

HOW CAN BEIQISHEN TEA CONSUMPTION INFLUENCE REDOX HOMEOSTASIS IN EXPERIMENTAL HYPERLIPIDEMY?

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In general, biologically active polyphenolic compounds have indirect lipid lowering effects, antioxidant properties and they can also eliminate the accumulated toxic metal elements in the liver in cases of hyperlipidemy. Because of all these, we studied the effect of Beiqishen tea on altered lipid metabolisms, redox parameters and on liver metal contents. Although it was expected, there was not any liver protecting activity of the Beiqishen tea extract in the hyperlipidemic rat model, which was proved by biochemical and morphological studies. The reason for our expectation was the significant polyphenol content of Beiqishen tea. Although, the detoxifying activity of tea polyphenols could be observed in hyperlipidemy, at the same time toxic metal element content (As, Mo, Ni, Pb and Cr) of the tea infusion accumulated in the liver in both normo- and hyperlipidemic rats.

Keywords: antioxidants, trace elements, redox homeostasis, Beiqishen tea

Hyperlipidemy, fatty liver and gallstone disease are significant problems in Western countries (TROTMAN, 1985; SZENTMIHÁLYI et al., 2002). Redox homeostasis is impaired in fatty liver and the balance between free radicals and antioxidant defence tips over in the direction of free radicals (BLÁZOVICS et al., 1992). In general, antioxidant and immunostimulant natural polyphenols and flavonoids may act as lipid lowering factors through immune reactions and modulating prostaglandin biosynthesis, therefore, the consumption of tea mixtures, as a natural source of bioactive compounds has become popular (POLYA et al., 2002). In fatty liver, due to the change in metal homeostasis, toxic elements are accumulated. Toxic metal load can be prevented by polyphenols, as well (BLÁZOVICS et al., 2003; SZENTMIHÁLYI et al., 2004). The hyperlipidemic rat model may also be suitable for studying the problem depending on fattening (BLÁZOVICS et al., 1992).

In our previous papers, the composition of Beiqishen tea has been determined (HÉTHELYI et al., 2001; BLÁZOVICS et al., 2003; 2004). According to the information sheet of Beiqishen tea, it may be assumed that its active compounds have antioxidant

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and/or free radical scavenger properties and these molecules may influence or modify the regulation of the cell cycle.

The root and stalk of *Astragalus membranaceus*, *Ganoderma lucidum* (mushroom) and the fruit of *Lycium barbarum* (Chinese wolfberry) as ingredients were identified by Medline analysis (HÉTHELYI et al., 2001).

Total polyphenol content of the tea drug was 20.77 ± 0.52 g/100 g drug, total flavonoid content 0.485 ± 0.036 g/100 g drug and tannins 9.063 ± 0.782 g/100 g drug. Total polyphenol content of the tea extract was 83.2 mg/100 ml. Significant amount of quercetin and kaempferol could be detected (0.75 and 1.99 mg/100 ml, respectively). Antioxidant or scavenger properties of the Beiqishen tea infusion were identified as a function of concentration. The tea exerted proton-donating ability in the presence of 1,1-diphenyl-2-picryl-hydrazyl (DPPH) stable radical. The amount required for 50% inhibition of the colour development of the DPPH is $I_{50} = 0.10$ ml of tea extract. In the same system the value for ascorbic acid is $I_{50} = 0.013$ mg and for trolox $I_{50} = 0.018$ mg. The exhibited reducing power of the tea sample was measured on the basis of the Fe(III) to Fe(II) redox reaction. Reducing power was expressed in ascorbic acid equivalent (ASE/ml). ASE/ml=1 ml sample exhibited the same reducing power as 7.78 mmol ascorbic acid. The chelating activity of 1 ml of Beiqishen tea extract was 1.86 ± 0.01 absorbance ratio at 484 vs. 530 nm. Strong scavenger capacity against oxygen free radicals was also verified ($>99\%$ in 0.1 ml volume) in a medium consisting of hydrogen peroxide and hydroxyl radicals. Total antioxidant status was 8.7 mmol l^{-1} trolox equivalent, in correspondence with chemiluminescent results. Essential oils (+)-limonene (21%), *p*-cymene (1.7%), estragol (3.2%), *b*-ocimene (1.4%), thymol (2.6%) were identified by gas chromatography. Caffeine was the predominant compound responsible for its refreshing effect (HÉTHELYI et al., 2001; BLÁZOVICS et al., 2004).

Metal ion analysis showed high Al, As, B, Ba, Co, Cr, Cu, Fe, Mn, Ni and Ti content in tea drug sample and Al, B, Cr, Co, Fe, Mn and Ni content in aqueous extract compared to other tea extracts (BLÁZOVICS et al., 2003). Since metal ions take part in free radical reactions, accumulation of these elements may be harmful in vivo.

1. Materials and methods

1.1. Materials

1,1-Diphenyl-2-picryl-hydrazyl stable radical, luminol, microperoxidase and hydrogen peroxide were obtained from SIGMA (St. Louis, USA). Merck ICP standard was obtained from Merck (Darmstadt, Germany), and all other reagents were purchased from Reanal Chemical Co. (Budapest, Hungary).

1.2. Drug

Beiqishen tea (Daxing' anling Beiqishen Healthy Food Co. Ltd., Permission number No. 022; 1998) obtained from the commercial network was examined to determine its

antioxidant property and to control its main active components. Based on prescription, this product was made from the roots, tender stalks, leaves and flowers of wild *Astragalus mongolicus* (growing in Danxing' anling) supplemented with Glossy ganoderma and the fruit of Chinese wolfberry. Recommended dosage is one bag three times a day by immersing the bag in boiling water for five minutes. These active compounds have a beneficial effect in several diseases (both *Astragalus membranaceus* and *A. mongolicus* were given by Beiqishen Healthy Food Co. Ltd. in the same product).

1.3. Tea making

Ten packets of tea taken from different boxes of Beiqishen tea drug were mixed, and then 2 g were infused with 250 ml of boiling double distilled water and allowed to stand for 30 min.

1.4. Hyperlipidemic rats

Young male Wistar albino rats (150–200 g bw) were used in the experiment. The animals were divided into four groups, each consisting of 10 animals. The animals in group I were fed with normal diet obtained from BIOFARM PROMT Kft (BFP, Gödöllő, Hungary). The animals in group II were fed with the same normal diet and treated with Beiqishen tea infusion (2 g tea drug/kg bw). (See above!) The rats were kept on the diets for 14 days. The animals of the third group were fed with fat rich diet containing cholesterol (2.0%), sunflower oil (20%) and cholic acid (0.5%) added to the control BFP. The animals in the fourth group were fed with lipogenic diet and treated with Beiqishen tea (2 g tea drug/kg body weight).

1.5. Experimental protocol

The rats were anaesthetised with Nembutal (35 mg kg⁻¹). After laparotomy, blood was collected from the abdominal vein and then the animals were exsanguinated.

1.6. Histopathological method

Specimens of the liver were prepared for microscopy. Small pieces of the organ were fixed in 4% paraformaldehyde.

1.7. Determination of element concentration

Element concentration in liver samples was determined by an ICP-OES (inductively coupled plasma optical emission spectrometry). Type of instrument: Atom Scan 25 (Thermo Jarrell Ash), a sequential plasma emission spectrometer.

After digestion with a mixture of nitric acid (5 ml) and hydrogen peroxide (2 ml), the samples (1 g) were diluted to 25 ml with deionised water. The concentration of elements (Al, As, B, Ba, Ca, Cd, Co, Cr, Cu, Fe, Hg, K, Li, Mg, Mn, Mo, Na, Ni, P, S, Ti, V, Zn) was determined (SZENTMIHÁLYI et al., 2002).

1.8. H-donating ability

H-donating ability was determined by BLOIS's method (1958) in the presence of a DPPH radical. Absorbance of the methanolic DPPH-dye was assessed spectrophotometrically at 517 nm. For characterization of the activity, DPPH degradation was expressed in the percentage of inhibition.

1.9. Reducing power

OYAZU's method (1986) was adopted for analysis of the reducing power property. The change in absorbance was measured, which accompanied Fe(III)–Fe(II) transformation at 700 nm, and the reducing power property was compared to that of ascorbic acid.

1.10. Total scavenger capacity

Total scavenger capacity was determined by chemiluminescence method (BLÁZOVICS et al., 1999) in a $\text{H}_2\text{O}_2/\cdot\text{OH}$ -luminol-microperoxidase system. Total volume of the reaction mixture system was 1 ml. Chemiluminescence light intensity given in RLU (relative light unit) is reduced in the presence of free radical scavenger compounds.

1.11. Diene conjugates

Diene conjugates were measured by the A.O.A.C. method (1984).

1.12. Protein measurement

Protein concentration was measured by the method of LOWRY and co-workers (1951). Ten mg ml^{-1} protein content of tissue homogenate was used.

This study was approved by the Regional Committee of Science and Research Ethics Semmelweis University, Permission Number TUKEB 24/1996.

1.13. Mathematical statistical analysis

All values are presented as $\text{mean} \pm \text{SD}$. The differences between two groups were examined by Student's *t*-test. The differences were considered statistically significant at $P < 0.05$. Pearson's correlation matrix was used to evaluate the correlation between measured parameters. For comparison of the mean values of metal elements, one-way analysis ANOVA was used by Graph Pad software (version 1.14, 1990).

2. Results and discussion

Histopathological investigation of the liver in alimentary induced hyperlipidemy showed characteristic pathomorphological changes, centrilobular fatty degeneration, balloon cell degeneration, necrosis and vacuolisation in comparison to normal liver structure. Decrease in the glycogen content of the hepatic cells was considered as the

first sign of liver injury. Beiqishen tea treatment causes moderate changes in the liver tissue in normolipidemic animals and the necrotic process of the liver in hyperlipidemic animals was not arrested. Lymphocyte infiltration was observed during tea treatment (Fig. 1). The arrows point to the area infiltrated by lymphocytes.

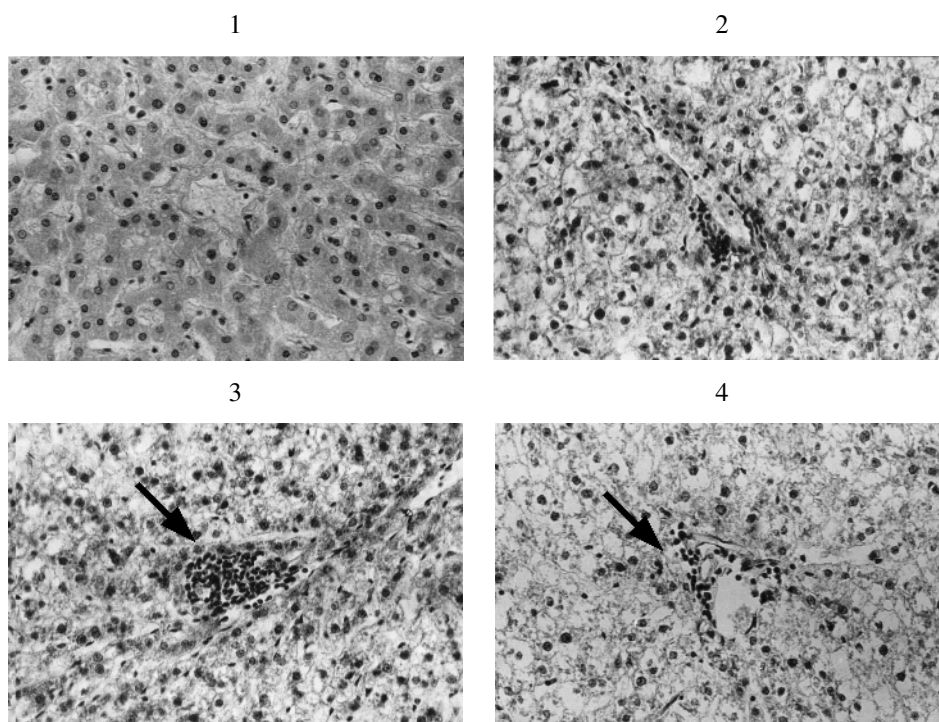


Fig. 1. Morphological picture. 1: Normolipidemy; 2: hyperlipidemy; 3: normolipidemy + tea; 4: hyperlipidemy + tea

Chromium and Mn concentration in the tissue was found to be elevated, whereas Fe, Mg, S, P and Zn concentration was reduced and As, Co, Mo, Ni and Pb appeared in the fatty liver. Parallel with these findings, lipid droplets accumulated in the tissue and necrotic lesions were observed. The concentration of toxic metal elements was under the detection limit in the normolipidemic group. As a result of Beiqishen tea consumption, As, Co, Mo and Ni were detected and the concentration of Mn and Fe was elevated in the liver of the normolipidemic group. In the liver of hyperlipidemic rats treated with Beiqishen tea, the concentration of Mg, S, P and Zn was favourably increased, while the liver was unable to eliminate totally toxic elements As, Ni and Pb. Elevated essential Mg, S, P and Zn content indirectly suggests the formation of metallothionein scavenger proteins (Table 1).

Table 1. Element concentration in liver homogenates

Elements	Normolipidemy	Normolipidemy + tea ($\mu\text{g g}^{-1}$)(mean $\pm$ SD)	Hyperlipidemy	Hyperlipidemy + tea	Significance P<0.05
As	<0.150	0.427 $\pm$ 0.152	0.438 $\pm$ 0.281	0.358 $\pm$ 0.101	s
Ca	27.78 $\pm$ 12.41	31.38 $\pm$ 3.70	30.23 $\pm$ 3.33	28.29 $\pm$ 11.44	ns
Co	<0.015	0.0484 $\pm$ 0.0097	0.216 $\pm$ 0.027	0.0487 $\pm$ 0.0054	s
Cr	0.103 $\pm$ 0.024	0.084 $\pm$ 0.033	0.156 $\pm$ 0.013	0.091 $\pm$ 0.018	s
Cu	2.03 $\pm$ 0.22	2.14 $\pm$ 0.12	2.10 $\pm$ 0.11	2.13 $\pm$ 0.17	ns
Fe	37.84 $\pm$ 8.41	52.83 $\pm$ 11.26	34.24 $\pm$ 12.86	39.71 $\pm$ 12.90	ns
K	4672 $\pm$ 198	4588 $\pm$ 125	4515 $\pm$ 126	4405 $\pm$ 174	ns
Mg	258.7 $\pm$ 11.9	254.1 $\pm$ 8.4	238.5 $\pm$ 14.2	392.3 $\pm$ 36.5	s
Mn	0.995 $\pm$ 0.113	1.161 $\pm$ 0.172	1.113 $\pm$ 0.125	1.314 $\pm$ 0.258	s
Mo	<0.025	0.353 $\pm$ 0.157	0.215 $\pm$ 0.022	0.202 $\pm$ 0.061	s
Na	201.9 $\pm$ 12.2	209.5 $\pm$ 3.1	116.2 $\pm$ 14.2	152.4 $\pm$ 22.9	s
Ni	<0.025	0.212 $\pm$ 0.132	1.270 $\pm$ 0.073	1.078 $\pm$ 0.057	s
P	2034 $\pm$ 65	2021 $\pm$ 28	1876 $\pm$ 41	3405 $\pm$ 157	s
Pb	<0.150	<0.150	0.436 $\pm$ 0.055	<0.150	ns
S	1531 $\pm$ 135	1457 $\pm$ 96	1288 $\pm$ 124	2322 $\pm$ 123	s
Zn	16.11 $\pm$ 3.17	16.71 $\pm$ 2.48	14.86 $\pm$ 1.51	26.89 $\pm$ 8.41	s

s: significant

ns: not significant

Table 2. Effect of Beiqishen tea on serum parameters in rats

Groups (n=10)	ALP (U l ⁻¹)	GPT (U l ⁻¹)	GOT (U l ⁻¹)	GGT (U l ⁻¹)
	(mean $\pm$ SD)			
Normolipidemy	785.2 $\pm$ 88.9	53.4 $\pm$ 12.2	192.4 $\pm$ 95.47	1.75 $\pm$ 0.96
Hyperlipidemy	1861.0 $\pm$ 239.9	78.2 $\pm$ 47.9	162.3 $\pm$ 42.0	2.25 $\pm$ 1.89
Normolipidemy + tea	710.0 $\pm$ 63.8	53.2 $\pm$ 14.7	156.0 $\pm$ 55.0	1.66 $\pm$ 0.57
Hyperlipidemy + tea	1756.6 $\pm$ 381.7	124.3 $\pm$ 46.2	201.0 $\pm$ 62.5	2.66 $\pm$ 0.57
Significance	1 vs. 2 s	1 vs. 2 ns	1 vs. 2 ns	1 vs. 2 ns
P<0.05	1 vs. 3 s	1 vs. 3 ns	1 vs. 3 ns	1 vs. 3 ns
	1 vs. 4 s	1 vs. 4 s	1 vs. 4 ns	1 vs. 4 s
	2 vs. 3 s	2 vs. 3 ns	2 vs. 3 ns	2 vs. 3 ns
	2 vs. 4 ns	2 vs. 4 s	2 vs. 4 ns	2 vs. 4 ns
	3 vs. 4 s	3 vs. 4 s	3 vs. 4 ns	3 vs. 4 s

s: significant

ns: not significant

After tea consumption for 2 weeks, the concentration of serum glucose and uric acid increased in normolipidemic rats, although these parameters did not change in two of the animals. In hyperlipidemy the significantly changed enzyme activities did not improve during tea consumption. The values of GPT, CHOL, TBIL and BUN were worse, but the difference was not significant (Tables 2–4). On the basis of literature data, positive correlation could be determined between the concentration of serum uric acid and reducing power, although no beneficial effect could be observed. The level of

diene conjugates of the liver was higher in Beiqishen treated animals, which impaired the total scavenger capacity in both dietary groups (Figs 2, 3). Reducing power and H-donating ability of the liver lowered in normolipidemic + tea treated animals, but some alterations were detected in hyperlipidemic + tea treated rats, for only the H-donating ability was lower (Figs 4, 5). In the serum, no alterations were observed in the redox parameters measured in tea treated animals compared to the (equivalent) controls in this “short term” experiment (Figs 6, 7).

Table 3. Effect of Beiqishen tea on serum parameters in rats

Groups (n=10)	CHOL (mmol l ⁻¹)	TG (mmol l ⁻¹)	GLU (mmol l ⁻¹)	AMY (U l ⁻¹)
	(mean ± SD)			
Normolipidemy	1.876±0.253	2.078±0.797	12.76±8.81	2909±826
Hyperlipidemy	7.144±1.431	0.690±0.135	9.73±1.21	2341±284
Normolipidemy + tea	2.056±0.282	1.432±0.519	19.17±9.73	3100±310
Hyperlipidemy + tea	8.010±1.920	1.209±0.889	14.77±9.37	2498±174
Significance	1 vs. 2 s	1 vs. 2 s.	1 vs. 2 ns	1 vs. 2 ns
P<0.05	1 vs. 3 ns	1 vs. 3 ns	1 vs. 3 ns	1 vs. 3 ns
	1 vs. 4 s	1 vs. 4 s	1 vs. 4 ns	1 vs. 4 ns
	2 vs. 3 s	2 vs. 3 s	2 vs. 3 s	2 vs. 3 s
	2 vs. 4 ns	2 vs. 4 s	2 vs. 4 ns	2 vs. 4 ns
	3 vs. 4 s	3 vs. 4 ns	3 vs. 4 s	3 vs. 4 s

s: significant

ns: not significant

Table 4. Effect of Beiqishen tea on serum parameters in rats

Groups (n=10)	TBIL (μmol l ⁻¹)	UA (μmol l ⁻¹)	BUN (mmol l ⁻¹)	CRE (μmol l ⁻¹)
	(mean ± SD)			
Normolipidemy	2.40±0.54	107±11	4.27±0.15	37.8±7.01
Hyperlipidemy	3.40±2.70	152±41	3.30±0.96	50.4±10.2
Normolipidemy + tea	2.40±0.54	364±219	4.46±0.63	49.2±8.2
Hyperlipidemy + tea	4.66±2.08	363±340	6.06±1.44	47.6±1.8
Significance	1 vs. 2 ns	1 vs. 2 s	1 vs. 2 s	1 vs. 2 s
P<0.05	1 vs. 3 ns	1 vs. 3 s	1 vs. 3 ns	1 vs. 3 s
	1 vs. 4 s	1 vs. 4 s	1 vs. 4 s	1 vs. 4 s
	2 vs. 3 ns	2 vs. 3 s	2 vs. 3 s	2 vs. 3 ns
	2 vs. 4 ns	2 vs. 4 ns	2 vs. 4 s	2 vs. 4 ns
	3 vs. 4 s	3 vs. 4 ns	3 vs. 4 s	3 vs. 4 ns

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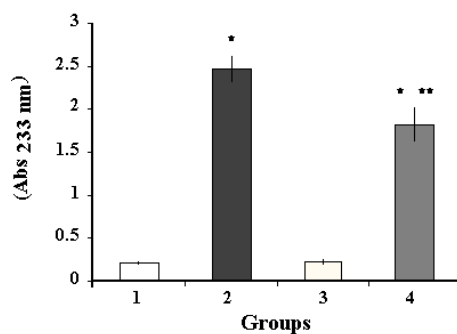


Fig. 2. Diene conjugates in the liver. 1: Normolipidemy; 2: hyperlipidemy; 3: normolipidemy + tea; 4: hyperlipidemy + tea (significance $P < 0.05$: * vs. normolipidemy; ** vs. hyperlipidemy)

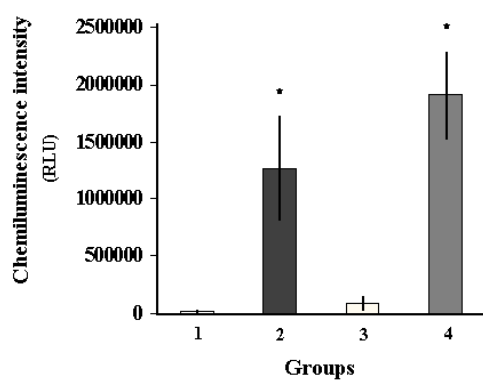


Fig. 3. Chemiluminescence intensity in the liver. 1: Normolipidemy; 2: hyperlipidemy; 3: normolipidemy + tea; 4: hyperlipidemy + tea (significance $P < 0.05$: * vs. normolipidemy)

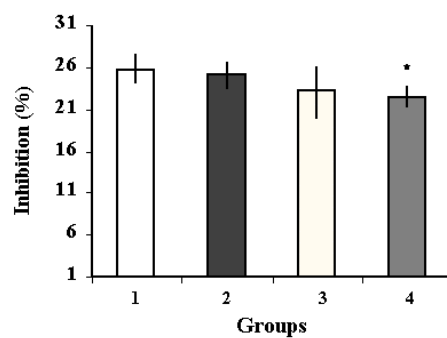


Fig. 4. H-donating ability in the liver. 1: Normolipidemy; 2: hyperlipidemy; 3: normolipidemy + tea; 4: hyperlipidemy + tea (significance $P < 0.05$: * vs. normolipidemy)

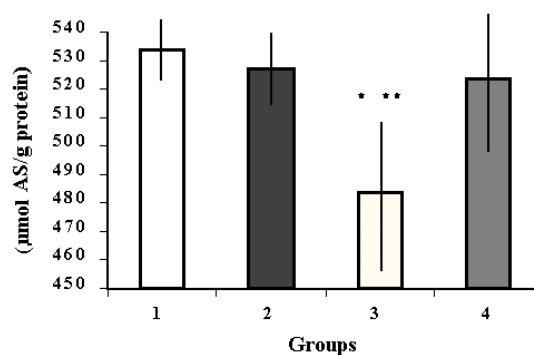


Fig. 5. Reducing power in the liver. 1: Normolipidemy; 2: hyperlipidemy; 3: normolipidemy + tea; 4: hyperlipidemy + tea (significance $P < 0.05$: * vs. normolipidemy; ** vs. hyperlipidemy)

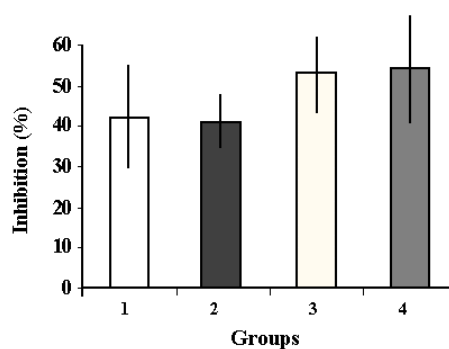


Fig. 6. H-donating ability in the sera. 1: Normolipidemy; 2: hyperlipidemy; 3: normolipidemy + tea; 4: hyperlipidemy + tea (not significant)

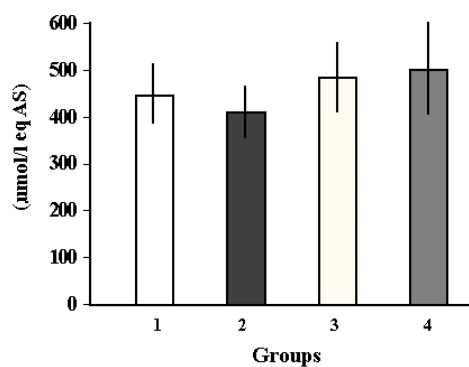


Fig. 7. Reducing power in the sera. 1: Normolipidemy; 2: hyperlipidemy; 3: normolipidemy + tea; 4: hyperlipidemy + tea (not significant)

In most cases, tea infusions contain a number of active molecules, antioxidants, immunostimulants as well as trace elements in high concentration. Therefore, serious complications may arise in the course of herbal medication (JADOUL et al., 1993; GARTNER et al., 1995).

Redox homeostasis is very important for normal tissue function, regeneration, proliferation and apoptosis, in which free radicals and metal elements are predominant, consequently the cell cycle is dependent on intracellular redox state. Oxidative stress can be considered as a pleiotrop modulator in signal transduction pathways (POWIS et al., 1997). Oxidative stress induces stress response genes, whereas moderate oxidative stress specifically down-regulates the expression of various genes (MOREL & BAROUKI, 1999).

DNA synthesis, selective gene expression, enzyme activation and regulation of cell proliferation are affected in redox signalling. The low level of free radicals may modulate various kinases or may directly activate transcription factors to affect gene regulation in the nucleus.

Heavy metal accumulation may inhibit enzyme activities, influence acute phase protein synthesis and gene expression as well as the pro-oxidant and antioxidant forms of scavenger molecules. Metal ions enhance the production of TNF- α , activate protein kinase C and affect the reduction of stress proteins. Zinc, Cu, Cd, Mn and Ni divalent cations can modulate the function of tumour suppressor protein p53 in vitro (KERNOHAN et al., 1996). DNA repair systems are very sensitive targets of Ni, Cr and As ions and the risk of tumour formation is increased in the presence of these elements (HARTWIG et al., 1994). These toxic metal elements compete with Mg ions in several biochemical pathways. Competition between Ni(II) and Mg(II) may provide an important mechanism for interfering with DNA-protein interactions involved in the repair process, because the inhibition of DNA repair is partly reversible by the addition of Mg (KASPRZAK et al., 1987). In addition, presumable Ni(II), Co(II) and As(II) ions displace Zn ion in the zinc-finger structure of DNA repair enzymes (HARTWIG et al., 1994). Nickel, Cr and As elements are established carcinogens in humans (HADFIELD et al., 1998). Nickel and Fe can jointly induce renal sarcomas (HIGINBOTHAN et al., 1992).

In in vitro studies epigallocatechin-3-gallate antioxidant treatments caused cell cycle deregulation and the apoptosis of several cancer cells may be mediated through NF- κ B inhibition, although not in the case of normal cells (AHMAD et al., 2000).

Phenolic type antioxidants activate mitogen-activated protein-kinase (MAPK), which may lead to the induction of genes producing protection and survival mechanisms leading to apoptosis (SCHULZE-OSTHOFF et al., 1997).

Several macrocyclic ellagtannin oligomers showed cytotoxic activity. These compounds induced apoptotic cell death characterized by DNA fragmentation, but macrocyclic compounds in higher concentrations produce their own radicals and become prooxidants (SAKAGAMI et al., 2000).

A lot of polyphenols, especially tannins precipitate proteins, depending on the dose, and therefore cannot penetrate the mucous membranes (LUGASI, 2000).

It may be established that the predominant compound of Beiqishen tea responsible for the refreshing effect is the well absorbing caffeine. Caffeine is a very effective inhibitor of apoptosis. Caffeine and metallic elements, especially As, Cr, Ni and Fe show effects opposite to those of phenolic antioxidants (BLÁZOVICS et al., 2003).

3. Conclusion

It may be assumed that there is a synergistic and antagonistic effect between tea-compounds. Natural preparations may themselves be toxic and especially dangerous while interacting with other medicines. Our results show that higher concentration of Pb, Ni and As cause mainly liver damage in which free radicals are involved. According to molecular-biological research, toxic elements and high concentration of polyphenolic agents participate in tumorigenesis. In addition to these effects, toxic elements may precipitate into gallstones (SZENTMIHÁYI et al., 2002).

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