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GREEN TEA, RED WINE AND LEMON EXTRACTS REDUCE EXPERIMENTAL TUMOR GROWTH AND CANCER DRUG TOXICITY

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Aim: **To evaluate antitumor effect of plant polyphenol extracts from green tea, red wine lees and/or lemon peel alone and in combination with antitumor drugs on the growth of different transplanted tumors in experimental animals.** ***Materials and Methods:*** **Green tea extract (GTE) was prepared from green tea infusion. GTE-based composites of red wine (GTRW), lemon peel (GTRWL) and/or NanoGTE as well as corresponding nanocomposites were prepared. The total polyphenolics of the different GTE-based extracts ranged from 18.0% to 21.3%. The effects of GTE-based extracts were studied in sarcoma 180, Ehrlich carcinoma, B16 melanoma, Ca755 mammary carcinoma, P388 leukemia, L1210 leukemia, and Guerin carcinoma (original, cisplatin-resistant and doxorubicin-resistant variants). The extracts were administered as 0.1% solution in drinking water (0.6–1.0 mg by total polyphenolics per mouse per day and 4.0–6.3 mg per rat per day).** ***Results:*** **Tumor growth inhibition (TGI) in mice treated with NanoGTE, cisplatin or cisplatin + NanoGTE was 27%, 55% and 78%, respectively, in Sarcoma 180%, 21%, 45% and 59%, respectively, in Ehrlich carcinoma; and 8%, 13% and 38%, respectively in B16 melanoma. Composites of NanoGTE, red wine, and lemon peel (NanoGTRWL) enhanced the antitumor effects of cyclophosphamide in mice with Ca755 mammary carcinoma. The treatment with combination of NanoGTE and inhibitors of polyamines (PA) synthesis (DFMO + MGBG) resulted in significant TGI of P388 leukemia (up to 71%) and L1210 leukemia. In rats transplanted with Guerin carcinoma (parental strain), treatment with GTRW or GTE alone resulted in 25–28% TGI *vs.* 55–68% TGI in cisplatin-treated animals. The inhibition observed in the case of combination of GTE or GTRW with cisplatin was additive giving 81–88% TGI. Similar effects were observed when combinations of the cytostatics with GTE (or NanoGTE) were tested against cisplatin- or doxorubicin-resistant Guerin carcinoma. Moreover, the plant extracts lowered side toxicity of the drugs. Treatment with GTE, NanoGTE, and NanoGTRW decreased the levels of malondialdehyde in heart, kidney and liver tissue of experimental animals, as well as the levels of urea and creatinine in blood serum, increased erythrocyte and platelet counts, hemoglobin content, and decreased leucocyte counts.** ***Conclusion:*** **The obtained data indicate the prospects for further development of GTE and corresponding nanocomposites as auxiliary agents in cancer chemotherapy.**

Key Words: **polyphenolic plant extracts, antitumor effect, cancer therapy.**

Cancer is one of the major causes of death in Ukraine and in Western countries. Only 5–10% of all cancer cases are associated with genetic factors and 90–95% of cases are influenced by lifestyle, such as smoking, diet, alcohol, sedentary lifestyle, obesity and sun exposure, as well as infections and environmental contaminants [1, 2]. These results argue that there are opportunities to prevent cancer through lifestyle changes, in particular, with changes in the diet. The strong interrelationship between nutrition and occurrence of malignant tumors is well described in the literature [2, 3]. For instance, plant-rich diets may prevent 30–40% of all cancer types [2, 4–6]. This statement is supported by numerous epidemiological studies, which demonstrate that in Eastern countries, such as Japan, Vietnam, and Philippines, where people traditionally consume green tea and soy products, the incidence of breast cancer and prostate cancer is significantly lower than in other countries [7–14].

The prophylactic properties of green tea, soy products and various fruits and vegetables are attributed to the presence of components such as polyphenols. The polyphenols are a large and diverse group of compounds contained in many plants and the antioxidant properties of polyphenols contribute to prevention of the diseases associated with oxidative stress, including cancer [15]. The catechins, contained in tea leaf, flavonones in citrus plants, anthocyanidins and resveratrol from the peel and seeds of red grapes are especially rich sources of polyphenols.

Polyphenols, such as catechins in green tea, especially epi(-)gallocatechin-3-gallate and resveratrol of red grapes, effectively suppress the growth of human and animal cancer cells, *in vitro*. In particular, growth of lung, intestinal, colorectal or prostate cancer cells, as well as hepatoma or leukemia cells is inhibited by tea catechins [16–18]. Studies *in vivo* have demonstrated that epi(-)gallocatechin-3-gallate in green tea and resveratrol in grapes have prophylactic properties and significantly reduce the yield of carcinogen-induced tumors in experimental animals [16, 19, 20]. Also, plant polyphenols inhibit the growth of different transplanted tumors in animals [20–25]. Further, the potential synergies between polyphenolic compounds in diverse diseases including

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Abbreviations used: DFMO – difluoromethylornithine; GTE – green tea extract; GTRW – GTE and red wine lees; GTRWL – GTE, red wine lees and lemon peels; MDA – malondialdehyde; MGBG – methylglyoxal bis(guanylhydrazone); NanoGTRWL – composites of NanoGTE, red wine, and lemon peel; ODC – ornithine decarboxylase; PA – polyamines; PMG – polyhexamethylenguanidin; TGI – tumor growth ingibition.

cancer [26], especially breast cancer [27], or liver cancer [28] are reported.

To evaluate the potential enhancement of antitumor activity of synthetic anticancer substances by plant polyphenols, we used chemotherapeutics widely applied in treatment of cancer, such as cisplatin, doxorubicin, and cyclophosphamide, as well as synthetic inhibitors of polyamine (PA) metabolism. Inhibitors of PA synthesis are effective as antitumor substances, since PAs are absolutely necessary for the growth and proliferation of cells, including malignant cells [29–31]. Specific blocking of PA synthesis stops cell growth. At present time, inhibitors of PA metabolism are widely studied with aim of their further use as antitumor drugs [32, 33].

In present research, the extracts of green tea polyphenols containing five catechins (catechin, epicatechin, catechin gallate, epicatechin gallate and epi(-)gallocatechin-3-gallate) were used. Besides the extract of green tea, we have also studied antitumor effect of bio-composites developed on the basis of extracts of green tea, red wine lees and lemon peel. In order to improve bioavailability of polyphenols, we also used the nanosized forms of the extracts. The pharmacological efficacy of the teas pulverized into nanograde has been shown to be greatly enhanced [34]. This effect may be explained by the large increase of the absorbing area in fine nanotea particles and the tunnel effect allowing for quantum particles to overcome otherwise insurmountable energy barriers. Quantum-mechanical tunneling highly increases the corresponding rate constant value, in such a way that catechins become able to trap the lipid peroxyl radicals competing with damaging free-radical chain-lipid peroxidation [34].

The current research was aimed at the evaluation of the effects of plant polyphenols extracts derived from green tea, red wine lees and/or lemon peel alone and in combination with antitumor drugs on the growth and development of different transplanted experimental tumors.

MATERIALS AND METHODS

Preparation of green tea extract (GTE) and plant composites. Green tea leaves were purchased from a tea producer company ("Geoplant", Tbilisi, Georgia). Tea leaves were extracted with hot (80 °C) water (1:6 w/v) for 20 min under constant stirring; the water extract was filtered through "fine grade" filter sheet of "Vigo Sheet Filter" under pressure at a flow rate of 420 liters per hour. The filtrate was concentrated under vacuum to 15% dry matter, followed by spray drying in a lab scale Mini Spray Dryer Y015 ("Shanghai Pharmaceutical", China). Inlet and outlet temperatures were 190 °C and 95 °C, respectively. Plant composites were produced from GTE, according to the patents of Georgia [35, 36]. Briefly, a GTE and red wine lees (GTRW) composite was prepared by mixing liquid red wine lees, produced from Georgian variety of Vitis vinifera v. Saperavi, and dry powder of GTE. Wine lees were incubated overnight at 4 °C; after sedimentation, the supernatant was obtained by decantation and filtered through a dense filter under vacuum. The filtered wine lees were heated on hot plate to 60 °C and dry GTE (1:2 v/v) was added gradually, with constant stirring and spray dried; the mixture was subsequently spray-dried. The composites from GTE, red wine lees and lemon peels (GTRWL) were also prepared. The filtered supernatant of the red wine lees was poured into a vessel containing pressed lemon peel (Georgian variety "Kartuli"), at a ratio of 6:1 v/w. The mixture was heated to 80 °C, held for 15 min and filtered through a dense filter under pressure. The filtered extract of red wine lees and lemon peel was added to GTE at a ratio of 1:2 based on dry matters content, filtered again through fine grade filter sheet and spray-dried. Plant extracts were further treated with 50% ethanol and the alcohol extract was centrifuged at 5,000 g, 10 min. The supernatant was collected and concentrated under vacuum. The extract was filtered under vacuum through 0.45 μm filters ("Pall Corporation", USA). Dry powder was obtained by spray-drying method. Nanoextracts were produced from ordinary plant extracts by reconstitution of dry powder into distilled water, filtration under vacuum through 0.45 μm filters ("Pall Corporation", USA) and spray drying. Bioactivity of nanoextracts is greatly enhanced because of increased absorbing area and quantum tunneling effect. An electron microscope ("Tesla BS-500", Czech Republic) was used to estimate particle size.

Chemical analysis of tea extracts. Chemical analysis of plant materials and extracts was accomplished by the conventional procedures as described. The colorimetric assay using Folin — Ciocalteu reagent was used to determine the total polyphenol content in plant materials [37]. Pectic substances were quantitatively analyzed by the reaction of galacturonic acid with carbazole in sulphuric acid [38]. Amino acids were determined by colorimetric reaction of free amino acids with ninhydrin reagent with minor modifications [38]. For analysis of reducing sugars, the standard reducing-sugar assay of Nelson and Somogyi was applied [40, 41]. The content of organic acid was determined according to ISO 750:1998 [42]. Total ash was determined according to ISO standard #7517–1990 (Instant tea in solid form — Determination of total ash) [43].

Experimental animals and care. Mice and rats were obtained from the vivarium of the R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology of the NAS of Ukraine (IEPOR) and used in experiments. At the start of each experiment, the age of the animals was about 2–2.5 months; weight of animals was 20–24 g for mice and 120–150 g for rats. Species, strain and sex of animals varied depending on the tumor model of interest; specifics are reported in the description of experimental tumors. The animals of control and experimental groups were housed in plastic cages with wire tops and maintained at the temperature of 22 °C, with a 12 h light–12 h dark cycle. In each group, 8–12 animals were used. The animals of control groups had standard lab diet (PK-120–4, Phoenix, Kyiv) and drank water ad libitum. The animals of experimen-

tal groups had standard lab diet and drinking water containing 0.1% of indicated polyphenol extracts. All experiments on animals were carried out according to international requirements for work with experimental animals as described in: http://www.nc3rs.org.uk.

Experimental tumors. Tumor strains were obtained from the Bank of Cell Lines from Human and Animal Tissues of R.E. Kavetsky IEPOR. The following transplanted experimental tumors were used: sarcoma S180 (transplanted to the non-inbred mice via s/c injection of 0.2 ml of 25% suspension of shredded tissue per animal); Ehrlich carcinoma (solid form, transplanted to non-inbred mice via s/c injection of 10^6 tumor cells per animal); melanoma B16 (transplanted to C57Bl/6 mice via injection of $5 \cdot 10^5$ tumor cells in muscle of lower leg); Ca755 mammary carcinoma (transplanted to female C57Bl/6 mice via s/c injection of 0.2 ml of 20% suspension of shredded tissue); lymphocytic leukemia P388 and lymphocytic leukemia L1210 (transplanted to F1(C57Bl/6×DBA/2) BDF1 mice or F1(Balb/C×DBA/2) CDF1 mice via i/p injection of $3–5 \cdot 10^5$ leukemia cells); Guerin carcinoma wild-type strain and its cisplatin- or doxorubicin-resistant variants generated and maintained in R.E. Kavetsky IEPOR. Guerin carcinoma was transplanted to non-inbred female rats via s/c injection of 0.5 ml of 25% suspension of shredded tissue.

Animals were euthanized under the state of narcosis and tumors were excised and weighed. Homogenates for biochemical studies were prepared from the liver, heart and kidneys.

Animals with ascitic tumors were guillotined and blood was collected. Thereafter, ascites were washed from the abdominal cavity with an isotonic solution of sodium chloride and the tumor cells were counted in the hemocytometer.

Administration of therapeutic agents. Polyphenol extracts were dissolved in water, previously boiled and cooled to room temperature (near 20–22 °C). Since the second or third day after tumor transplantation and until the end of experiment, animals in the treatment group received 0.1% solutions of plant extracts (GTE, GTRW, NanoGTE, NanoGTRW or NanoGTRWL) in the drinking water. Consumption by mice and rats averaged 3–5 ml and 20–25 ml, respectively, per animal per day. Control animals with transplanted tumors received ordinary drinking water.

The following inhibitors of PA metabolism were used in the study: α-difluoromethylornithine (DFMO) — an inhibitor of ornithine decarboxylase (ODC); polyhexamethylenguanidin (PMG) — inhibitor of ODC and PA oxidase; methylglyoxal bis(guanylhydrazone) (MGBG) — inhibitor of S-adenosyl-L-methionine decarboxylase (S-AMDC) and also known as antitumor drug mitoguazone. DFMO and MGBG were purchased from the Aldrich (http://www.sigmaaldrich.com). PMG was kindly granted by a member of Latvia SA Marger Lidak (Institute of Organic Synthesis, Riga, Latvia).

In experiments, we have used the following antitumor drugs: Cisplatin and Doxorubicin ("Ebewe", Austria), and Cyclophosphan (JSC "Kievmedpreparat", Ukraine). Administration regimens and dosages are described in corresponding Table and Figure footnotes in Results.

To evaluate an effect of the GTE and GTE-based composites on side toxicity of anticancer drugs, creatinine and urea contents in blood serum were measured using standard kits according to the manufacturer's recommendations (Felicit Diagnostica, Dnepropetrovsk, Ukraine); malondialdehyde (MDA) in tissues was measured as described by Stalnaya and Garishvili [44]; blood cells were counted in hemocytometer. Blood was treated with heparin and diluted 1:20 with 3% solution of concentrated acetic acid in saline to count leucocytes or diluted 1:400 in saline alone to count erythrocytes. Blood serum for biochemical studies has been prepared by standard method.

Statistical analysis of the results was conducted [45]. For the evaluation of significance of differences between groups, the Student's *t*-test was used. Significant differences were set at $p < 0.05$. The data in Figures and Tables are presented as M ± m.

RESULTS

Chemical composition and particle size of the extracts. The chemical composition of the extracts is presented in Table 1. The extracts contain polyphenols as well as pectin, amino acids, organic acids, and mineral elements. The total phenolic composition for the extracts ranged from 18.0 to 21.3 g/100 g and was nearly the same for all extracts regardless of the treatment. A wider variation was observed in the total sugars, which ranged from 20.2 to 30.0 g/100 g. There was a lesser amount of pectic substances, organic acids and amino acids in the tea extracts and composites (see Table 1). Total ash was less than 8 g/100 g in all samples.

Table 1. Chemical composition of green tea, wine, lemon peel extracts

Sample	Poly-phenolics	Reducing sugars	Pectic substances	Organic acids	Amino acids	Total ash
GTE	20.1 ± 0.5	28.0 ± 0.9	10.0 ± 0.8	10.8 ± 0.4	7.5 ± 0.3	8.0 ± 0.4
NanoGTE	20.0 ± 0.6	27.5 ± 0.9	10.0 ± 0.8	9.0 ± 0.4	7.3 ± 0.4	8.3 ± 0.4
GTRW	18.2 ± 0.5	30.0 ± 0.8	10.3 ± 0.5	9.5 ± 0.4	10.3 ± 0.4	8.2 ± 0.5
NanoGTRW	20.5 ± 0.7	29.5 ± 0.9	9.8 ± 0.5	8.5 ± 0.4	10.0 ± 0.3	8.4 ± 0.4
GTRWL	21.3 ± 0.8	20.2 ± 0.7	13.0 ± 0.7	13.9 ± 0.5	7.0 ± 0.4	8.0 ± 0.3
NanoGTRWL	18.0 ± 0.5	22.5 ± 0.6	12.5 ± 0.8	9.0 ± 0.3	7.3 ± 0.5	8.2 ± 0.4

Note: Units are g/100g. Data are presented as a mean of three measurements ± standard deviation. Green tea extract (GTE); GTE with particle size of 10–45 nm (NanoGRE); composite of NanoGTE and red wine phenolics (NanoGTRW); composite of GTRW and lemon peel phenolics (GTRWL); composite of NanoGTRW and lemon peel phenolics (NanoGTRWL).

Particle size of nanoextacts under electronic microscope was between 10–45 nm.

Antitumor effect in mice. The antitumor properties of polyphenolic extracts and biocomposites were tested in murine transplanted tumors: sarcoma 180, solid Ehrlich carcinoma, Ca755 mammary carcinoma, B16 melanoma, P388 lymphocytic leukemia, and L1210 lymphoid leukemia. NanoGTE suppressed the growth of sarcoma 180 by 27%, compared to 55% inhibition of growth by cisplatin. It is important to emphasize that use of NanoGTE in combination with cisplatin increased tumor growth inhibition (TGI) up to 78% (Table 2).

Table 2. TGI effect of NanoGTE, cisplatin and their combined application in mouse sarcoma 180

Group	No. animals per group	Tumor mass, g	TGI, %
Control	10	3.70 ± 0.20	–
NanoGTE	10	2.70 ± 0.20*	27
Cisplatin	10	1.66 ± 0.16**	55
Cisplatin + NanoGTE	10	0.80 ± 0.13**,***	78

Note: Analysis was conducted on 21st day after tumor transplantation. NanoGTE was administered as 0.1% solution in drinking water on the third day after tumor transplantation. Cisplatin was administered as 5 i/p injections (1.2 mg/kg), once per two days, starting from 10th day after tumor transplantation (*$p < 0.01$; **$p < 0.001$ as compared to control; ***$p < 0.001$ as compared to cisplatin).

Similar results were obtained for solid Ehrlich carcinoma (Table 3). TGI was 21%, 45% and 59% in mice treated with NanoGTE, cisplatin, or their combination, respectively. Also, the absolute tumor weight reduction and percentage inhibition, showed an enhancement of cisplatin effect by NanoGTE.

Table 3. TGI effect of NanoGTE, cisplatin and their combined application in solid Ehrlich carcinoma in mice

Group	No. animals per group	Tumor mass, g	TGI, %
Control	10	2.90 ± 0.20	–
NanoGTE	8	2.30 ± 0.13*	21
Cisplatin	8	1.60 ± 0.12**	45
Cisplatin + NanoGTE	8	1.22 ± 0.11**,***	59

Note: Analysis was conducted on 21st day after tumor transplantation. NanoGTE and cisplatin were administered as described in Table 2. *$p < 0.05$; **$p < 0.001$ as compared to control; ***$p < 0.001$ as compared to cisplatin.

NanoGTE, NanoGTRW, and NanoGTRWL showed TGI (Fig. 1). On the 24th day after B16 transplantation in animals treated with NanoGTE, NanoGTRW, and NanoGTRWL the tumor weight was 3.51 ± 0.39; 3.34 ± 0.65, and 2.36 ± 0.57 g, respectively. Relative to the control value of 3.82 ± 0.39 g, this represents an inhibition rate of 8%, 12%, and 38%, respectively. The inhibition by the NanoGTRWL extract was statistically significant ($p < 0.05$). The results evidence on synergistic B16-suppressing effect of red wine lees and lemon peel polyphenols included to the GTE.

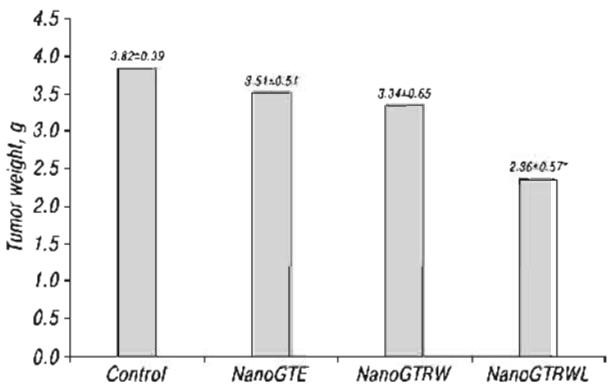

Fig. 1. TGI effects of NanoGTE, NanoGTRW and NanoGTRWL in mouse melanoma B16. Plants extracts were administered as 0.1% solutions in the drinking water. Tumor mass was measured on 24th day after tumor transplantation. *Note*: *Significantly different from the control ($p < 0.05$).

Cyclophosphamide, NanoGTRWL, or their combination resulted in slower growth of Ca755 carcinoma in C_{57}Bl/6 female mice (Fig. 2) and lower tumor weight on 23rd day after Ca755 transplantation (Fig. 3). At this time point, the average tumor weight was 2.59 ± 0.29; 0.98 ± 0.26; 1.65±0.45 g, in the control, cyclophosphamide-, and NanoGTRWL-administered groups, respectively. Treatment with cyclophosphamide and NanoGTRWL reduced the tumor weight by 62 or 36%, respectively, compared to the control. Administration of NanoGTRWL and cyclophosphamide combination led to the most effective reduction of tumor weight up to 0.67 ± 0.32 g (by 74% *vs.* control group; see Fig. 3). The cyclophosphamide-dependent inhibition was significantly enhanced by NanoGTRWL. Moreover, treatment with NanoGTRWL prolonged the lifespan of Ca755-bearing animals: on the 23rd day after Ca755 transplantation all animals in NanoGTRW-treated group were alive *vs.* 33% in the control group.

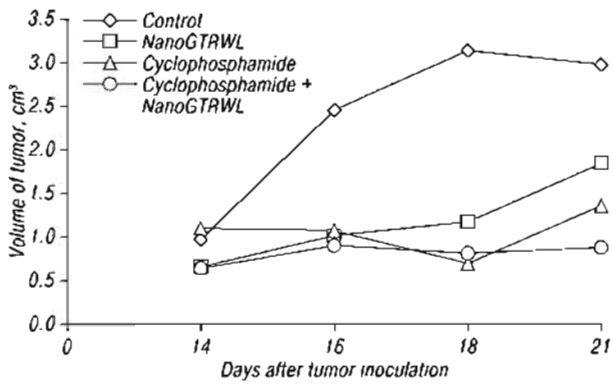

Fig. 2. TGI effects of cyclophosphamide, NanoGTRWL and their combined application in Ca755 mammary carcinoma growth dynamics in C57Bl/6 female mice. NanoGTRWL was administered as 0.1% solution in the drinking water. Cyclophosphamide was administered intraperitoneally (i/p): 45 mg/kg, 5 injections (on the 14th, 15th, 17th, 18th and 20th days after tumor transplantation)

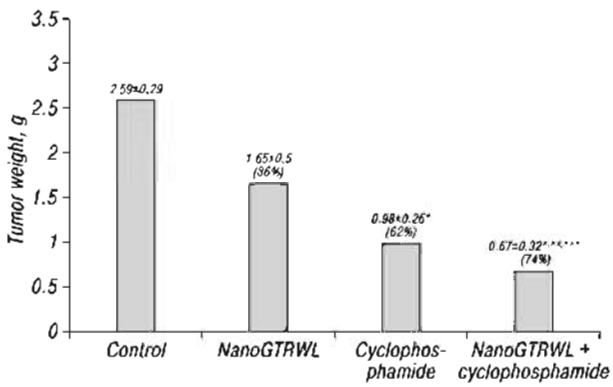

Fig. 3. TGI effects of cyclophosphamide, NanoGTRWL and their combined application in Ca755 mammary carcinoma in C57Bl/6 female mice. NanoGTRWL was administered as 0.1% solution in the drinking water. Cyclophosphamide was administered intraperitoneally (i/p): 45 mg/kg, 5 injections (on the 14th, 15th, 17th, 18th and 20th days after tumor transplantation; tumor mass was measured at 23rd day after transplantation). *Note*: *$p < 0.001$ as compared to the control animals; **$0.05 < p < 0.08$ (NanoGTRWL + cyclophosphamide group as compared to the GTRWL group); ***$0.1 < p < 0.2$ (NanoGTRWL + cyclophosphamide group as compared to the cyclophosphamide group)

In another series of experiments, we have studied effects of NanoGTE and NanoGTRW and their combination with inhibitors of PA metabolism — DFMO, MGBG, PMG on the growth of lymphocytic leukemia P388 and lymphoid leukemia L1210 in mice. The results are shown in Fig. 4–6. The PA synthesis inhibitors and

NanoGTE suppressed the growth of P388 lymphocytic leukemia in mice (see Fig. 4). The tumor cells count was reduced by 11%, 27%, or 39% when NanoGTE, DFMO + MGBG or PMG were administered. However, when the combination of DFMO, MGBG and NanoGTE was administered, the count of tumor cells decreased by 58%. When the combination of PMG and NanoGTE was administered, the tumor cell count decreased by 50% reiative to control and by 40% as compared to PMG treatment only. The combined application of NanoGTE and inhibitors of PA synthesis was the most effective in inhibiting growth of transplantable leukemia.

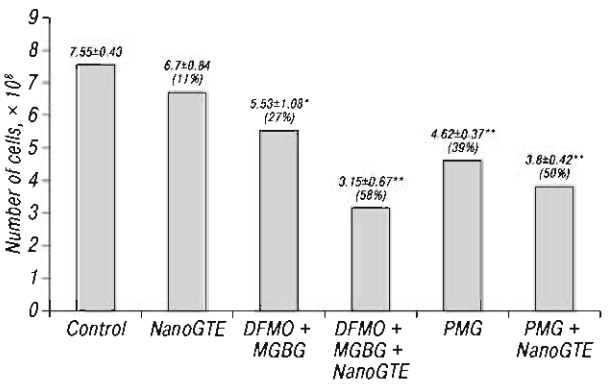

Fig. 4. TGI effects of NanoGTE, α-DFMO + MGBG, PMG and their combined application in P388 leukemia in CDF1 mice. P388 leukemia cells ($5 \cdot 10^5$ cells per mouse) were transplanted; NanoGTE was administered as 0.1% solution in the drinking water, α-DFMO — 800 mg/kg (3 injections), MGBG — 10 mg/kg (3 injections), and PMG — 1.5 mg/kg (3 injections); the number of P388 leukemia cells was counted on the 7th day after P388 transplantation). *Note*: *$p < 0.1$; **$p < 0.001$ as compared to the control animals

In addition, the combined effect of DFMO + MGBG and NanoGTE exceeded the sum of their individual effects. The differences between DFMO + MGBG + NanoGTE group and DFMO + MGBG group, or between DFMO + MGBG + NanoGTE group and NanoGTE group were statistically significant ($p < 0.05$ and $p < 0.005$).

In contrast, the joint effect of PMG and NanoGTE was lower than the sum of their individual effects: $0.05 < p < 0.1$ for PMG + NanoGTE group *vs.* PMG group; $p < 0.005$ for PMG + NanoGTE *vs.* NanoGTE group.

In animals with transplanted P388 leukemia cells, similar TGI was observed in the cases of combined application of PMG and NanoGTE or PMG and NanoGTRW (see Fig. 5) or combined application of DFMO, MGBG with NanoGTE (see Fig. 6). The synergistic effect of PMG and NanoGTE or PMG and NanoGTRW combination is illustrated by the *p* values where $0.1 < p < 0.2$ — control group *vs.* PMG group; $p < 0.005$ — control group *vs.* PMG + NanoGTE group; $p < 0.005$ — control group *vs.* PMG + NanoGTRW group (TGI was 26.5%, 54%, and 57% in PMG, PMG + NanoGTE, and PMG + NanoGTRW-treated animals, respectively). The differences between DFMO + MGBG + NanoGTE and DFMO + MGBG groups were significant ($p < 0.05$) as well as between DFMO + MGBG + NanoGTE and NanoGTE groups ($p < 0.02$) and demonstrated an enhancement of DFMO + MGBG antitumor effects by NanoGTE (see Fig. 6).

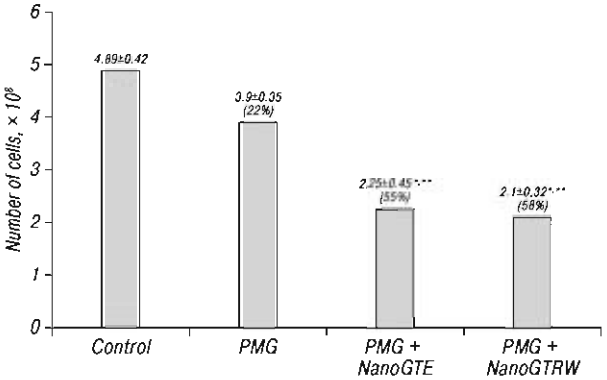

Fig. 5. TGI effects of PMG and combined application of PMG with NanoGTE or PMG with NanoGTRW in P388 leukemia in CDF1 mice. P388 leukemia cells ($3 \cdot 10^5$ cells per mouse) were transplanted; NanoGTE and NanoGTRW were administered as 0.1% solutions in the drinking water; PMG — 1.5 mg/kg (3 injections); the number of P388 cells was counted on the 7th day after P388 transplantation). *Note*: *$p < 0.001$ as compared to the control animals; **$p < 0.01$ and ***$p < 0.001$ as compared to the PMG group

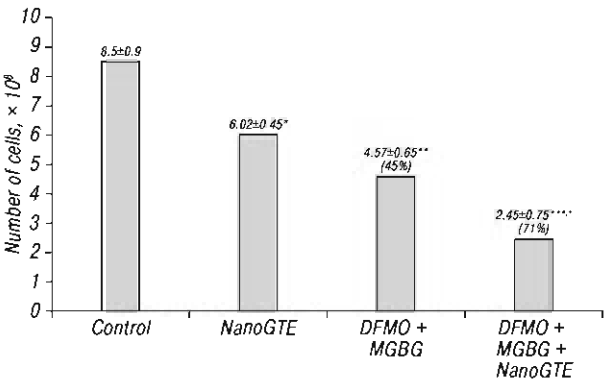

Fig. 6. TGI effects of NanoGTE, α-DFMO + MGBG and their combined application on P388 leukemia in CDF1 mice ($5 \cdot 10^5$ tumor cells transplanted per mouse; NanoGTE administered as 0.1% solution in the drinking water; α-DFMO was administered at 800 mg/kg (4 injections); MGBG — 10 mg/kg (4 injections); the number of cells was counted 7 days after transplantation). *Note*: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ as compared to the control animals; *$p < 0.05$ compared to the α-DFMO + MGBG group

The influence of NanoGTE, NanoGTRW and their combination with inhibitors of PA metabolism (DFMO, MGBG and PMG) on the growth of L1210 lymphoid leukemia in mice, is presented in Fig. 7, 8 and Table 4. 12% decrease in leukemic cell count in NanoGTE-treated CDF1 mice was observed. Higher inhibition (by 28%) was observed after 3 injections of α-DFMO (800 mg/kg) and MGBG (10 mg/kg) (see Fig. 7). In the case of combined application of α-DFMO + MGBG + NanoGTE, decrease in leukemia cell count in mice was 30%, and the combined application of α-DFMO + MGBG + NanoGTRW resulted in 54% of reduction of cell count. Significant enhancement of the antitumor effect was observed in the case of NanoGTRW + α-DFMO + MGBG ($p < 0.001$) (see Fig. 7) and PMG + NanoGTRW ($p < 0.02$) (Fig. 8). Likewise, an increased antitumor effect of NanoGTE and synthetic inhibitors of the PA metabolism was observed in BDF1 mice transplanted with L1210 leukemia (see Table 4). The statistical analysis ($p < 0.02$ for DFMO + MGBG + NanoGTE group *vs.* DFMO + MGBG group; $p < 0.0005$ for DFMO + MGBG + NanoGTE group *vs.*

NanoGTE group) showed that the combined application of NanoGTE with the inhibitors of PA metabolism significantly enhances antitumor effect.

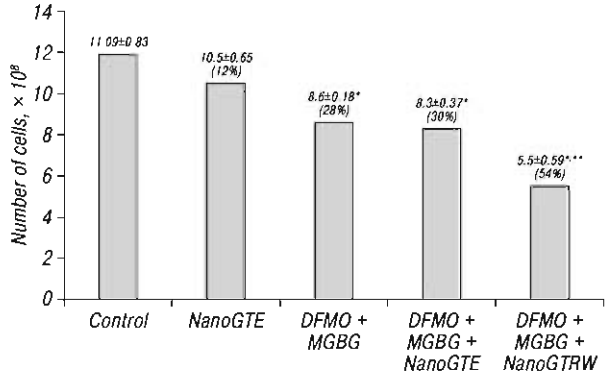

Fig. 7. TGI effects of NanoGTE, α-DFMO + MGBG and combined application of α-DFMO + MGBG with NanoGTE and NanoGTRW on L1210 leukemia in CDF1 mice. L1210 leukemia cells ($5 \cdot 10^5$ cells per mouse) were transplanted; NanoGTE and NanoGTRW were administered as 0.1% solutions in the drinking water; α-DFMO — 800 mg/kg (3 injections); MGBG — 10 mg/kg (3 injections); the number of L1210 cells was counted on 10th day after L1210 transplantation. *Note*: *$p < 0.001$ as compared to the control animals; **$p < 0.001$ as compared to the α-DFMO + MGBG group

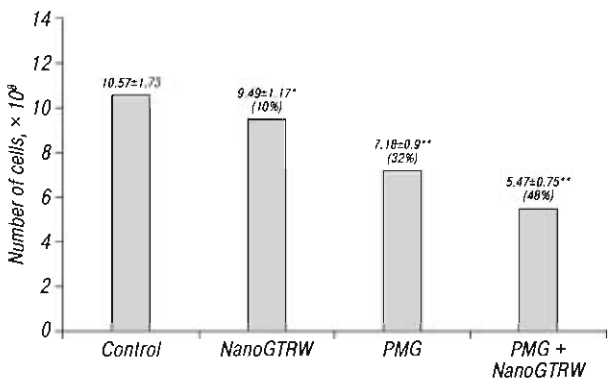

Fig. 8. TGI effects of NanoGTRW, PMG and their combined application on L1210 leukemia in CDF1 mice. L1210 leukemia cells ($5 \cdot 10^5$ cells per mouse) were transplanted; NanoGTRW was administered as 0.1% solution; PMG — by 1.5 mg/kg in 5 injections; the number of L1210 cells was counted on 11th day after L1210 transplantation. *Note*: *$p < 0.1$; **$p < 0.02$ as compared to the control animals

Table 4. TGI effect of DFMO + MGBG, NanoGTE and their combined application in BDF mice with L1210 leukemia

Group of animals	No. animals per group	Cell number, $\times 10^8$ cells/animal	TGI, %
Control	12	15.5 ± 1.4	–
DFMO + MGBG	9	7.8 ± 0.5*	50
NanoGTE	9	13.1 ± 0.8	15
DFMO + MGBG + NanoGTE	9	4.2 ± 1.5*,**	73

Note: Analysis was conducted on 12th days after L1210 transplantation ($5 \cdot 10^5$ cells per BDF1 mouse). DFMO (800 mg/kg) and MGBG (10 mg/kg) were administered as 5 i/p injections each (*$p < 0.001$ compared to the control; **$p < 0.05$ as compared to α-DFMO + MGBG effect)

Similar results have been obtained in the case of combined application of NanoGTRW and another inhibitor of PA metabolism, PMG (see Fig. 8). The combined application of PMG and NanoGTRW resulted in 48% decrease in leukemic cell number. In summary, the results indicate that polyphenol extracts alone manifest growth-inhibiting effect in relation to lymphoid leukemia L1210 and P388, with their effect being significantly enhanced in combination with inhibitors of PA biosynthesis.

Antitumor effect in rats. The antitumor activity of polyphenol extracts (GTE and GTRW) was studied in rats transplanted with wild-type Guerin carcinoma (Tables 5, 6) and its cisplatin- and doxorubicin-resistant variants (Fig. 9, 10). Consumption of 0.1% solutions of GTE (see Table 5) or GTRW (see Table 6) by animals caused the Guerin carcinoma growth inhibition by 28 and 25%, correspondingly. The combined treatment with GTE or GTRW with cisplatin increased antitumor effect of cisplatin used alone.

Table 5. TGI effect of cisplatin, GTE and their combined application in parental strain of Guerin carcinoma

Group of animals	No. of animals per group	Tumor weigh, g	TGI, %
Control	7	25.8 ± 3.1	–
GTE	5	18.7 ± 5.6	28
Cisplatin	5	11.6 ± 1,0*	55
GTE + cisplatin	5	3.04 ± 0.9**, ***	88

Note: Analysis was conducted on 16th day after transplantation of Guerin carcinoma. Cisplatin was administered in 5 i/p injections (1.2 mg/kg), every other day, starting from 10th day after tumor transplantation; GTE was administered as 0.1% in drinking water. *$p < 0.01$; **$p < 0.001$ as compared to the control; *** $p < 0.0005$ – GTE + cisplatin as compared to the cisplatin; $p < 0.0005$ – GTE + cisplatin as compared to the GTE

Table 6. TGI effect of cisplatin, GTRW and their combined application in parental strain of Guerin carcinoma

Group of animals	No. of animals per group	Tumor weight, g	TGI, %
Control	7	45.4±3.1	–
GTRW	7	34.0±3.6*	25
Cisplatin	7	14.7±4.0**	68
Cisplatin + GTRW	7	8.6±1.1**, ***	81

Note: Analysis was conducted on 19th day after transplantation of Guerin carcinoma. Cisplatin and GTRW were administered as described in Table 5. *$p < 0.05$; **$p < 0.001$ as compared to the control; ***$p < 0.1$ as compared to the cisplatin-treated group.

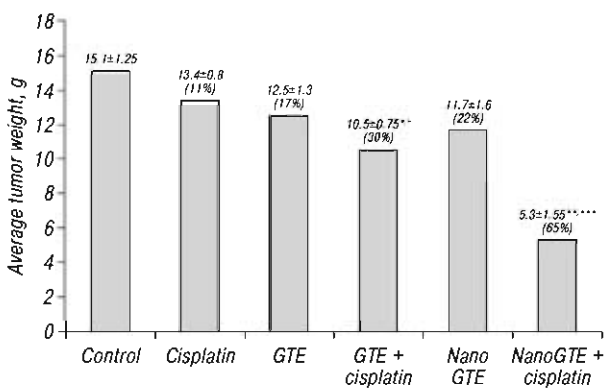

Fig. 9. TGI effects of cisplatin, GTE, NanoGTE and their combined application on cisplatin-resistant Guerin carcinoma in rats. GTE, NanoGTE were administered as 0.1% solutions; cisplatin was administered via 5 i/p injections by 1.2 mg/kg; the tumor mass was measured on 21st day after tumor transplantation. *Note*: *$p < 0.01$; **$p < 0.001$; ***$p < 0.001$; as compared to the control animals; †$p < 0.02$ as compared to the cisplatin group; $p < 0.01$ when GTE + cisplatin group to the cisplatin group; $p \approx 0.1$ when GTE + cisplatin group to the GTE group; $p < 0.01$ when NanoGTE + cisplatin group was compared to the cisplatin group; $p < 0.01$ when NanoGTE + cisplatin group was compared to the NanoGTE group

The effects of GTE and NanoGTE, or their combination with cisplatin (see Fig. 9) or doxorubicin (see Fig. 10) were evaluated in drug-resistant variants of Guerin carcinoma. When rats were treated with cis-

platin or consumed 0.1% solutions of GTE or NanoGTE, the decrease of tumor weight was not significant (by 11%, 17%, and 22%, correspondingly, $p > 0.05$). When cisplatin and GTE were administered in combination, TGI reached 30% ($p < 0.01$). The highest growth inhibition of cisplatin-resistant Guerin carcinoma was observed in rats treated with cisplatin with NanoGTE ($p < 0.001$); the TGI index in this case was 65% (see Fig. 9).

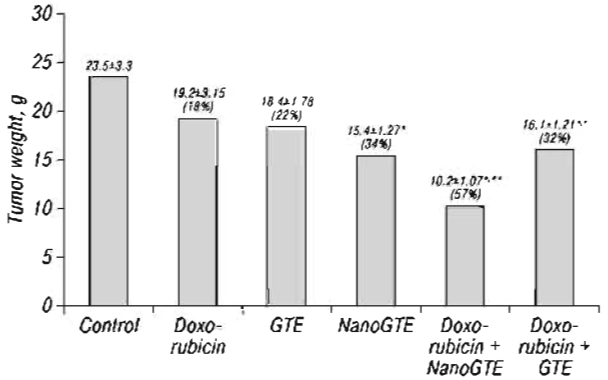

Fig. 10. TGI effects of doxorubicin, GTE, NanoGTE and their combined application on doxorubicin-resistant Guerin's carcinoma in rats. GTE, NanoGTE were administered as 0.1% solutions; doxorubicin was administered via 4 i/p injections by 1.5 mg/kg; the tumor mass was measured on 17th day after tumor transplantation. *Note*: *$p < 0.05$; **$p < 0.001$ as compared to the control animals; ***$p < 0.001$; ·$p > 0.1$ as compared to the doxorubicin group; $0.05 < p < 0.1$ when doxorubicin + GTE group was compared to the doxorubicin group; $0.1 < p < 0.25$ when doxorubicin + GTE group was compared to the GTE group; $p < 0.005$ when doxorubicin + NanoGTE group was compared to the doxorubicin group; $p < 0.05$ when doxorubicin + NanoGTE group was compared to the NanoGTE group

In the case of administration of doxorubicin and GTE, TGI was 18 and 22%, without significant difference from the control ($p > 0.05$) (see Fig. 10). Administration of NanoGTE or GTE and doxorubicin reduced tumor weight by 34% ($p < 0.05$) and 32% ($p < 0.05$), respectively. As single agents, neither doxorubicin, nor GTE had a significant effect on tumor weight. The highest TGI (57%; $p < 0.001$) was observed in the case of the combined application of doxorubicin and NanoGTE. The results demonstrate that consumption of 0.1% solution of GTE (and especially NanoGTE) increased therapeutic activity of cisplatin and doxorubicin in chemoresistant Guerin carcinoma variants.

Anti-toxic properties of tea extracts. In cancer therapy with drugs like doxorubicin or cisplatin, an increase in MDA, urea or creatinine as well as the changes in blood count (increased leukocyte and reduced erythrocyte counts, hemoglobin and platelets) are indicative of their adverse effects. The potential of plant extracts and composites to reduce toxicity associated with doxorubicin and cisplatin was of particular interest.

Therefore, changes in MDA content in tissues of heart (Fig. 11), liver (Fig. 12, 13), and kidneys (Fig. 14) of rats were evaluated. Tissue MDA levels were higher in animals after tumor transplantation and after chemotherapy while the plant extracts and composites diminished this effect. In rats with doxorubicin-resistant Guerin carcinoma, MDA levels in control rats and untreated rats were 2.7 and 5.9 μM/g of heart tissue respectively, and increased up to 6.3 μM/g of heart tissue in animals treated with doxorubucin. Administration of GTE, NanoGTE, doxorubicin and GTE or doxorubicin and NanoGTE, significantly decreased ($p < 0.001$) MDA level (see Fig. 11).

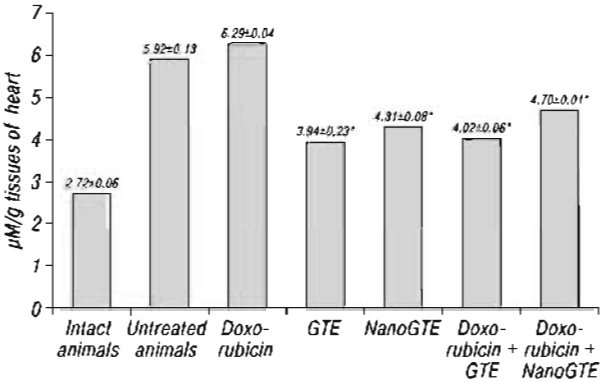

Fig. 11. Effect of GTE, NanoGTE, doxorubicin and their combination on heart MDA level in the animals with grafted doxorubicin-resistant Guerin carcinoma. *Note*: *$p < 0.001$ as compared to the untreated and doxorubicin-treated rats

In rats with doxorubicin-resistant Guerin carcinoma, MDA levels in control rats and untreated rats were 1.7 and 3.0 μM/g liver tissue, respectively, and increased up to 3.2 μM/g liver tissue in animals treated with doxorubucin. Administration of GTE, NanoGTE, doxorubicin and GTE or doxorubicin and NanoGTE, significantly decreased ($p < 0.001$) MDA level (Fig. 12).

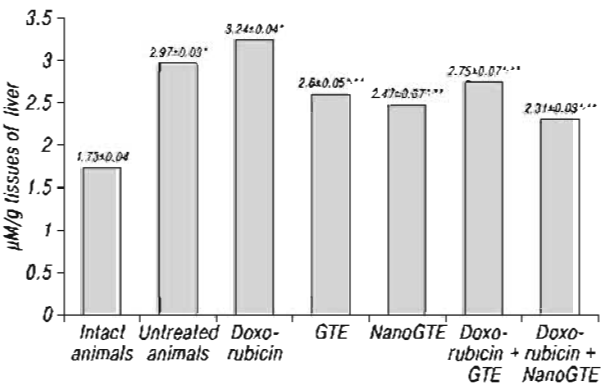

Fig. 12. Effect of GTE, NanoGTE, doxorubicin and their combination on liver MDA level in the animals with doxorubicin-resistant Guerin carcinoma. *Note*: *$p < 0.001$ as compared to the intact animals; **$p < 0.001$ as compared to the untreated and doxorubicin-treated animals

In rats with cisplatin-resistant Guerin carcinoma, MDA levels in control rats and untreated rats were 1.7 and 2.6 μM/g liver tissue, respectively, and increased up to 2.7 μM/g liver tissue in animals treated with cisplatin. Administration of GTE significantly decreased ($p < 0.001$) the level of MDA to 1.8 μM/g liver tissue (Fig. 13).

In rats with cisplatin-resistant Guerin carcinoma, MDA levels in kidneys of control rats and untreated rats were 7.3 and 8.0 μM/g liver tissue, respectively, and increased to 9.5 μM/g liver tissue in animals treated with cisplatin. Administration of GTE significantly decreased ($p < 0.001$) the level of MDA to 7.1 μM/g kidney tissue (Fig. 14).

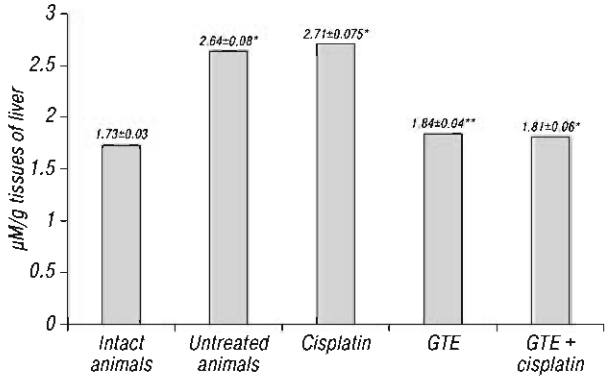

Fig. 13. Effect of GTE, cisplatin and their combined application on liver MDA level in the animals with cisplatin-resistant Guerin carcinoma. *Note*: *p < 0.001 as compared to the intact animals; **p < 0.001 as compared to the untreated and cisplatin-treated animals

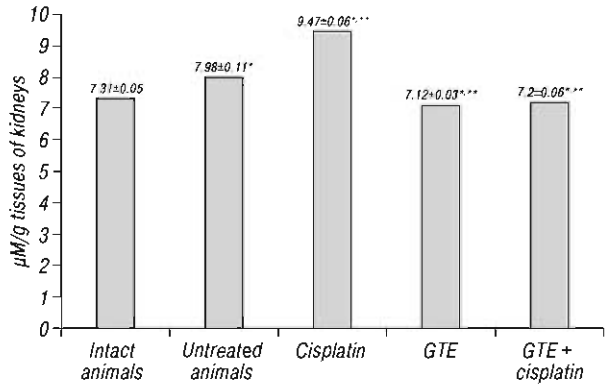

Fig. 14. Effect of GTE, cisplatin and their combined application on kidney MDA level in the animals with cisplatin-resistant Guerin carcinoma. *Note*: *p < 0.01 as compared to the intact animals; **p < 0.001 as compared to the untreated animals; ***p < 0.001 as compared to the cisplatin-treated animals

The urea and creatinine levels in blood serum of Sarcoma 180-bearing mice and solid Ehrlich carcinoma-bearing mice after administration of NanoGTE and cisplatin were also studied. Cisplatin administration increased blood serum urea and creatinine content by 37 and 31%, respectively. NanoGTE in combined therapy decreased the level of urea and creatinine by approximately 13 and 11%, respectively, as compared to cisplatin (Table 7). The urea and creatinine levels in blood serum of solid Ehrlich carcinoma-bearing mice after administration of NanoGTE and cisplatin were studied. Cisplatin administration resulted in an increase of blood serum urea and creatinine content by 43 and 41%, respectively. The NanoGTE use in combined therapy decreased the level of urea and creatinine practically to the control values (Table 8).

Some of the plant extracts and composites improved the peripheral blood values (Table 9), especially they increased erythrocyte and platelet counts, hemoglobin content and decreased leukocyte counts. In mice with L1210 leukemia treated with NanoGTE or NanoGTE, DFMO and MGBG, leukocyte counts were reduced from $30.5 \cdot 10^3$/ml to $17.8 \cdot 10^3$/ml. Notably, platelet counts increased ($p < 0.05$) in mice that received NanoGTE, NanoGTRW, or composites of these plant extracts with DFMO and MGBG. Platelet counts increased from $260 \cdot 10^3$/ml to a range of $438–594 \cdot 10^3$/ml, depending on extract (see Table 9). In mice with P388 lymphocytic leukemia, leukocytes decreased significantly ($p < 0.01$) from 65 to 24 or $33 \cdot 10^3$/ml when treated with NanoGTE, NanoGTRW or composites (Table 10). Also, hemoglobin content increased significantly ($p < 0.01$) in mice treated with NanoGTE (see Table 10).

Table 7. Levels of urea and creatinine in blood serum of sarcoma 180-bearing mice after administration of NanoGTE, cisplatin and their combination

Group of animals, n	Urea level, mmol/l	Creatinine, μmol/l
Control, n = 10	7.14 ± 0.28	71.07 ± 2.26
NanoGTE, n = 10	7.34 ± 0.31	70.00 ± 2.11
Cisplatin, n = 10	9.80 ± 0.23*	92.96 ± 3.12*
Cisplatin + NanoGTE, n = 10	8.56 ± 0.47**	82.41 ± 2.47***

Note: *p < 0.001 as compared to the control; **p < 0.05; ***p < 0.02 as compared to the cisplatin-treated group.

Table 8. Levels of urea and creatinine in blood serum of solid Ehrlich carcinoma-bearing mice after administration of NanoGTE, cisplatin and their combination

Group of animals, n	Urea, mmol/l	Creatinine, μmol/l
Control, n = 10	7.2 ± 0.2	71.3 ± 1.8
NanoGTE, 0.1% solution, n = 10	7.2 ± 0.2	70.0 ± 2.3
Cisplatin, n = 10	10.3 ± 0.5*	100.3 ± 3.6*
Cisplatin + NanoGTE, 0.1% solution, n = 10	7.6 ± 0.4**	73.7 ± 3.4***

Note: *p < 0.001 as compared to the control; **p < 0.01; ***p < 0.001 as compared to the cisplatin-treated group.

Table 9. Some hematological parameters in mice with L1210 leukemia after administration of NanoGTE, NanoGTRW, DFMO + MGBG and their combinations

Group of animals, n	Leukocytes, $\times 10^3$/ml	Erythrocytes, $\times 10^6$/ml	Hemoglobin, g/dl	Platelets, $\times 10^3$/ml
Control animals with L1210, n = 5	30.5 ± 5.2	8.2 ± 2.8	15.6 ± 1.1	260.3 ± 22.6
NanoGTE, n = 5	17.8 ± 1.7*	10.6 ± 0.4	16.3 ± 1.2	544.3 ± 18.8**
NanoGTRW, n = 5	23.5 ± 4.0	11.7 ± 1.2	17.0 ± 1.1	594.6 ± 10.1**
DFMO + MGBG + NanoGTE, n = 5	18.4 ± 1.0*	10.4 ±0.5	15.8 ± 1.1	485.0 ± 8.1**
DFMO + MGBG, n = 5	17.1 ± 3.9	13.0 ± 0.5	19.5 ± 0.3*	305.3 ± 32.8
DFMO + MGBG + NanoGTRW, n = 5	22.5 ± 4.4	11.2 ± 0.8	16.3 ± 1.0	438.6 ± 9.3**

Note: *p < 0.05; **p < 0.001 as compared to the control.

Table 10. Some hematological parameters in mice with P388 leukemia after administration of NanoGTE, DFMO + MGBG and their combinations

Group of animals, n	Leukocytes, $\times 10^3$/ml	Erythrocytes, $\times 10^6$/ml	Hemoglobin, g/dl	Platelets, $\times 10^3$/ml
Control animals with P388, n = 5	65.2 ± 5.6	6.5 ± 1.10	9.1 ± 0.35	326.0 ± 29.0
NanoGTE, n = 5	54.5 ± 9.8	8.6 ± 0.1	10.7 ± 0.25**	343.0 ± 20.1
DFMO + MGBG, n = 5	33.2 ± 7.0**	9.5 ± 0.6*	12.5 ± 0.8**	424.3 ± 45.8
DFMO + MGBG + NanoGTE, n = 5	23.9 ± 4,6***	9.4 ± 1.1	11.5 ± 1.2	339.3 ± 32.4
DFMO + MGBG + NanoGTRW, n = 5	25.9 ± 5.0***	9.5 ± 0.6*	12.1 ± 0.6**	358.3 ± 37.7

Note: *p < 0.05; **p < 0.01; ***p < 0.001 compared to the control.

DISCUSSION

The results of this study support the observation that plant polyphenolic extracts derived from green tea possess antitumor activity. Our results are in a good agreement with similar *in vivo* data obtained by other authors [20, 28, 32].

Furthermore, the favorable influence of GTE was observed regardless of cancer cell type evaluated. Moreover, composites with green tea and red wine lees and/or lemon peel green tea (NanoGTRW and NanoGTRWL) manifest antitumor activity and suppress growth of solid and ascitic tumors in experimental

animals. GTE and composites of GTE with particle size less than 45 nm were also effective. This study documents that NanoGTE and NanoGTRW enhance the antitumor activity of doxorubicin, cisplatin and inhibitors of PA synthesis.

Particularly for drug-resistant experimental tumors, the synergism of doxorubicin or cisplatin with plant extracts is reflected in an enhanced efficacy of chemotherapy. Further impact of the beneficial effect of plant extract in cancer therapy is a decrease of some indices of doxorubicin-related cardiotoxicity and cisplatin-related nephrotoxicity. Plant extracts decrease the levels of creatinine and urea in blood serum, decrease the levels of MDA in tissues of heart, kidneys and liver of animals with transplanted tumors. Finally, some of the plant extracts increase the lifespan [22] and improve hematological values in animals with experimental tumors.

So, the results demonstrate that extracts and nanoextracts of green tea (GTE and NanoGTE) and corresponding composites (GTRW, GTRWL and their nanosized modifications) themselves essentially retard growth of different experimental tumors. Moreover, these agents enhance therapeutic activity of cisplatin and doxorubicin in different experimental tumor models, especially in drug-resistant ones. Therefore, the obtained data indicate the prospects of further development of GTE and corresponding nanocomposites as auxiliary agents in cancer chemotherapy.

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