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EFFECTS OF GREEN AND BLACK TEA BIOCOMPOSITES ON ENDOGENOUS SYNTHESIS, METABOLISM AND GENOTOXIC EFFECT OF CARCINOGENIC *N*-NITROSODIMETHYLAMINE

O.A. Glavin[1,] *, P.M. Mikhailenko[1], S.P. Zaletok[1], L. Gulua[2]

[1]R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology NAS of Ukraine, Kiev 03022, Ukraine

[2]S.Durmishidze Institute of Biochemistry and Biotechnology of NAS of Georgia, Tbilisi 0159, Georgia

Aim: **To study the modifying effect of green and black tea biocomposites on endogenous synthesis and genotoxic action of the carcinogenic *N*-nitrosodimethylamine.** ***Methods:*** **Green and black tea biocomposites were administered to the white inbred rats *in vivo*. Amidopyrine and sodium nitrite were used as *N*-nitrosodimethylamine precursors and 4-methylpyrazol as an inhibitor of its metabolism. *N*-nitrosodimethylamine (blood, daily urine and reaction mixture), nitrites and nitrates (daily urine) levels were measured. Genotoxic action was tested by formation of DNA single-strand breaks in hepatocytes.** ***Results:*** **In *in vitro* system, biocomposites increased *N*-nitrosodimethylamine synthesis in neutral medium and decreased in acid conditions. *In vivo,* black tea biocomposite consumption resulted in enhanced background level of DNA single-strand breaks in rats hepatocytes and higher genotoxic effect upon administration of *N*-nitrosodimethylamine precursors. The levels of *N*-nitrosodimethylamine in blood and urine of experimental animals were increased after precursors' administration. In contrast, green tea biocomposite significantly decreased background level of DNA single-strand breaks. However, there was no protective action of this food supplement at the *N*-nitrosodimethylamine, precursors' administration. 4-methylpyrazol administration did not increase *N*-nitrosodimethylamine excretion in urine, while this effect was observed in control and black tea biocomposite groups.** ***Conclusions:*** **The effects of green tea and black tea biocomposites on *N*-nitrosodimethylamine synthesis in *in vitro* system are unidirectional and depend on biocomposites' concentration and acidity of the medium. Long-term consumption of black tea biocomposite resulted in intensification of endogenous *N*-nitrosodimethylamine synthesis and increased damage of the hepatocytes' DNA. As to the green tea biocomposite, the obtained results allow us to suggest that this biocomposite enhanced *N*-nitrosodimethylamine metabolism.**

Key Words: **biocomposites, green tea, black tea, *N*-nitrosodimethylamine, endogenous synthesis, genotoxicity.**

Nowadays many studies are devoted to the development of bioactive plant-derived substances with useful biological properties, possessing protective action against harmful environmental factors. Different types of tea, food products and specific bioactive compounds, manufactured on their basis, are also in the scope of interest of many researchers.

Green and black teas possess protective activity against toxic action of NO-radicals. Some of their constituent compounds could be considered as molecular traps for NO-radicals and peroxynitrite. They inhibit production of NO-radicals by inducible NO-synthase (iNOS) and depress the LPS-induced activation of iNOS [1]. Green tea has anti-inflammatory action [2], and its extract decreases NO concentration in blood of patients with diabetes [3]. Catechins of green tea extracts reduce the range of ischemic damage areas in brain and partially suppress growth of NOx concentration and level of lipid peroxydation in blood [4]. In Ames test, polyphenols of green tea reduce the mutagenicity of tobacco smoke extract in a dose-depended manner [5]. Constituent compound of green tea epigallocatechin gallate suppresses cytokine-induced pancreatic beta-cell death, induces a significant reduction in IL-1β and IFN-γ -induced nitric oxide (NO) production and reduces levels of the iNOS and its mRNA [6]. This active component of tea is also the molecular trap for nitrite [7]. Use of green tea extracts as a molecular trap for nitrates has several considerable advantages compared to traditional treatments like ascorbic acid, e. g. green tea extracts do not cause DNA damage of gastric tissue [8].

As a result, models with carcinogenic nitrosamines (NA) are one of the prevalent test systems for investigation of protective features of different types of teas and tea-derived products [9–12]. On the model of *N*-nitrosodimethylamine (NDMA) formation in human stomach, it is demonstrated that tea reduces formation of this carcinogen [13]. Similar results were obtained on experimental animals [14]. Inhibiting effect of different types of tea on endogenous formation of nitrosoamines in humans is demonstrated with the use of nitrosoproline, which is not a carcinogen [15].

Regardless of great number of studies, devoted to the different types of tea and tea-derived bioactive substances, there are many uncertain points of the technological processes and application conditions. It was demonstrated that green tea properties changed under the far-infrared irradiation and also depend on the temperature regimen. Treatment below 90 °C increases phenol and flavanol excretion from the tea leaves and changes quantity of epigallocatechin and epigallocatechin gallate. Activity of tea, as the nitrites' trap, increases for temperatures up to 110 °C [16]. Epigallocatechin gallate inhibits genesis of cancer in-

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*Correspondence: Fax: +38 (044) 258-16-56
E-mail: veterok61@mail.ru

Abbreviations used: AP – amidopyrine; BT – biocomposite from black tea; DNA SSB – DNA single-strend breaks; iNOS – inducible NO-synthase; GT – biocomposite from green tea; NA – nitrosamines; NDMA – *N*-nitrosodimethylamine; NO – nitric oxide; MP – 4-methylpyrazole; SN – sodium nitrite.

duced by *N*-nitrosomethylbenzylamine action, but this effect disappears if hot water is used [17]. Reduction of the NDMA excretion with urine in humans in case of amines- and nitrates-rich diet takes place only on the fourth day of green tea consumption [18].

It is worth mentioning that the results of tea administration significantly depend on the selected experimental model. For instance, it was shown that the cell death of the primary culture of rat mesencephalic cells under the action of NO-synthase inductor, 6-hydroxydopamine modifying effect of catechins from tea, could be attributed solely to their antioxidant action. NO-synthases inhibitory effect was not observed [19].

Thus, the development of new bioactive food supplements on the basis of different tea extracts could be perspective. At the same time, it is important to explore the positive and negative effects of these supplements on the metabolism of toxic compounds. In this paper we studied the modifying effect of green and black tea biocomposites on endogenic synthesis and genotoxic action of the carcinogenic *N*-nitrosodimethylamine.

MATERIAL AND METHODS

Biocomposites from green tea and black tea. Bioomposites from green tea (GT) and black tea (BT) were developed and produced in S.Durmishidze Institute of Biochemistry and Biotechnology of NAS of Georgia [20]. These bioactive food supplements have the following chemical composition in % of dry matter. The GT includes polyphenols — 23.5 ± 0.9 (among them catechins — 9.0 ± 0.5; the catechins found are: 1 — (–) epicatechin galate, 2 — (–) epigallocatehin-3-gallate, 3 — (–) epicatechin, 4 — (±) gallocatechin, 5 — (–) epigallocatechin); amino acids — 14.3 ± 0.5; pectin — 9.5 ± 0.6; organic acids — 5.3 ± 0.4. The BT includes polyphenols — 15.0 ± 0.5 (among them catechins — 3.6 ± 0.1; the catechins found are: 1 — (–) epicatechin galate, 2 — (–) epigallocatehin-3-gallate, 3 — (–) epicatechin, 4 — (±) gallocatechin, 5 — (–) epigallocatechin); amino acids — 15 ± 0.4; pectin — 7.0 ± 0.3; organic acids — 5.99 ± 0.4.

Research in vivo. Experiment was conducted on 100–150 g weight male white inbred rats from the vivarium of Institute of Experimental Pathology, Oncology and Radiobiology (IEPOR). Experimental procedures were approved by the Ethical Committee of IEPOR.

Three groups of experimental animals were studied. The animals drank clean drinking water or 1 mg/ml solutions of GT or BT biocomposites during 35 days *ad libitum*. The animals of experimental groups, which consumed GT and BT solutions, did not receive drinking water. Solutions of tea biocomposites and water had been renewed every day. Amount of consumed liquids was measured daily. After 35 days some animals were exposed to chemicals and 12 small groups (6–12 experimental animals in each) were formed from the three big experimental groups of animals: 1) control; 2) 4-methylpyrazol (MP); 3) amidopyrine (AP) + sodium nitrite (SN); 4 MP + AP + SN; 5) GT; 6) GT + MP; 7) GT + AP + SN; 8) GT + MP + AP + SN; 9) BT; 10) BT + MP; 11) BT + AP + SN; 12) BT + MP + AP + SN.

AP and SN served as NDMA precursors, and MP as inhibitor of NDMA metabolism [21, 22] were administrated on 36th day from the beginning of experiment. Water solutions of AP, SN and MP (or equivalent quantity of water) were administrated into the stomach of experimental animals by the metallic probe. AP (250 mg/kg of the body weight) and SN (125 mg/kg of the body weight) were administered simultaneously prior to transferring animals to exchange chambers. MP (75 mg/kg of the body weight) was administrated twice — 20–30 min before transfer of animals in exchange chambers and 7 h after that.

Experimental animals were killed with anesthesia 24 h after their transfer to exchange chambers. The samples of blood (5–8 ml) were collected in glass test tubes moistened by the 5% solution of sodium citrate. Samples of daily urine were collected in glass bottles with 2.5 ml 1 N sodium alkaline to inhibit possible NDMA synthesis in excreted urine. Urine sample volume was measured.

The 0.5 ml of urine was used for nitrites determination and the same volume was used for nitrates determination. Remaining urine was used for volatile NA determination. NA and nitrites determination was conducted immediately after urine collection. Samples for determination of nitrates were kept at –18 °C. The livers of experimental animals were perfused with cold (4 °C) 0.27 M citrate buffer, pH 7.4. Samples of liver for determination of DNA single-strand breaks (DNA SSB) levels in hepatocytes were frozen and stored in liquid nitrogen.

Model experiments. The effects of GT and BT biocomposites on NDMA synthesis from AP and SN were investigated in model experimental system. Experiments were conducted at the different acidity of reagent mixture and GT or BT biocomposites concentrations. Maximum final concentration of HCl in reagent mixture was close to its concentration in the stomach content of control rats [23]. To simulate hypoacidity and dilution of the stomach content in the process of AP, SN and MP administration, in this experiments either the lower HCl concentration was used or distillated water was added in reagent mixture instead of HCl. Considering that the average volume of rat's stomach is 6 ml and taking into account AP and SN solutions volumes and concentrations that were administered in *in vivo* investigation, the reagent mixture was composed of AP (50 mg/ml), SN (25 mg/ml), GT/BT in concentrations 2 or 0.5 mg/ml and HCl (0.06, 0.012 and 0.006 M)/H_2O at the ratio — 1 : 1 : 3 : 1 v/v. The samples were incubated for 45 min, at 37 °C in darkness. Distilled water was used to simulate strong hypoacidity conditions in stomach. The influence of the GT and BT biocomposites on acidity of reagents' mixtures was studied before the investigations of NDMA synthesis, because there is direct association

between the decrease of reaction medium pH and SN nitrosation reaction rate. Value of pH of reagents' mixtures was measured twice: immediately after preparation of reaction mixture and in 45 min. During incubation of mixtures, pH lowered. For the highest HCl concentration, difference between pH values at the beginning and the end of incubation was less than 0.1. For other samples difference was more significant — 0.2–0.4. These changes were more significant (up to 0.5 pH un.) in the case of higher biocomposites concentration, 2 mg/l. At the final concentration of HCl 0.001 M/l in reagent mixtures, pH value didn't vary significantly from mixtures with distilled water instead the HCl. According to results of pH measurements, HCl concentrations of 0.06 N and 0.012 N were selected for NDMA synthesis investigation. Reaction was stopped by adding 1 N NaOH (final pH 7–9). NDMA extraction was conducted immediately after end of reagent mixtures incubation.

Determination of NA in blood and urine samples was conducted by means of double distillation with an aquatic pair from a neutral and alkaline medium followed by NA extraction in a dichloromethane and consequent concentration of water-free extract under the stream of gaseous nitrogen and light heating [24]. The internal standard (1 ml alcohol solution of nitrosodipropyleamine, 2 μg/ml) was added to the samples before distillation for determination of the degree of NA extraction. Extracts of NA in dichloromethane were stored at 4–7 °C before their concentration. Concentrated NA extracts were stored at –18 °C.

For the blood samples, NA was extracted with ethylacetate in proportion 1 : 1 (v : v) three times with vigorous mixing for 30 s before distillation. Anhydrous sodium sulfate (1–2 g) was added to combined extract and it was stored in a refrigerator at –18 °C before standard procedure. Treatment of urine samples was conducted immediately using the method of NA distillation with the water steam. NA level was evaluated using a system of gas-liquid chromatograph with "TEA-502A" analyzer as a detector [25]. Determination of individual NA and computation of their amount were performed using retention times and area of standard NA peaks.

During *in vitro* investigation we used a modified method for NDMA measurement. According to this modified method, we did not add NDPA as an internal standard. The final volume of NA extracts was increased to 25 ml by dichloromethane, and NDMA measuring was conducted without extracts concentration. These modifications in the method were used because volume of reagent mixtures was not limited, and the main losses of NA take place on the step of concentration of dichloromethane extracts. Therefore, it was difficult to predict the amount of internal standard for samples with very high variation of NDMA amount.

Determination of nitrites and nitrates in urine. Analysis of nitrites concentration was conducted using the method of Green [26, 27]. 1.5 ml of the distilled water was added to urine sample (0.5 ml) and was centrifugated for 20 min at 3000 rpm. The samples cleared from urocheras were used for determination of nitrites. Since Green reagent and samples of urine had the individual color, the obtained results needed correction. For this purpose we measured absorption of control solutions in which only distilled water was added instead of the urine or Green reagent.

For determination of nitrates in the urine samples, nitrates were reduced to nitrites by cadmium column [28]. 0.5 ml of the distilled water was added to the sample of urine (0.5 ml) and centrifugated for 20 min at 3000 rpm. The samples cleared from feculence were used for nitrates reduction. 0.25 ml ammonium buffer, pH 9.6, was added to the sample and passed through the column with metallic cadmium (1 drop at 20–40 s). The sample was washed from a column by distilled water. The final volume of the sample with the reduced nitrates was 3 ml. Then the content of nitrites was determined as described above.

Genotoxic test. DNA SSB determination was conducted according to the method of G. Ahnstrom and K. Erixon [29] with modification of A.I. Gaziev *et al.* [30]. Granular hydroxyapatite was prepared. Its physical and chemical properties were verified by A. Mazin *et al*. [31]. The frozen samples of liver were thawed in a refrigerator before the isolation of hepatocytes for analysis.

Statistical analysis. Analysis of results was conducted using Student's *t*-criterium. Linear regressive analysis was used for the calculation of coefficients in calibrate formulas for determination of nitrites and for estimation of consumption of drinking-water and solutions of GT and BT by experimental animals [32].

RESULTS

Consumption of drinking water and solutions of BT and GT by experimental animals. Effects of bioactive substances is dependent on the regimen of their consumption by experimental animals. The experimental animals obtained the solutions of GT- or BT- biocomposites *ad libitum*, and amount of consumed liquids was measured daily. Average daily consumption of GT solution (28.62 ± 0.97 ml/animal/day, 36 animals per group) and BT solution (26.32 ± 1.14 ml/animal/day, 36 animals per group) for the 35 days term was reliably greater than the consumption of drinking water (22.79 ± 1.12 ml/animal/day, 38 animals per group). For control animals that consumed drinking water, the volume of liquid consumption practically did not change for the whole experiment. For BT and GT groups, there was reverse correlation between time and volume of the tea biocomposites consumption. Liquid consumption was decreased 1.3-fold for GT group and 1.6-fold for BT group at the 35 day of biocomposites administration in comparison with the first day of experiment.

Genotoxic action of endogenous NDMA may be changed by altered excretion of carcinogen and/or its precursors with urine. The average urine excretion was measured one day after putting rats in exchange

chambers. Differences were statistically unreliable: 39.8 ± 1.9 ml/kg of the body weight for control animals, 33.9 ± 3.4 ml/kg body weight for BT group and 35.6 ± 1.5 ml/kg body weight for GT group. In addition, in group 8 three animals died during the first 6 h after administration of precursors.

Endogenous synthesis and metabolism of carcinogenic NDMA in vivo. Levels of the NDMA contents in blood of experimental animals and its excretion with urine are shown in Table 1. In blood and in daily urine of control groups of animals that drank only water or only GT or BT solutions, NA was not found (groups 1, 5 and 9). Administration of MP to the experimental animals also did not lead to appearance of this carcinogen in blood and urine (groups 2, 6 and 10).

24 h after administration of NDMA precursors and its metabolism inhibitor MP to the animals of control group (drinking water), this carcinogen was excreted with urine in amounts over detection limit, and some amount of the formed NDMA continued to circulate in blood (group 4). Without MP action (group 3), most of NDMA generated from AP and SN was metabolized. NDMA in blood was found in small concentration only in 1 of 10 animals of the group, in small concentration. The NDMA excretion with urine was 13-fold lower (Table 1). In group 12 that consumed BT biocomposite, NDMA excretion with urine at MP action was nearly 10 times higher than in the control animals (group 4). The contents of NDMA in blood after AP and SN administration was also 1.5-fold higher. These effects can be attributed to the intensified synthesis of carcinogen in stomach. In the absence of MP (group 11), NDMA level in urine decreased by two orders of magnitude. In group 11, as well as in the control group 3, NDMA was found in blood of 1 animal out of 10 (see Table 1).

However, most striking difference was observed in GT-receiving groups *versus* control animals (groups 1–4). After AP and SN with MP administration (group 8), increase of NDMA excretion with urine was 3.7 times lower than in control group 4. Moreover, concentration of NDMA in blood decreased 34.4-fold. At the same time, without MP (group 7), the NDMA excretion with urine did not change. Furthermore, this carcinogen was not detected in blood even after administration of its precursors (see Table 1).

Effects of GT and BT consumption on genotoxic action of endogenous NDMA synthesis. The genotoxic action of endogenous NDMA was estimated by the level of DNA SSB in hepatocytes. The consumption of BT and GT changed the level of DNA damage in hepatocytes. In the BT group level of DNA SSB was 1.3-fold higher than in control group. In contrary, GT exhibited cell-protective action. In GT-consuming groups DNA SSB level was decreased 2.7-fold (significant differences, $P < 0.05$). MP administration did not influence on these indexes (Figure).

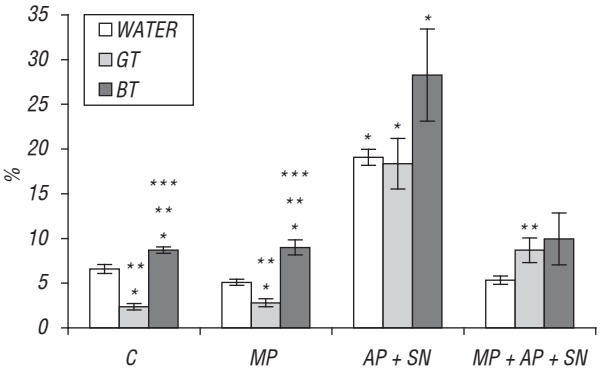

Figure. Rate of DNA SSB (%) in the hepatocytes of rats, which consumed extracts of BT or GT at the endogenous NDMA synthesis. C (control) — animals without MP, AP and SN administration. Changes in DNA SSB were significant in comparison with: *control group; **with group, which received water in C, MP, AP + SN and MP + AP + SM groups; ***GT group in C, MP, AP + SN and MP + AP + SM groups ($P < 0.05$, n = 9 for group 1, n = 5 for group 2, and n = 6 for groups 3–12).

In the case of endogenous NDMA synthesis as a result of AP and SN administration, level of DNA SSB in control group of animals increased almost 3-fold. MP administration completely annihilated this negative effect, caused by action of the endogenously synthesized carcinogen. In experimental groups, the level of DNA SSB was increased by 15.1% for the GT group and by 19.5% for BT group. These rates were higher than in the control group — in the latter only 12.61% of DNA SSB level increase was detected. MP practically canceled the negative influence of NDMA synthesis in the water and BT groups, but MP action in the GT group was less effective (see Figure).

Thus, BT influence on the genetoxic action of NDMA was negative, because during its consumption background level of DNA SSB in hepatocytes was elevated. GT action, in contrary, was positive. A level of damage at the NDMA endogenous synthesis in GT-receiving group was the lowest among all the groups. Such effect can

Table 1. NDMA contents in the blood of experimental animals and its excretion with daily urine

	Control	MP	AP + SN	MP + AP + SN
		NDMA contents in a blood, μg/l		
Drinking-water	$0 \pm 0^{4, 8, 12*}$	$0 \pm 0^{4, 8, 12}$	$1.51 \pm 1.51^{4, 8, 12}$	$350.51 \pm 72.07^{1, 2, 3, 5, 6, 7, 8, 9, 10, 11}$
	(group 1, n = 8)	(group 2, n = 8)	(group 3, n = 10)	(group 4, n = 10)
Green tea	$0 \pm 0^{4, 8, 12}$	$0 \pm 0^{4, 8, 12}$	$0 \pm 0^{4, 8, 12}$	$10.20 \pm 2.49^{1, 2, 3, 4, 5, 6, 7, 9, 10, 11, 12}$
	(group 5, n = 8)	(group 6, n = 8)	(group 7, n = 10)	(group 8, n = 9)
Black tea	$0 \pm 0^{4, 8, 12}$	$0 \pm 0^{4, 8, 12}$	$2.12 \pm 2.12^{4, 8, 12}$	$514.56 \pm 161.46^{1, 2, 3, 5, 6, 7, 8, 9, 10, 11}$
	(group 9, n = 8)	(group 10, n = 6)	(group 11, n = 10)	(group 12, n = 9)
		NDMA excretion with daily urine, μg/kg of the body weight		
Drinking-water	$0 \pm 0^{3, 4, 7, 8, 11, 12}$	$0 \pm 0^{3, 4, 7, 8, 11, 12}$	$2.78 \pm 0.28^{1, 2, 4, 5, 6, 8, 9, 10, 12}$	$36.46 \pm 9.00^{1, 2, 3, 5, 6, 7, 8, 9, 10, 11, 12}$
	(group 1, n = 9)	(group 2, n = 8)	(group 3, n = 9)	(group 4, n = 10)
Green tea	$0 \pm 0^{3, 4, 7, 8, 12}$	$0 \pm 0^{3, 4, 7, 8, 12}$	$10.17 \pm 3.47^{1, 2, 4, 5, 6, 9, 10, 12}$	$9.87 \pm 1.66^{1, 2, 3, 4, 5, 6, 9, 10, 12}$
	(group 5, n = 8)	(group 6, n = 8)	(group 7, n = 10)	(group 8, n = 10)
Black tea	$0 \pm 0^{3, 4, 7, 8, 12}$	$0 \pm 0^{3, 4, 7, 8, 12}$	$3.50 \pm 1.34^{1, 2, 4, 12}$	$354.08 \pm 112.10^{1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11}$
	(group 9, n = 7)	(group 10, n = 6)	(group 11, n = 10)	(group 12, n = 9)

Notes: *in up right angle indicated numbers of experimental groups, to which are statistical differences, $P < 0.05$; n – number of animals in groups.

probably be attributed to the general protective effect of the GT consumption and considerable decrease of background level of DNA damage.

Excretion of nitrites and nitrates with the urine of experimental animals. Endogenous NA synthesis and its genotoxic action can depend on intensity of nitrosation agents excretion from organism. Therefore, the excretion of nitrites and nitrates with daily urine of experimental rats was studied.

For animals that consumed solutions of tea biocomposites instead of water, there was a tendency for the decrease of nitrites excretion with urine and statistical decrease of nitrates excretion approximately 2-fold. After MP administration, we observed increase of nitrites excretion with urine for control animals (2.7 times) and for BT-receiving rats (1.4 times). Excretion of nitrates for control animals, on the contrary, reduced 1.7-fold (see Table 2).

Intragastric administration of SN at high doses considerably increased nitrites excretion with urine (9–15 times). For animals that drank solutions of the tea biocomposites, nitrites excretion with urine was more intensive — statistically significant increase for GT and tendency of increase for BT. Administration of MP with SN resulted in decrease of nitrites excretion for the animals of GT group (1.4 times) and nitrites increased excretion for the animals of the control group (at the level of tendency) and, especially, for the rats of BT group (1.5 times) (see Table 2).

It should be noted that the main fraction of SN administered to the animals was excreted with urine in form of nitrates. Considerable difference between the control group (drinking water) and GT- and BT-groups was not observed. On the average, 18% of SN, which was administrated to the experimental animals, was excreted with daily urine in the form of nitrates. After administration of SN with MP, for animals that consumed tea biocomposites, nitrates excretion with urine decreased approximately by 30%. For control animals there was only an insignificant tendency toward increase (see Table 2).

Effects of BT and GT on NDMA synthesis in in vitro model system. Modifying effects of GT and BT on endogenous synthesis and genotoxic action of NDMA can be attributed to both changes in the repair and metabolic systems of organism, and its influence on formation of the above mentioned carcinogen from its precursors. To clarify the precise mechanism, we conducted an *in vitro* investigation, focused on the influence of GT and BT on the NDMA synthesis from its precursors, AP and SN.

Results of NDMA synthesis in model mixtures are presented in Table 3. In the control mixture with distilled water (instead of GT or BT biocomposites without HCl) there was no NDMA synthesis. When the concentration of HCl increased from 0.002 to 0.01 M, the amount of NDMA, generated during 45 min, raised by order of magnitude.

Replacement of distilled water in the reaction mixtures by GT or BT biocomposites resulted in NDMA synthesis changes that depended on pH. If pH values were neutral, the presence of GT and BT biocomposites led to low NDMA formation, which was not observed in control mixture. There were no statistical changes in NDMA synthesis if different biocomposites or their concentrations were used (see Table 3).

Quite different influence of tea biocomposites was observed in the case of acidic conditions in the mixtures. In mixtures containing 0.002 M HCl, the NDMA synthesis decreased significantly (8.3–15.7-fold). There was no statistical difference between GT and BT action. However, we observed correlation between tea biocomposites concentration and levels of NDMA synthesis: higher concentration of the tea biocomposites inhibited NDMA synthesis more evidently (see Table 3).

Under the highest concentration of HCl in reaction mixture (0.01 M), inhibition of NDMA synthesis was observed only at higher BT or GT biocomposites con-

Table 2. Excretion of nitrites and nitrates with daily urine for experimental animals, which consumed BT and GT extracts

	Control	MP	AP + SN	MP + AP + SN
		Nitrites, mg nitrite-ion/kg of the body mass		
Drinking-water	$0.021 \pm 0.002^{2, 3, 4, 7, 8, 11, 12*}$ (group 1, n = 9)	$0.057 \pm 0.017^{1, 3, 4, 5, 7, 8, 11, 12}$ (group 2, n=8)	$0.194 \pm 0.015^{1, 2, 5, 6, 7, 9, 10, 12}$ (group 3, n=9)	$0.225 \pm 0.032^{1, 2, 5, 6, 9, 10, 12}$ (group 4, n=10)
Green tea	$0.018 \pm 0.001^{2, 3, 4, 7, 8, 10, 11, 12}$ (group 5, n = 8)	$0.020 \pm 0.002^{3, 4, 7, 8, 11, 12}$ (group 6, n = 8)	$0.252 \pm 0.016^{1, 2, 3, 5, 6, 8, 9, 10, 12}$ (group 7, n = 10)	$0.178 \pm 0.015^{1, 2, 5, 6, 7, 9, 10, 11, 12}$ (group 8, n = 10)
Black tea	$0.017 \pm 0.002^{3, 4, 7, 8, 10, 11, 12}$ (group 9, n = 7)	$0.024 \pm 0.001^{3, 4, 5, 7, 8, 9, 11, 12}$ (group 10, n = 6)	$0.269 \pm 0.035^{1, 2, 5, 6, 8, 9, 10, 12}$ (group 11, n = 10)	$0.412 \pm 0.039^{1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11}$ (group 12, n = 9)
		Nitrates,|nitrate,azotate| mg nitrate-ion/kg of the body weight		
Drinking-water	$1.02 \pm 0.12^{2, 3, 4, 5, 6, 7, 8, 9, 11, 12}$ (group 1, n = 8)	$0.59 \pm 0.06^{1, 3, 4, 7, 8, 11, 12}$ (group 2, n=8)	$28.68 \pm 1.36^{1, 2, 5, 6, 8, 9, 10, 11}$ (group 3, n = 8)	$25.59 \pm 2.25^{1, 2, 5, 6, 8, 9, 10}$ (group 4, n = 8)
Green tea	$0.55 \pm 0.04^{1, 3, 4, 7, 8, 11, 12}$ (group 5, n = 8)	$0.59 \pm 0.05^{1, 3, 4, 7, 8, 11, 12}$ (group 6, n=8)	$30.62 \pm 1.65^{1, 2, 5, 6, 8, 9, 10, 12}$ (group 7, n = 8)	$19.37 \pm 1.35^{1, 2, 3, 4, 5, 6, 7, 9, 10, 11}$ (group 8, n = 8)
Black tea	$0.49 \pm 0.12^{1, 3, 4, 7, 8, 11, 12}$ (group 9, n = 8)	$0.51 \pm 0.08^{3, 4, 7, 8, 11, 12}$ (group 10, n=8)	$31.48 \pm 2.96^{1, 2, 5, 6, 8, 9, 10, 12}$ (group 11, n = 8)	$20.03 \pm 3.05^{1, 2, 3, 5, 6, 7, 9, 10, 11}$ (group 12, n = 8)

*In up right angle indicated numbers of experimental groups, to which are statistical differences, $P < 0.05$; n – number of animals in groups..

Table 3. NDMA synthesis (μg/l of reaction mixture) from AP and SN after 45 min incubation

	mg/ml	H_20	Final HCl concentration	
			0.002 M/l	0.01 M/l
H_20		$0^{2–15*}$ (variant 1)	$3929.0 \pm 322.8^{1–5, 7–15}$ (variant 6)	$40163.3 \pm 4063.5^{1–10, 13–15}$ (variant 11)
BT	0.5	$79.0 \pm 7.1^{1, 4–15}$ (variant 2)	$476.8 \pm 103.0^{1–6, 11–15}$ (variant 7)	$54241.1 \pm 5186.6^{1–10, 13, 15}$ (variant 12)
	2.0	$153.0 \pm 40.0^{1, 6, 7, 9–15}$ (variant 3)	$250.5 \pm 28.5^{1, 2, 4–6, 11–15}$ (variant 8)	$14234.3 \pm 229.3^{1–12, 14}$ (variant 13)
GT	0.5	$122.7 \pm 24.6^{1, 2, 6–15}$ (variant 4)	$339.5 \pm 60.7^{1–6, 11–15}$ (variant 9)	$52241.1 \pm 5186.6^{1–11, 13, 15}$ (variant 14)
	2.0	$136.0 \pm 2.3^{1, 2, 6–15}$ (variant 5)	$281.8 \pm 14.3^{1–6, 11–15}$ (variant 10)	$13261.8 \pm 1166.0^{1–12, 14}$ (variant 15)

Notes: *in up right angle indicated numbers of variants of experience, to which are statistical differences , $P < 0.05$, for each variant n = 4.

centration (2.8–3.0-fold). At lower concentration of GT and BT, *vice versa*, there was an insignificant trend to the increase of NDMA synthesis (see Table 3).

Thus, the biocomposites of GT and BT changed the synthesis of NDMA from AP and SN. Their effects depended on acidity of reaction medium. However, there was no direct relation between changes in the synthesis of NDMA and changes of pH of reaction mixture as a result of presence of GT or BT. Most plausible explanation may be suggested as follows: changes of protons' concentration lead to alteration of the activity of tea compounds as nitrates "traps".

DISCUSSION

Administration of biocomposites from tea instead of drinking-water to the experimental animals results in clear increase of liquid consumption. On the other hand, according to the analysis of the approximate results of the liquid consumption by the animals from experimental groups, we conclude, that this increase is characteristic mainly for the initial stages of the experiment. However, complete replacement of water by solutions of biocomposites from tea leads to the gradual decrease of the liquid consumption by animals. In a control group such tendency is not observed. Most considerable changes are observed for BT-group, where consumption of liquid at the end of experiment is lower than that for control animals.

Replacement of drinking water by solutions from the tea biocomposites causes insignificant (at the level of tendency) decrease of nitrites' excretion with urine and reliable decrease of nitrates' excretion (approximately 2 times). This is an expected result, taking into account available data on the properties of tea and its components as nitrites' traps and inhibitors of iNOS [1, 3, 7]. For BT and GT experimental groups decrease of the level of nitrates' excretion, but not that of nitrites, can be explained by conversion of main fraction of nitrites into more stable nitrates.

The long-term consumption of tea biocomposites causes modification at the background level of DNA SSB in hepatocytes of experimental animals. In the GT-group DNA SSB level was 2.75 times lower than that of the control group, which means that GT extract either has protective action against genotoxic factors, or enhances reparative activity in hepatocytes. In contrast, in the BT group the level of DNA SSB increased 1.35 times. Possibly, this negative effect of long-term BT consumption resulted in the decrease of the BT consumption by animals at the end of experiment.

At 24 h after administration of SN and AP to the experimental animals, we observed an obvious increase in DNA damage level in hepatocytes as a result of genotoxic action of endogenously synthesized NDMA. Under the administration of MP to the experimental animals, this genotoxic effect of NDMA was suppressed almost completely.

The most negative effect was observed for animals consuming BT (the level of DNA SSB 1.48 times exceeds that of the control group). Under MP administration genotoxic effect of NDMA was practically absent. In that case excretion of the carcinogen with urine was almost an order of magnitude higher than that in the control group. Concentration of remaining NDMA in blood (24 h after SN and AP administration) was 1.48 times higher. Probably this effect can be attributed to enhanced endogenous synthesis of NDMA from precursors. Influence of BT on the reaction of nitrosation could be both direct and indirect, that is why for the animals of this group excretion of nitrites with urine was essentially enhanced. Rates of transformation of nitrites to nitrates decreased possibly as a result of BT consumption.

For animals of GT-group and BT-group, effects of administration of NDMA precursors differed greatly. Namely, the level of DNA SSB in hepatocytes after administration of NDMA precursors was even below that of the control animals. At the same time, taking into account lower background level of DNA SSB, action of NDMA was not less acute than in the control group. Administration of MP to the animals reduced genotoxic effect of NDMA, but the level of DNA SSB in group 8 remained enhanced. As for the level of NDMA excretion with urine and its final concentration in blood 24 h after administration of precursors, it differed considerably from those of control animals and animals of BT-group. NDMA excretion with urine after AP and SN administration was enhanced, but administration of MP to the animals did not influenced its intensity (group 8), and the remaining level of this carcinogen in blood sharply decreased. Also, in contrast to the cases of the control group and BT-group, under MP and precursors administration to the experimental animals of GT group, excretion of nitrites with urine decreased. Obtained results showed that the simultaneous action of GT and MP suppressed inhibition of NDMA metabolism and enhanced the reduction of nitrites.

This might be a reason for decrease of the endogenous synthesis of NDMA and its excretion with urine. There can be other or additional explanation of such effects of GT biocomposite. Y. Kuroiwa *et al* [33] showed that green tea catechins alone showed a weak chemopreventive effect on gastric carcinogenesis, but in the presence of sodium nitrite they, in contrary, exerted a promoting effect. Proving their hypothesis the researchers carried out experiments, showing that level of 8-hydroxydeoxyguanosine in DNA after short term combined action of green tea catechins and SN was significantly increased.

Model investigations *in vitro* reflect that BT and GT biocomposites modify NDMA synthesis from its precursors, AP and SN. However, their effects depend on acidity of reaction medium and extracts concentration. In neutral reaction medium GT and BT biocomposites somewhat enhance NDMA synthesis. In contrary, presence of GT and BT in acidic reaction medium lead to decrease of NDMA formation. Increase of HCl concentration in reagent mixtures to the concentration equal to that in stomach of control rats [23] partly eliminated this effect of GT and BT. Results obtained

in vitro explain greater differences of NDMA excretion with urine between individual animals in experimental groups with GT and, especially, BT administration.

On the other hand, changes observed in *in vivo* study could not be explained only by GT and BT action as chemical compounds that had modified reaction of nitrosation in stomach. Unlike *in vitro* research, the effects of tea extracts action in case of *in vivo* dramatically differed one from another, both due to genotoxic action and NDMA excretion with urine and levels of this carcinogen in blood. Observed effects are possibly caused by modifying action of GT and BT on the function of the systems, which are responsible for metabolism and reparation processes in animals organism. Young with co-authors [34] have shown, that green tea extracts induce some isoforms of cytochrome P-450 and raise the level of its mRNA. On the other hand, R. Krishnan and G.B. Maru [35] have shown that polyphenols of black tea decrease activity of CYP 1A1 and 1A2. This data is in accordance with our results. However, contradictory results are received in many works, and effects significantly differ for various kinds of tea or tea extracts, and model chosen for analysis [36, 37].

Results of NDMA excretion with urine and residual NDMA content in the blood from the animals of group 12 (BT consumption) allow us to assume that long-term consumption of BT extract could result in hyperacidity of gastric content. This hypothesis requires additional study, because hypersecretion of gastric juice can result in intensification of NDMA synthesis due to two mechanisms: 1) enhancement of nitrosation reaction as a result of decrease of the pH of reactionary medium [38, 39]; 2) partial suppression of BT action as nitrites trap and an inhibitor of NDMA synthesis. Alternative hypothesis, related to NDMA synthesis as a result of gastric hypoacidity, growth of gastric microflora, and reduction of nitrates to nitrites, seems less plausible (see [40]). In the case of BT, it is necessary also to take into account information about its bactericidal action against gastric and intestinal microflora [41, 42], although there are contradictory results about action of BT against *Helicobacter pylori* [41, 43].

Summarizing the obtained results, it is possible to make the following conclusions. Biocomposites from both BT and GT modify the reaction of NDMA synthesis from AP and SN in a similar way. Their action depends on acidity of reaction medium and concentration of extracts. In neutral reaction medium GT and BT extracts intensify synthesis of NDMA from AP with SN, but in acid conditions their effect reverses.

Long-term consumption of BT has negative effect on experimental animals. Background level of DNA SSB in hepatocytes was enhanced, endogenous NDMA synthesis from precursors was intensified and its genotoxic action was increased.

Biocomposite from GT, on the contrary, had positive effect on experimental animals. Under the long-term GT consumption, background level of DNA SSB in hepatocytes decreases. This can be explained either by upregulation of reparation in liver, stability of membranes of hepatocytes or by suppression of the genotoxic factors due to the GT action. Consumption of GT does not protect the liver cells from genotoxic action of NDMA under its endogenous synthesis. Results on the NDMA excretion with urine and its residual levels in blood of experimental animals allow us to suggest that GT modifies action of MP, as the inhibitor of NDMA metabolism. It can be assumed that GT biocomposite activates xenobiotics metabolism and its protective action is most possibly achieved through influence on carcinogenic, genotoxic and toxic compounds that require metabolic activation.

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ВЛИЯНИЕ БИОКОМПОЗИТОВ ИЗ ЗЕЛЕНОГО И ЧЕРНОГО ЧАЯ НА ЭНДОГЕННЫЙ СИНТЕЗ, МЕТАБОЛИЗМ И ГЕНОТОКСИЧЕСКОЕ ДЕЙСТВИЕ КАНЦЕРОГЕННОГО *N*-НИТРОЗОДИМЕТИЛАМИНА

Цель: **изучить модифицирующий эффект биокомпозитов из зеленого и черного чая на эндогенный синтез и генотоксическое действие канцерогенного *N*-нитрозодиметиламина.** ***Методы:*** **в эксперименте *in vivo* белые нелинейные крысы получали биокомпозиты из зеленого и черного чая. Амидопирин и нитрит натрия использовали в качестве предшественников *N*-нитрозодиметиламина и 4-метилпиразол в качестве ингибитора его метаболизма. Измеряли содержание *N*-нитрозодиметиламина (кровь, суточная моча и реакционная смесь), нитритов и нитратов (суточная моча). Генотоксическое действие оценивали по образованию однонитевых разрывов ДНК в гепатоцитах.** ***Результаты:*** **в системе *in vitro* биокомпозиты повышали синтез *N*-нитрозодиметиламина при нейтральной кислотности реакционной среды и снижали его в кислых условиях. В эксперименте *in vivo* употребление крысами биокомпозита из черного чая приводило к повышению уровня однонитевых разрывов ДНК в гепатоцитах и повышало генотоксический эффект от введения предшественников *N*-нитрозодиметиламина. После введения предшественников содержание *N*-нитрозодиметиламина в крови и моче было повышено. Наоборот, биокомпозит из зеленого чая значительно снижал фоновый уровень однонитевых разрывов ДНК. Однако не отмечали защитного действия этой пищевой добавки при введении предшественников *N*-нитрозодиметиламина. Введение 4-метилпиразола не приводило к повышенной экскреции *N*-нитрозодиметиламина с мочой, хотя этот эффект отмечали в контрольной группе и группе с назначением биокомпозита из черного чая.** ***Выводы:*** **в системе *in vitro* биокомпозиты из зеленого и черного чая однонаправлено влияли на синтез *N*-нитрозодиметиламина, а их эффекты зависели от кислотности реакционной среды и концентрации в ней биокомпозитов. Длительное употребление биокомпозита из черного чая приводило к интенсификации эндогенного синтеза *N*-нитрозодиметиламина и повышало уровень повреждений ДНК в гепатоцитах. Результаты, полученные относительно биокомпозита из зеленого чая, позволяют нам предположить, что он повышал метаболизм *N*-нитрозодиметиламина.**

Ключевые слова: **биокомпозиты, зеленый чай, черный чай, *N*-нитрозодиметиламин, эндогенный синтез, генотоксичность.**