethanol}$ of 53.1% and in $AUC_{5\ g\ of\ clinoptilolite\ +\ 25\ g\ of\ ethanol}$ of 169.1% (*Fig. 3*). Therefore, in males, mean AUC values were lower than in females, independent of clinoptilolite intake. The different distributions of ethanol levels resulted from the different behaviour of clinoptilolite in males and females. For males, statistically significant differences were observed between the following:

a) The mean $AUC_{5\ g\ of\ clinoptilolite\ +\ 25\ g\ of\ ethanol}$ (20.15 ± 7.0) and $AUC_{25\ g\ of\ ethanol}$ (46.63 ± 9.2; P = 0.0002), resulting in a percentage difference of 56.79%;

b) The mean $AUC_{5\ g\ of\ clinoptilolite\ +\ 25\ g\ of\ ethanol}$ (20.15 ± 7.0) and $AUC_{2.5\ g\ of\ clinoptilolite\ +\ 25\ g\ of\ ethanol}$ (42.67 ± 7.7; P = 0.0004), resulting in a percentage difference of –57.78%.

In females, there was instead a statistically significant difference only between $AUC_{5\ g\ of\ clinoptilolite\ +\ 25\ g\ of\ ethanol}$ (54.22±16.3) and $AUC_{25\ g\ of\ ethanol}$ (72.97±19.4; p = 0.002), resulting in a percentage difference of –5.69%.

Table 3 reports the Cmax of ethanol in various phases of the study after a logarithmic transformation of the data. Statistically significant differences were observed between the following:

a) The mean$Cmax_{5\ g\ of\ clinoptilolite\ +\ 25\ g\ of\ ethanol}$ (25.36 ± 13.2) and $Cmax_{25\ g\ of\ ethanol}$ (42.82 ± 13.5; P = 0.0002), resulting in a percentage difference of 40.8%;

b) The mean$Cmax_{5\ g\ of\ clinoptilolite\ +\ 25\ g\ of\ ethanol}$ (25.36 ± 13.2) and $Cmax_{2.5\ g\ of\ clinoptilolite\ +\ 25\ g\ of\ ethanol}$ (40.98 ± 10.3; P = 0.0002), resulting in a percentage difference of 38.1%.

Table 4 reports the times at which the Cmax of ethanol (Tmax) was observed in various phases of the study. Statistically significant differences were observed between the following:

a) The mean$Tmax_{2.5\ g\ of\ clinoptilolite\ +\ 25\ g\ of\ ethanol}$ and $Tmax_{25\ g\ of\ ethanol}$ (P = 0.046), resulting in a percentage difference of 25.4%;

b) The mean$Tmax_{2.5\ g\ of\ clinoptilolite\ +\ 25\ g\ of\ ethanol}$ and $Tmax_{5\ g\ of\ clinoptilolite\ +\ 25\ g\ of\ ethanol}$ (P = 0.046), resulting in a percentage difference of 24.1%;

b) The mean$Tmax_{5\ g\ of\ clinoptilolite\ +\ 25\ g\ of\ ethanol}$ and $Tmax_{2.5\ g\ of\ clinoptilolite\ +\ 25\ g\ of\ ethanol}$ (P = 0.011), resulting in a percentage difference of 66%.

An analysis of Cmax values in relation to gender showed a statistically significant difference in male subjects (P = 0.011)

but not in females (P = 0.069). In males, there was a statistically significant difference (P = 0.028) between $Cmax_{5\ g\ of\ clinoptilolite\ +\ 25\ g\ of\ ethanol}$ (14.7 ± 5.1) and $Cmax_{25\ g\ of\ ethanol}$ (35.90 ± 10.9) only, resulting in a percentage difference of 59.1%.

A gender evaluation of each dose showed a statistically significant difference in $Cmax_{5\ g\ of\ clinoptilolite\ +\ 25\ g\ of\ ethanol}$ (p=0.004) only, with a mean Cmax in males (14.7±5.1) that was less than the mean Cmax in females (36.0 ± 9.2), resulting in a percentage difference of 59.2%.

A Tmax analysis by gender showed no statistically significant differences between individual doses.

DISCUSSION

Alcohol abuse is currently a major cause of morbidity and mortality due to two types of acute effects: acute poisoning and accidents at work or on the road. Furthermore, alcohol abuse causes chronic diseases involving nearly all parts of the body (19). Recently, Bebarova *et al.* (20) reported alterations of cardiac electrophysiology related to alcohol consumption in rat ventricular myocytes.

Many clinical and epidemiological studies have investigated the mechanisms of action and the effects of alcohol on the body, the resulting damage, and the types and efficacies of treatments (21, 22). The consumption of alcoholic beverages has a direct impact on the digestive system due to its contact with the mucous membranes, the absorption and metabolism of ethanol and its interference with digestive function and the intestinal flora. For example, a recent study by Kasicka-Jonderko *et al.* (23) showed that alcoholic beverages were emptied from the stomach significantly more slowly than isotonic glucose. Moreover, alcoholic beverages produced by fermentation only (beer, red wine) were emptied from the stomach more slowly than ethanol solutions of identical proof, while the gastric evacuation of whisky (a distillation product) and matching alcohol solution was similar.

Alcohol intake is rapidly growing among women, and consequently, in recent years, this topic of gender medicine has brought about increased attention from researchers (24, 25).

The concentration of alcohol in the blood is determined by the volume of distribution in the body, which indicates the ability of alcohol to spread and of drugs to penetrate various organs and tissues of the body, and the rate of elimination. The effects of alcohol consumption vary depending on the amount of ethanol that reaches the organs. The rate of absorption depends on several factors, including the concentration and amount of alcohol ingested, the amount and type of food in the stomach and the mode of consumption. Experimental evidence shows that when ingesting equal doses of alcohol under equal conditions, blood concentrations or blood levels are higher in women than in men (26, 27). This phenomenon may be due to more than one cause, one of which is the lower body water content of women (17). In this study, we assessed the ability of clinoptilolite to reduce the plasma concentration and bioavailability of ethanol acutely administered to healthy subjects who were habitual drinkers of moderate amounts of ethanol. Clinoptilolite, when used in the dose range recommended for human subjects (5 – 15 g), is able to absorb a constant amount of ethanol from water-alcohol solutions, regardless of the alcohol content. Previous preliminary experiments showed that the amount absorbed is approximately 5 mL of ethanol (equivalent to 4 g of ethanol) per gram of clinoptilolite (9). In our study, the subjects were matched by age and sex, and no statistically significant differences were observed in anthropometric parameters or in the results of routine blood tests performed at T0. The only significant difference between the sexes was found in body water content, mainly in the extracellular content, as assessed by bioelectrical impedance.

The administration of two drinks of a 12% water-alcohol solution resulted in normal absorption kinetics at various observation times, with proportionally higher absorption in females than in males compared with standard reference curves (27). No significant difference was found between the levels of ethanolaemia determined by biochemical blood tests and the levels determined by breath tests, demonstrating the complete reproducibility of the data, as shown in *Table 1*. The administration of the device delivering 2.5 g of clinoptilolite (phase 2) and 5 g of clinoptilolite (phase 3) after the intake of two drinks of a 12% water-alcohol solution resulted in statistically significant reductions in the levels of ethanolaemia at various time points, especially with a dose of 5 g of clinoptilolite. This reduction was much more evident in males (*Fig. 1*).

The evaluation of AUC values for the plasma ethanol concentration with respect to time in the various phases showed that the dose of 5 g of clinoptilolite caused a statistically significant reduction in the AUC (P = 0.0002). In male subjects, the mean AUC value was lower than the mean AUC in female subjects (P = 0.0002; *Table 2*). After the administration of clinoptilolite, we observed this same pattern as well (*Fig. 3*).

The difference in the kinetics of ethanol between the two sexes is known in the literature. Women have proportionately more fat and less body water than men do, and because ethanol diffuses through the water in the body, for an equivalent intake of alcohol, the volume of distribution in women is lower. Thus, the resulting blood alcohol concentration is higher in women. This phenomenon is confirmed by the fact that by normalising the blood concentrations based on body water content, the gender difference decreases, and the consumption of equivalent amounts of alcohol results in similar adjusted blood concentrations.

To confirm the data supporting the differences in body composition, we evaluated total body water content, in which ethanol is diffusible, in both males and females. There was a significant negative correlation between total body water and ethanolaemia. The impedance analysis allowed us to highlight that the water content in most females is mainly extracellular, and this difference helps to explain the different distribution kinetics of ethanol as well (data not shown).

The comparison between the mean Cmax values of the three study phases showed that the average $Cmax_{5\ g\ of\ clinoptilolite\ +\ 25\ g\ of\ ethanol}$ was lower than the average Cmax for the other two doses, and this difference was statistically significant (P = 0.0002), confirming the greater effectiveness of the device at a dose of 32.5 mL (*Table 3*). In particular, the statistically significant difference in the mean Cmax values only in male patients (P = 0.011; data not shown) confirms the different kinetics of ethanol distribution in relation to gender. The doses differed in Cmax, with a lower mean $Cmax_{5\ g\ of\ clinoptilolite\ +\ 25\ g\ of\ ethanol}$ (14.7 ± 5.1) observed than the average $Cmax_{25\ g\ of\ ethanol}$ (35.90 ± 10.9) only in males. This difference was statistically significant (P = 0.028) and resulted in a percentage change of 59.1%.

The use of this device is only to reduce the absorption of ethanol and it cannot be used as an antidote (i.e., after ethanol intoxication). Clinoptilolite does not affect ethanol blood levels if taken after ethanol ingestion. Therefore, clinoptilolite works only if given prior to ethanol consumption. In particular, the time between ingestion of the medical device and alcohol consumption was 2 – 3 minutes.

In conclusion, our study shows that clinoptilolite at a dose of 5 g is able to decrease the absorption of ethanol *in vivo*. Other key results are that a) ethanolaemia data obtained by breath testing and by a spectrophotometric method overlap, and b) body composition, and particularly body water, influence plasma ethanol levels; this concept is well-known.

The present research represents only a pilot study that requires further validation in a larger study sample and under different experimental conditions, such as after meals, after chronic administration of the product and after a greater intake of ethanol. The relevance of the results is exclusively related to the demonstration that the mineral clinoptilolite may function as an absorbent of low quantities of ethanol *in vivo* in social drinkers.

Authors' contributions: Conception and design of the work, acquisition of data, or analysis and interpretation of data: Alessandro Federico, Marcello Dallio, Antonietta Gerarda Gravina, Carmela Paura, Giuseppa Rossetti, Francesco Somalvico and Concetta Tuccillo, Carmela Loguercio. Drafting the article or revising it critically for important intellectual content: Alessandro Federico, Marco Romano and Carmela Loguercio. Final approval of the version to be published: Alessandro Federico, Marcello Dallio, Antonietta Gerarda Gravina, Carmela Paura, Marco Romano, Giuseppa Rossetti, Francesco Somalvico, Concetta Tuccillo and Carmela Loguercio.

Acknowledgements: This study was supported by HF Europe Srl, Bassano del Grappa (VI), Italy.

Conflict of interest: none declared.

REFERENCES

1. Breck DW. Crystalline molecular sieves. *J Chem Educ* 1964; 41: 678-689.
2. Flanigen EM. Molecular sieve zeolite technology: the first twenty-five years. In: Proceedings of the 5th International Conference on Zeolites, Rees LVC (ed). London, 1980, pp. 760-780.
3. Sersale R. Natural zeolites: processing, present and possible applications. In: Studies in Surface Science and Catalysis Vol.24. 1985, 503-512.
4. Naber JE, De Jong KP, Stork WH, Kuipers HP, Post MF. Industrial application of zeolite catalysis. In: Studies in Surface Science and Catalysis 1994; 84: pp. 2197-2220.
5. Garces JM. Observations on zeolite applications. In: Proceedings of the 12th International Conference on Zeolites, Treacz MMJ, Marcus BK, Misher ME, Higgins JB, (eds). Warrendale. Materials Research Society, 1999, pp. 551-566.
6. Colella C. Natural zeolites in environmentally friendly processes and applications. In: Studies in Surface Science and Catalysis Vol. 125. 1999, pp. 641-655.
7. Concepcion-Rosabal B, Rodriguez-Fuentes G, Simon-Carballo R. Development and featuring of the zeolitic active principle FZ: a glucose adsorbent. *Zeolites* 1997; 19: 47-50.
8. Rodriguez-Fuentes G, Barrios MA, Iraizoz A, Perdomo I, Cedre B. Enterex - anti-diarrheic drug based on purified natural clinoptilolite. *Zeolites* 1997; 19: 441-448.
9. Pavelic K, Katic M, Sverko V, *et al.* Immunostimulatory effect of natural clinoptilolite as a possible mechanism of its antimetastatic ability. *J Cancer Res Clin Oncol* 2002; 128: 37-44.
10. Ueki A, Yamaguchi M, Ueki H, *et al.* Polyclonal human T-cell activation by silicate in vitro. *Immunology* 1994; 82: 332-335.
11. Aikoh T, Tomokuni A, Matsukii T, *et al.* Activation-induced cell death in human peripheral blood lymphocytes after stimulation with silicate in vitro. *Int J Oncol* 1998; 12: 1355-1359.
12. Lim Y, Kim SH, Kim KA, Oh MW, Lee KH. Involvement of protein kinase C, phospholipase C, and protein tyrosine kinase pathways in oxygen radical generation by asbestos-stimulated alveolar macrophages. *Environ Health Perspect* 1997; 105: 1325-1327.
13. Simeonova P, Torium W, Kommineni C, *et al.* Molecular regulation of IL-6 activation by asbestos in lung epithelial cells: role of reactive oxygen species. *J Immunol* 1997; 159: 3921-3928.
14. Martin LD, Krunkosky TM, Dye JA, *et al.* The role of reactive oxygen and nitrogen species in the response of airway epithelium to particulates. *Environ Health Perspect* 1997; 105: 1301-1307.
15. Pavelic K, Hadzija M, Bedrica LJ, *et al.* Natural zeolite clinoptilolite: new adjuvant in anticancer therapy. *J Mol Med* 2001; 78: 708-720.
16. Babor TF, de la Fuente JR, Saunders J, Grant M. The alcohol use disorders identification test: guidelines for use in primary health care. Geneva, World Health Organization AUDIT, 1989.
17. Marshall AW, Kingstone D, Boss M, Morgan MY. Ethanol elimination in males and females: relationship to menstrual cycle and body composition. *Hepatology* 1983; 3: 701-706.
18. Bendtsen P, Hultberg J, Carlsson M, Jones AW. Monitoring ethanol exposure in a clinical setting by analysis of blood, breath, saliva, and urine. *Alcohol Clin Exp Res* 1999; 23: 1446-1451.
19. Bellavia A, Bottai M, Wolk A, Orsini N. Alcohol consumption and mortality: a dose-response analysis in terms of time. *Ann Epidemiol* 2014; 13: 1047-2797.
20. Bebarova M, Matejovic P, Pasek M, Simurdova M, Simurda J. Dual effect of ethanol on inward rectifier potassium current Ik1 in rat ventricular myocytes. *J Physiol Pharmacol* 2014; 65: 497-509.
21. Loguercio C, Federico A. Oxidative stress in alcoholic liver disease: clinical studies. In: Comprehensive Handbook of Alcohol Related Pathology, Vol 2, VR Preedy, R Watson, (eds). Elsevier Academic Press, 2005, pp. 775-783.
22. Roerecke M, Gual A, Rehm J. Reduction of alcohol consumption and subsequent mortality in alcohol use disorders: systematic review and meta-analyses. *J Clin Psychiatry* 2013; 74: 1181-1189.
23. Kasicka-Jonderko A, Jonderko K, Gajek E, Piekielniak A, Zawislan R. Sluggish gallbladder emptying and gastrointestinal transit after intake of common alcoholic beverages. *J Physiol Pharmacol* 2014; 65: 55-66.
24. Uijtdewilligen L, Twisk JW, Singh AS, Chinapaw MJ, van Mechelen W, Brown WJ. Biological, socio-demographic, work and lifestyle determinants of sitting in young adult women: a prospective cohort study. *Int J Behav Nutr Phys Act* 2014; 11: 7.
25. Mereish EH, Bradford JB. Intersecting identities and substance use problems: sexual orientation, gender, race, and lifetime substance use problems. *J Stud Alcohol Drugs* 2014; 75: 179-188.
26. Seidl S, Jensen U, Alt A. The calculation of blood ethanol concentrations in males and females. *Int J Legal Med* 2000; 114: 71-77.
27. Baraona E, Abittan CS, Dohmen K, *et al.* Gender differences in pharmacokinetics of alcohol. *Alcohol Clin Exp Res* 2001; 25: 502-507.

Received: September 19, 2014
Accepted: March 9, 2015

Author's address: Dr. Alessandro Federico, Department of Hepato-Gastroenterology, Faculty of Medicine, Second University of Naples, 5 Via Pansini, 80131 Naples, Italy.
E-mail: alessandro.federico@unina2.it