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The influence of beer with different antioxidant potential on plasma lipids, plasma antioxidant capacity, and bile excretion of rats fed cholesterol-containing and cholesterol-free diets

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Abstract

The aim of this investigation was to assess the influence of beers with different antioxidant potentials on plasma lipid metabolism, plasma antioxidant capacity, and bile excretion of rats fed cholesterol-containing and cholesterol-free diets. Four types of beers were investigated *in vitro*. Two of them (designated as BeerHigh and BeerLow) with the highest and lowest antioxidant potentials (34.5% and 21.4% and 2.07 mmol/L and 1.65 mmol/L according to β-carotene assay and Trolox equivalent antioxidant coefficient, respectively), were chosen for the experiment on rats. A total of 60 male Wistar rats were divided into 6 dietary groups of 10 rats each; the groups were designated as Control, BeerA, BeerB, Chol, Chol/BeerA, and Chol/BeerB. The rats in the Control group were fed a basal diet (BD) only, which included wheat starch, casein, soybean oil, vitamin, and mineral mixtures. To the BD of the other five groups were added the following: BeerHigh (BeerA), BeerLow (BeerB), 1% of cholesterol (Chol), 1% of cholesterol and BeerHigh (Chol/BeerA), and 1% of cholesterol and BeerLow (Chol/BeerB). After 4 weeks of feeding, diets supplemented with BeerHigh and, to a lesser degree, with BeerLow (Chol/BeerA and Chol/BeerB groups) hindered a rise in plasma lipids and a decrease in plasma antioxidant capacity, and increased the bile excretion indices. Supplementation with BeerHigh and, to a lesser degree, with BeerLow in rats fed cholesterol-free diets increased their plasma antioxidant capacity. No significant changes in the plasma lipid levels, antioxidant capacity, and bile excretion indices were observed in the Control group. In conclusion, beer was found to have a positively effect on plasma lipid profile and plasma antioxidant capacity, and to increase the bile excretion indices in rats fed cholesterol-containing diets. The degree of this positive influence is directly connected to the contents of the bioactive components and the related antioxidant potential of beer. It is suggested that to achieve the best results, beer with the highest antioxidant potential must be consumed.

Keywords: Beer; Antioxidant compounds; Antioxidant potential; Plasma lipids and plasma antioxidant capacity; Bile excretion; Rats

1. Introduction

The cardioprotective role of alcoholic beverages in general and of beer in particular has been widely described [1–7]. In our previous reports based on the results of experiments on laboratory animals [8,9] and investigations on patients with heart disease [10,11], we came to the conclusion that moderate beer consumption improves plasma lipid levels and increases plasma antioxidant and plasma anticoagulant activities. However, we did not investigate the influence of beers with different antioxidant potentials on the above-mentioned indices in experiments on laboratory animals. Therefore, we decided to investigate *in vitro* several samples of beers and then to use two of them with the highest and lowest contents of the bioactive compounds and related antioxidant potentials in an experiment on laboratory animals fed cholesterol-containing and cholesterol-free diets.

As mentioned, we found that consumption of beer led to a decrease in plasma levels of lipids [8–11]. We then questioned whether the cholesterol-lowering effect of beer

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is genuine of whether it is instead a redistribution of cholesterol in the animal body. To answer this question we decided to study the effect of beer on bile volume, bile cholesterol, and bile acids in rats fed cholesterol-containing and cholesterol-free diets.

There are many methods for total antioxidant potential determination; each has its limitations [12,13]. It was shown that some antioxidant assay methods give different antioxidant activity trends [14]. Therefore, in the present investigation *in vitro*, two antioxidant assays that complemented each other were used: 1) a total antioxidant potential test using the Trolox equivalent antioxidant coefficient (TEAC) [15]; and 2) an antioxidant assay using a β-carotene linoleate model system (β-carotene) [16]. As far as we know, there have been no such investigations published to date.

2. Methods and materials

2.1. Beer samples

In the in vitro experiment, four commercial beers from four different countries (Bulgaria, Czech Republic, Israel, and the Netherlands) were studied. The aim of this investigation was to choose among them one sample with the highest and another with the lowest content of the bioactive compounds and the related antioxidant potential for the future experiment *in vivo*.

2.2. Total polyphenols determination

The total polyphenols were extracted with ethanol as well as with methanol and ethyl acetate. The beer samples were cooled and filtered under vacuum using Whatman No. 1 (Whatman International Ltd., Whatman House, Ols, UK). The filtrates were evaporated under vacuum at 60°C until 10 mL and then made up to 100 mL by distilled water. Total polyphenols were measured at 765 nm using Folin-Ciocalteu reagent with gallic acid as a standard and were expressed as μg/mL of gallic acid equivalent [17].

2.3. Flavonoid content determination

Total flavonoid content was determined by a colorimetric method. A 0.25-mL quantity of the beer extracts was diluted with 1.25 mL of distilled water; then 75 μL of a 5% $NaNO_2$ solution was added to the mixture. After 6 minutes, 150 μL of a 10%$AlCl_3 \times 6H_2O$ solution was added, and the mixture was allowed to stand for another 5 minutes. A 0.5-mL quantity of 1 mol/L NaOH was added, and the total was made up to 2.5 mL with distilled water. The solution was well mixed, and the absorbance was measured immediately against the prepared blank at 510 nm using a spectrophotometer in comparison with the standards prepared similarly with known (+)-catechin concentrations. The results were expressed as μg/mL of catechin equivalent [18,19].

2.4. Total antioxidant potential determination

As already mentioned, the total antioxidant potential was determined by the following two tests.

2.4.1. Total antioxidant potential test using TEAC

TEAC was done using the relative ability of antioxidant substances to scavenge the 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonate) radical cation ($ABTS^{\bullet+}$). $ABTS^{\bullet+}$ was generated by the interaction of ABTS (250 μmol/L) and $K_2S_4O_8$ (40 μmol/L). After addition of 990 μL of $ABTS^{\bullet+}$ solution to 10 μL of beer (0.2 mg/mL) or Trolox standards (final concentration 0–20 μmol/L) in methanol or phosphate-buffered saline, the absorbance was monitored exactly 1 minute and 6 minutes at 734 nm and compared with Trolox. The higher the TEAC value of the sample the stronger the antioxidant ability. The results were expressed as mmol/L TEAC [15].

2.4.2. Antioxidant assay using β-carotene linoleate model system

According to the β-carotene linoleate model system, β-carotene (0.2 mg) in 0.2 mL of chloroform, linoleic acid (20 mg), and Tween-40 (polyoxyethylene sorbitan monopalmitate) (200 mg) were mixed. Chloroform was removed at 40°C under vacuum, and the resulting mixture was diluted with 10 mL of water and mixed well. To this emulsion was added 40 mL of oxygenated water. Aliquots (4 mL) of the emulsion were pipetted into different test tubes containing 0.2 mL of the beer extracts (50 and 100 ppm) and synthetic antioxidant butylated hydroxyanisole (BHA) in ethanol. BHA was used for comparative purposes. A control containing 0.2 mL of ethanol and 4 mL of the above emulsion was prepared. The tubes were placed at 50°C in a water bath, and the absorbance at 470 nm was taken at zero time ($t = 0$). Measurement of absorbance was continued until the color of β-carotene disappeared in the control tubes ($t =$ 180 minutes) at an interval of 15 minutes. A mixture prepared as above without β-carotene served as a blank. The antioxidant potential of the extracts was evaluated in terms of bleaching of the β-carotene using the following formula: antioxidant potential $= 100\ [1 - (A_0 - A_t)/(A°_0 - A°_t)]$, where A_0 and $A°_0$ are the absorbance values measured at zero time of the incubation for test sample and control, respectively, and At and $A°t$ are the absorbance measured in the test sample and control, respectively, after incubation for 180 minutes. The results were expressed as percentage of antioxidant potential [16].

2.5. Rats and diets

The Animal Care Committee of the Warsaw Agricultural University had approved this experiment, in which the previously successfully proven protocol was used [20].

Wistar rats ($n = 60$), with a mean weight of 100 g at the beginning of the experiment, were provided by the Institute of Animal Physiology and Nutrition of the Polish Academy of Sciences (Jablonna, Poland). They were housed in individual plastic cages in the air-conditioned room (temperature 21–22°C and relative humanity 55–65%). These rats were divided into six dietary groups of 10 animals each, designated as Control, BeerA, BeerB, Chol, Chol/BeerA, and Chol/BeerB. Rats in the Control group were fed the basal diet (BD) only, which included wheat starch, casein, soybean oil, vitamin and mineral mixtures. To the BD of the other five groups were added the following: BeerHigh (BeerA), BeerLow (BeerB), 1% of cholesterol (Chol), 1% of cholesterol and BeerHigh (Chol/BeerA), and 1% of cholesterol and BeerLow (Chol/BeerB). To acclimate rats to the maximal quantity of beer or water (2 mL), the first week every animal received 1 mL, the second week 1.5 mL, and the last 2 weeks of the trial 2 mL of beer (BeerA, BeerB, Chol/BeerA and Chol/BeerA) or of water (Control and Chol) per day.

Cholesterol of analytical grade (USP) was obtained from Sigma Chemical (St. Louis, MO) and checked with high-performance liquid chromatography [21]. No cholesterol oxides were found. The cholesterol batches were mixed carefully with the BD (1:99) just before the diets were offered to the rats.

The diets contained as percentage of energy 67% of carbohydrates, 24% of protein, and 9% of fat. The calculated energy of the used diets was from 394.5 to 399.1 kcal/100 g, a difference that was statistically not significant.

All rats were fed once a day at 10 AM ad libitum. Access to drinking water was unrestricted. The food intake and body gains were monitored daily.

It is generally accepted that the most reliable data for blood lipid levels can be obtained from fasting animals, 14–16 hours after the last feeding. Therefore, the food was removed from the cages at 6 PM 1 day before and the samples were collected at 9 AM the next day.

Two time points were used in this experiment: before and after 28 days of feeding. Before the experiment, blood samples were taken from the tail vein. At the end of the trial, the rats were anesthetized by intraperitoneal urethane narcosis and blood samples were taken from the left atrium of the heart. Plasma was prepared and used for laboratory investigations. A range of laboratory tests was performed to determine the following: plasma total cholesterol (TC), low-density lipoproteins (LDL-C), high-density lipoproteins (HDL-C), triglycerides (TG), and total phospholipids (TPH).

At these time points bile was collected as previously described [20]; using the same urethane narcosis and volume, bile acids and bile cholesterol concentrations were determined [20].

2.6. Statistical analysis

The results of the investigation in vitro were expressed as means ± SD of five measurements. To compare several groups analysis of variance was used. Values of $P < 0.05$ were considered to be statistically significant.

3. Results

3.1. In vitro experiments

The contents of the main components in the Beer1 and Beer4 were as follows: a) total proteins (g/L) 5.3 ± 0.5 and 5.2 ± 0.5, respectively; b) sugars (% on dry substance): glucose, 0.9 ± 0.1 and 0.8 ± 0.1, respectively; maltose, 38.5 ± 3.2 and 37.8 ± 3.1, respectively; maltotriose, 30.5 ± 3.1 and 30.7 ± 3.1, respectively; dextrins, 30.1 ± 3.2 and 30.7 ± 3.2 respectively; and c) alcohol (% volume) 5.3 ± 0.5 and 5.2 ± 0.5, respectively ($P > 0.05$ in all cases). Therefore, the contents of the studied compounds in two beers were comparable.

The results of the determination of the content of some other bioactive compounds and the related antioxidant potential of all four studied beers are summarized in Table 1. As can be seen, Beer 1 has the highest and Beer 4 the lowest content of bioactive compounds and antioxidant potential. Beers 1 and 4 were chosen for the experiment *in vivo* and named BeerHigh and BeerLow, respectively.

3.2. In vivo experiments

The weight gains, food consumption and food efficiency in all groups of rats are summarized in Table 2. As can be seen, all three of the above-mentioned indices were higher in the rats of the Chol/BeerA group, but the differences were no significant.

BeerHigh- and, to a lesser degree, BeerLow-supplemented

Table 1
Antioxidant compounds and antioxidant potential of four studied beers

Sample	Total polyphenols (μg/mL)	Flavonoids (μg/mL)	β-carotene (% AA)	ABTS (mmol/L TEAC)
Beer 1	668.0 ± 52.2[a]	52.6 ± 4.1[a]	34.5 ± 2.8[a]	2.07 ± 0.2[a]
Beer 2	647.4 ± 51.9[a]	29.5 ± 2.6[b]	33.6 ± 2.7[a]	2.00 ± 0.2[a]
Beer 3	552.6 ± 47.3[b]	27.8 ± 2.4[b]	23.3 ± 2.1[b]	1.72 ± 0.1[a]
Beer 4	442.0 ± 38.9[c]	13.4 ± 1.2[c]	21.4 ± 2.1[b]	1.65 ± 0.1[b]

Values are means ± SD of five measurements. Means in columns without superscript letters in common differ significantly.

Table 2
Weight gains, food consumption, and food efficiency in all groups of rats

Group	Weight Gain (g/day)	Food Consumption (g/day)	Beer or Water Consumption (mL/day)*	Feed Efficiency Ratio
Control	5.05 ± 0.51[a]	16.33 ± 2.44[a]	1–2	0.31 ± 0.03[a]
Chol	4.76 ± 0.48[a]	15.85 ± 0.97[a]	1–2	0.30 ± 0.03[a]
Beer A	4.29 ± 0.43[a]	15.31 ± 2.33[a]	1–2	0.28 ± 0.03[a]
Beer B	5.31 ± 0.53[a]	16.50 ± 2.06[a]	1–2	0.32 ± 0.03[a]
Chol/Beer A	5.61 ± 0.99[a]	16.71 ± 0.89[a]	1–2	0.33 ± 0.03[a]
Chol/Beer B	5.41 ± 1.07[a]	17.69 ± 1.77[a]	1–2	0.31 ± 0.03[a]

Values are means ± SD (n = 10). Means in columns without superscript letters in common differ significantly.

* To get rats used to the maximal quantity of beer and water (2 mL) the first week every animal got 1 mL, the second week 1.5 mL, and the last 2 weeks of the trial 2 mL of beer for BeerA, BeerB, Chol/BeerA and Chol/BeerB groups and water for Control and Chol groups, respectively, per day.

Chol = cholesterol.

diets in rats fed added cholesterol significantly hindered the rise of plasma lipids (Table 3): a) TC, 25.7% and 23.2%, respectively; b) LDL-C, 39.6% and 35.5%, respectively; c) TG, 16.3% and 14.0%, respectively; and d) TPH, 24.9% and 21.4%, respectively. No significant changes in the concentrations of HDL-C were found.

Differences in bile volume, bile cholesterol, and bile acid concentrations before the experiment in all groups of rats were not significant (Figs. 1, 2, and 3, respectively). The changes in these indices after 4 weeks of different feeding are shown in Figs. 1, 2, and 3, respectively. As can be seen, after 4 weeks of the experiment, bile volume was increased significantly in all groups of rats whose diets were supplemented with either beer (Fig. 1).

The increases in bile cholesterol concentration were significant in groups of rats fed cholesterol (Chol, Chol/BeerA, and Chol/BeerA) and in the BeerA group. However, the most significant increase was in the rats in the Chol/BeerA group, whose diet was supplemented with the beer with the highest antioxidant potential (Fig. 2).

The increase in bile acid concentration was significant in groups of rats fed cholesterol (Chol, Chol/BeerA, and Chol/BeerA) and in the BeerA group. As in the case of bile cholesterol, the most significant increase in the bile acid concentration was in the rats of the Chol/BeerA group, whose diet was supplemented with the beer of the highest antioxidant potential (Fig. 3).

The plasma antioxidant capacity in rats of all groups before the experiment was without significant difference (Figs 4 and 5).

The changes in the plasma antioxidant capacity in rats of all groups are presented in Figs. 4 and 5. As can be seen in Fig. 4, supplementation of BeerHigh and, to a lesser degree, of BeerLow to the diets of rats fed cholesterol-free diet (BeerA and BeerB diet groups) increased the plasma antioxidant capacity (an increase in the TRAP and a decrease in MDA values).

Figure 5 shows the changes in the plasma antioxidant capacity in the groups of rats fed cholesterol. As can be seen, after 4 weeks of feeding, the plasma antioxidant capacity in the rats of all three groups (Chol, Chol/BeerA, and Chol/BeerB) was decreased (a decrease in the TRAP and an

Table 3
Plasma lipids (mmol/L) in of rats fed diets with and without 1% cholesterol (Chol) and with and without beers

Diet	TC	LDL-C	HDL-C	TG	TPH
Control	2.81 ± 0.15[c]	1.17 ± 0.05[c]	1.63 ± 0.07[a]	0.70 ± 0.04[b]	1.70 ± 0.08[a]
Chol	4.78 ± 0.21[a]	3.13 ± 0.12[a]	1.64 ± 0.07[a]	0.86 ± 0.05[a]	1.73 ± 0.08[a]
Beer A	2.77 ± 0.15[c]	1.12 ± 0.05[c]	1.65 ± 0.07[a]	0.71 ± 0.05[b]	1.70 ± 0.08[a]
Beer B	2.80 ± 0.15[c]	1.15 ± 0.05[c]	1.64 ± 0.07[a]	0.70 ± 0.05[b]	1.71 ± 0.08[a]
Chol/Beer A	3.55 ± 0.18[b]	1.89 ± 0.05[b]	1.65 ± 0.07[a]	0.72 ± 0.05[b]	1.30 ± 0.06[b]
Chol/Beer B	3.67 ± 0.18[b]	2.02 ± 0.05[b]	1.64 ± 0.07[a]	0.74 ± 0.05[b]	1.36 ± 0.06[b]
P value (2-way ANOVA)					
Beer A	NS	NS	NS	NS	NS
Beer B	NS	NS	NS	NS	NS
Chol	<0.001	<0.001	NS	<0.001	NS
Chol/Beer A	<0.050	<0.050	NS	<0.050	<0.01
Chol/Beer B	<0.050	<0.050	NS	<0.050	<0.01

Values are means ± SD (n = 10). Means in columns without superscript letters in common differ significantly ($P < 0.05$).

Chol = cholesterol; HDL-C = HDL cholesterol; LDL-C = LDL cholesterol; NS = not significant; TC = total cholesterol; TG = triglycerides; TPH = total phospholipids.

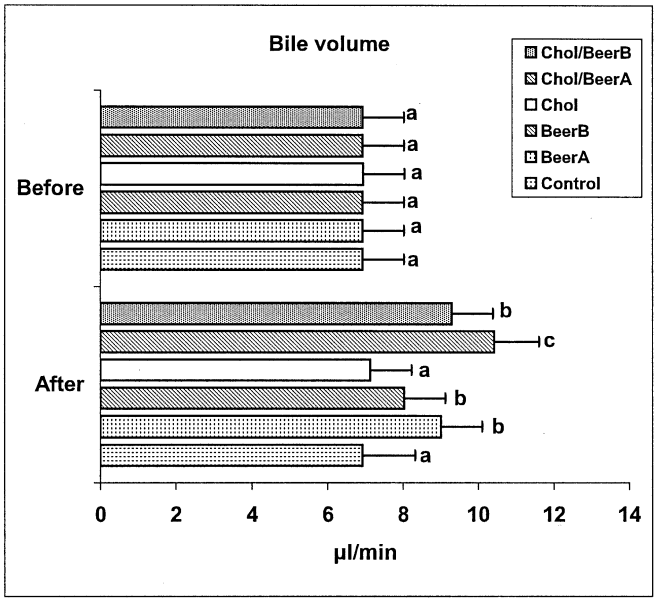

Fig. 1. Bile volume changes in rats fed cholesterol-free and cholesterol-containing diets. Mean ± SD (horizontal lines). Bars with different letters are significantly different ($P < 0.05$).

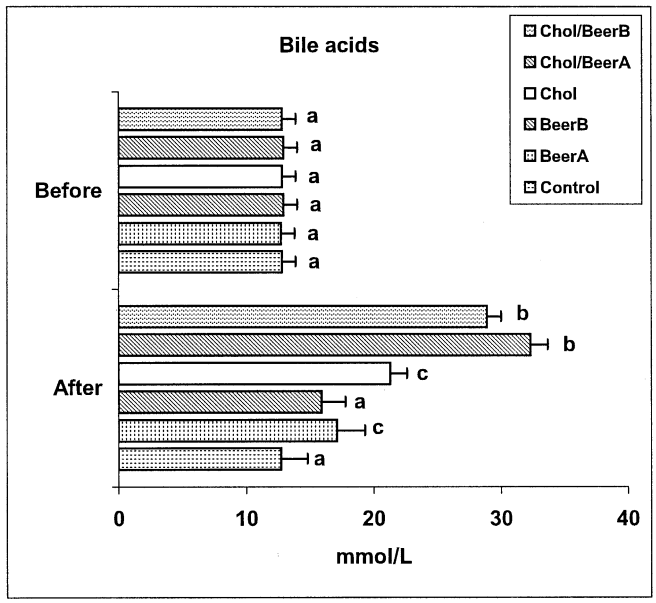

Fig. 3. Bile acids concentration changes in rats fed cholesterol-free and cholesterol-containing diets. Mean ± SD (horizontal lines). Bars with different letters are significantly different ($P < 0.05$).

increase in MDA values). However, this decrease was less significant in the groups of rats whose diets were supplemented with BeerHigh and, to a lesser degree, with BeerLow.

4. Discussion

It was shown in numerous epidemiological studies [22–26], in some experiments on laboratory animals [8,9,27] as well as investigations of human subjects [10,11,28,29] that moderate consumption of alcoholic beverages leads to an increase in the level of TC, LDL-C, TG, and apolipoprotein B, and to increase in plasma anticoagulant and antioxidant activities. It was also shown that the phenolics are the main bioactive components of alcoholic beverages and that these compounds contribute substantially to the antioxidant potential [29–31] of the beverages. Therefore, we selected for the *in vitro* study different kinds of beer: one with the highest and one with the lowest antioxidant potential, and investigated their influence on plasma lipid levels, plasma antioxidant activity, and bile excretion indices in rats fed cholesterol-containing and cholesterol-free diets. As far as we know, no such experiments have been conducted.

We found that the results of beer samples investigated *in vitro* were in full greement with the data of others [32,33].

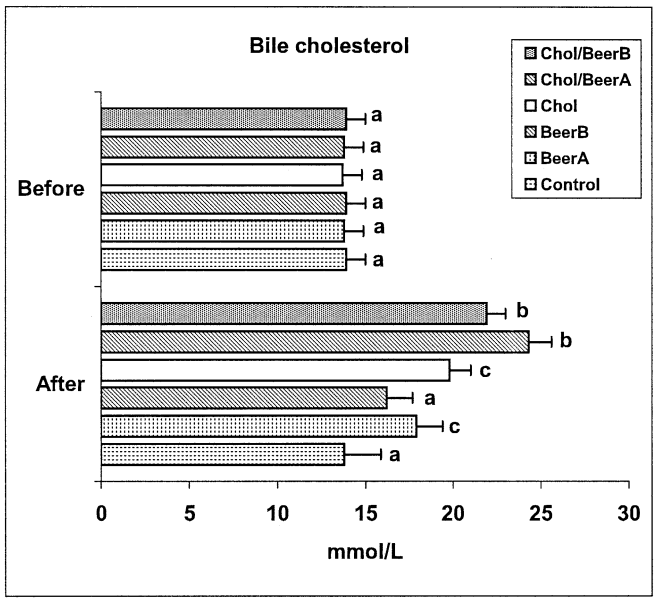

Fig. 2. Bile cholesterol concentration changes in rats fed cholesterol-free and cholesterol-containing diets. Mean ± SD (horizontal lines). Bars with different letters are significantly different ($P < 0.05$).

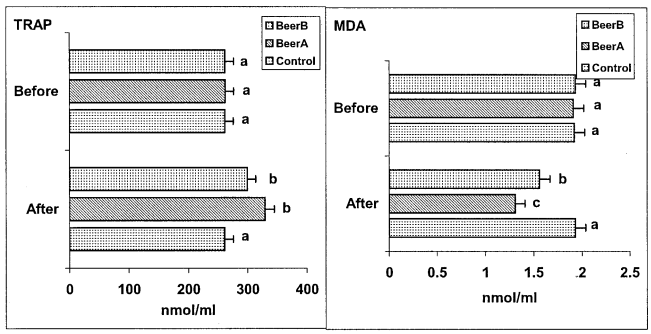

Fig. 4. Total radical-trapping antioxidative potential (TRAP) and malondialdehyde lipid peroxidation test (MDA) in rats fed diets without added cholesterol before and after the 4-week feeding period. Mean ± SD (horizontal lines). Bars with different letters are significantly different ($P < 0.05$).

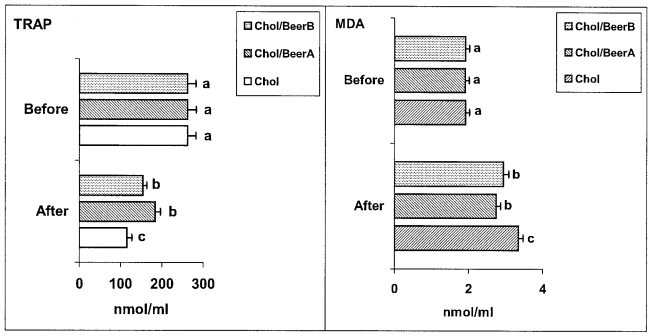

Fig. 5. Total radical-trapping antioxidative potential (TRAP) and malondialdehyde lipid peroxidation test (MDA) values in rats fed with added cholesterol before and after 4 weeks of feeding period. Mean ± SD (horizontal lines). Bars with different letters are significantly different ($P < 0.05$).

We found that BeerHigh- and, to a lesser degree, BeerLow-supplemented diets significantly hindered the rise in the plasma lipid levels in the Chol/BeerA and Chol/BeerB groups versus the Chol group, which were increased due to dietary cholesterol.

These results were predictable. It could be expected that beer-supplemented diet containing a high concentration of antioxidant compounds would positively influence plasma lipid levels [8,9].

It must be pointed out that improvement in the plasma lipid levels was observed only in the groups of rats fed cholesterol-containing diets. These results are consistent with those obtained by others [34]. It was shown [34] that natural, lipid-lowering products are effective only in cases of hyperlipidemia, both in experiments on laboratory animals [8,9] and in investigations on human subjects [10,11,35]. The results of our previous experiments on laboratory animals and investigations in human subjects are in accordance with these data [8–11].

The cholesterol-lowering effect in groups of rats fed cholesterol-free diets (BeerA and BeerB) was statistically not significant. Also these results are in accordance with the results of others, who have found that the cholesterol lowering effect could be observed only in animals fed cholesterol [34].

In our previous investigations we have demonstrated that alcohol beverages positively influenced the plasma lipid levels in rats [8,9]. However, we could not prove whether this effect is genuine or whether it is a redistribution of cholesterol in the animal body. Therefore, in this experiment we studied the changes in bile volume, bile cholesterol, and bile acid concentrations.

A significantly increase in bile cholesterol and bile acids concentrations in the Chol/BeerA and Chol/Beer groups versus the Chol group was noted. Therefore it can be supposed that the cholesterol-lowering effect of beer is genuine.

It was found that both beer used have increased the plasma antioxidant capacity in rats fed cholesterol-free diets (BeerA and BeerB). These results are also in accordance with the results of others and our own previous investigations [8,9,28,33].

Some researchers have shown that the cholesterol-containing diets leads to a decrease in the plasma antioxidant activity [36,37]. The results of our present investigation are in accordance with the results of the above-mentioned authors: the plasma antioxidant capacity in the rats of Chol, Chol/BeerA, and Chol/BeerB dietary groups after completion of the experiment was decreased. However, in the Chol/BeerA and Chol/BeerB groups versus the Chol groups, this decrease was less significant due to the antioxidant potential of the supplementary beers.

No significant changes in all studied indices were found in the Control group.

In conclusion, in this study, beer was found to have a positive effect on plasma lipid profile and plasma antioxidant capacity, and to increase bile volume, bile cholesterol, and bile acid concentrations mainly in rats fed cholesterol-containing diets. The degree of this positive influence of beer is directly connected to the bioactive compounds in beer. Therefore, it could be suggested that to receive the best results in terms of health benefits, beer with highest antioxidant potential must be consumed.

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