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SHORT COMMUNICATIONS

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SEA–BUCKTHORN JUICE PROTECTS MICE AGAINST GENOTOXIC ACTION OF CISPLATIN

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Aim: **To study the influence of sea-buckthorn (SB) juice on the micronucleus (MN) frequency in bone marrow cells and sperm abnormality induced by cisplatin (CP, cis-dichlordiammineplatinum-II).** ***Materials and methods:*** **The experiments were performed with male Swiss albino mice. SB juice (0.3 ml) prepared e*x tempore* was given to mice by gavage during 5 or 10 days. 3 h after the last gavage, mice received CP at doses either 1.2 mg/kg or 2.4 mg/kg i.p. MN frequency was studied in bone marrow polychromatic erythrocytes 24 h after the injection of the drug. The abnormality of sperm heads was studied by microscopy.** ***Results*****: The SB juice decreased significantly the number of MN in bone marrow cells induced by CP at dose of 1.2 mg/kg by 36.5% and 47.9% (when it was given 5 and 10 days, consequently), and by 19.0% ($p > 0.05$) at dose of 2.4 mg/kg.The SB juice decreased significantly also the damaging effect of CP on sperm head at low dose and not significantly at the higher one. Antigenotoxic effect of juice was 45.0% and 16.6%, respectively.** ***Conclusion:*** **SB juice decreased significantly the genotoxic effect of CP at dose of 1.2 mg/kg on somatic (bone marrow) and germ (sperm) cells of mice. At higher dose of the drug the effects was not statistically significant.**
Key Words**: cisplatin, sea-buckthorn juice, antigenotoxic effect, micronucleus test, sperm abnormality, mice.**

Cisplatin (CP, cis–dichlordiammineplatinum–II) is one of the most potent chemotherapeutic drugs used for treatment of a variety of human malignancies [1]. Along with high therapeutic activity this drug has a lot of side effects including high nephrotoxicity and genotoxicity [1, 2]. CP is a potential human carcinogen and increased carcinogenic risk with the development of secondary malignancy in patients has been reported [1]. In some recent publications antimutagenic (antigenotoxic) effect of some agents of natural origin against genotoxic effect of CP has been reported including vitamins C and E [3–6].

Sea–buckthorn (SB) berries appear to be an unsurpassed natural source of vitamins C, A (and several other carotenes), E (and several other tocopherols), B1, B2, K, and P, as well as numerous flavonoids, microelements, essential fatty acids and phytosterols [7]. Since many compounds of berries have antimutagenic properties, it would be of interest to study the possible protective action of juice obtained from SB berries on micronucleus–(MN)–inducing activity of CP.

The experiments were performed with Swiss albino male mice (25 g) bred in the Institute of Fine Organic Chemistry (Yerevan, Armenia). All animal procedures were carried out according to the rules of Ethic committee.

SB berries were collected in September from ecologically clean areas of Armenia, near lake Sevan. Juice was prepared *ex tempore*. The berries were crushed gently (not to damage the seeds which are also a source of a variety of biologically active agents), and the liquid was let pass trough the gauze. Each mouse received 0.3 ml of fresh juice by gavage during 5 days (in the morning, before access to food). One group of mice received juice during 10 days. 3 h after the last gavage of the juice, some mice received CP (Ebewe, Austria) at doses 1.2 mg/kg and 2.4 mg/kg i.p. As positive control cyclophosphamide (ASTA Medica, Germany) at dose of 30 mg/kg, i.p was used. Each experimental group consisted of 5 mice. As negative controls, juice and PBS were used (0.3 ml). 24 h after injection the mice were sacrificed by cervical dislocation. Bone marrow preparations were processed and stained as described earlier [8]. Each slide was assessed for MN in 2,000 polychromatic erythrocytes (PCE). The percentage of PCE among the erythrocytes was also calculated to register possible toxicity for bone marrow cells.

To study the abnormality of sperm heads, mice were injected with CP at doses of 1.2 mg/kg and 2.4 mg/kg i.p. Mice received SB juice during 10 days. Ten days after the injections the cauda epididymis of mice was removed and minced into pieces in physiological saline. It was left undisturbed for 20 min for diffusion of spermatozoa. The spermatozoa were spread on a microscope slide, air–dried and stained with 1% aqueous eosin–Y on the next day. 500 sperms from each animal were examined for the abnormalities in sperm head shapes following the criteria of Wyrobeck and Bruce [9].

The juice did not induce any increase of the level of MN compared with negative control. Cyclophosphamide used in our laboratory as a permanent positive control induced 2.44% of PCE with MN which is comparable with our previous results [2, 8], whilst CP applied at a dose of 1.2 mg/kg (1/10 of LD_{50}) — 1.92%, and at a dose of 2.4 mg/kg (1/5 of LD_{50}) — 3.48% of cells with MN, close to our previous results [2]. At the same time CP decreased the num–

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Abbreviationes used: CP — cis–dichlordiammineplatinum–II; MN — micronucleus; PCE — polychromatic erythrocytes; SB — sea–buckthorn.

ber of PCE by 10.3% ($p > 0.05$) and 17.3% ($p < 0.02$) at the doses of 1.2 and 2.4 mg/kg, respectively, indicating the toxicity for haemopoietic cells. SB juice showed antimutagenic activity and decreased the number of MN induced by CP at a dose of 1.2 mg/kg by 36.5% and 47.9% when it was given for 5 and 10 days, consequently. Despite such a difference, it was not statistically significant ($p > 0.05$). Juice decreased the MN–inducing effect of CP at higher dose (2.4 mg/kg) by 19.0%, but it was not significant ($p > 0.05$).

CP induced 4.9% and 8.4% of sperms with abnormal heads at low and high used doses, respectively. It is in agreement with the data of Khryshan and Prasad [4]. The most frequent abnormalities were amorphous (50%) and hooked headed (about 25%) sperms. Banana, triangular, dwarf, double headed, round headed, and double tailed sperms were much less frequent. Similar data were received by other investigators [4, 5]. SB juice decreased significantly the effect of CP at low dose and not significantly — at higher one. Antigenotoxic effect of juice in this case was close to bone marrow data and was 45.0% and 16.6%.

Hence, we showed that SB juice intake decreased significantly the genotoxic effect of CP at a dose of 1.2 mg/kg (1/2 of LD_{50}) on somatic (bone marrow) and germ (sperm) cells. At higher dose of the drug the effects was not statistically significant. The cause of the antigenotoxic effect of the SB juice is the presence of a lot of biologically active components in it, mainly antioxidants. It has been shown that almost all antioxidants can decrease the genotoxic effect of the CP, namely vitamins C and E [5, 10]. It is well known that CP upon hydrolysis in aqueous solution forms various reactive hydroxyl species [11] which can be destroyed by antioxidants [4–6, 10]. The oxygen radicals scavenging ability of antioxidants is the main suggested mechanism responsible for antigenotoxic effect described here. Antigenotoxic effect of SB juice is much more higher than those of vitamins C and E [5, 6] because vitamins induced less pronounced antigenotoxic effects only at doses equal to 21.0–25.0 g per person/day (300–360 mg/kg b.w.). Our findings are in agreement with data of Li and Liu [12] about higher antigenotoxic efficacy of SB juice compared to vitamin C.

Table 1. The influence of sea–buckthorn juice on micronucleus–inducing activity of cisplatin

Experimetal groups (dose in mg/kg b.w.)	Polychromatic erythrocytes with MN, %	Antimutagenic effect, %	Content of polychromatic erythrocytes, %
Cisplatin (1.2)	1.92 ± 0.49	—	48.6 ± 5.4
Juice[1] + cisplatin (1.2)	1.22 ± 0.40*	36.5	51.6 ± 4.8
Juice[2] + cisplatin (1.2)	1.00 ± 0.28*	47.9	55.4 ± 3.9
Cisplatin (2.4)	3.48 ± 0.81	—	44.8 ± 5.0**
Juice + cisplatin (2.4)	2.82 ± 0.63	19.0	48.8 ± 5.0
Cyclophosphamide	2.44 ± 0.40	—	50.8 ± 3.9
Juice	0.10 ± 0.09	—	54.8 ± 4.6
PBS	0.16 ± 0.09	—	54.2 ± 4.1

Mice received sea–buckthorn juice by gavage at volume of 0.3 ml during 5 ([1]) and 10 ([2]) days before cisplatin administration; *$p < 0.01$ compared with action of cisplatin; **$p < 0.02$ compared with negative controls (juice and PBS).

Table 2. The influence of sea–buckthorn juice on sperm abnormality induced by cisplatin

Experimetal groups (dose in mg/kg b.w.)	Total number of damaged sperms	Damaged sperms (%)
Cisplatin (1.2)	122	4.9 ± 0.8
Cisplatin (1.2) + juice	68	2.7 ± 0.6*
Cisplatin (2.4)	209	8.4 ± 1.4
Cisplatin (2.4) + juice	175	7.0 ± 1.2
Juice	48	1.9 ± 0.8

Mice received sea–buckthorn juice by gavage at volume of 0.3 ml 10 days after cisplatin administration; *$p < 0.01$ compared with action of cisplatin.

Antigenotoxic activity of SB juice is not sudden. It is well known that consumption of fresh fruits and vegetables is associated with decline in cancer incidence [13]. It is due to many biologically active compounds which can trap the aggressive metabolites of carcinogens. It is well known that many carcinogens/mutagens act via radical mechanisms and hence damaging biologically important molecules, DNA in the first turn [14]. Many vegetables and fruits are known to prevent chromosomal and DNA damage in animals [15, 16].

Our results may have a practical use in decline of genotoxic effects of CP in cancer patients which may decrease a risk of development of secondary malignancy due to it antimutagenic and antioxidant (antiradical) activity. Radicals which can induce damage in biologically important molecules can be trapped by antioxidants and hence prevent cancer [13]. Usually antimutagens acting in rodents are active in humans, too [3]. The calculation have shown that 0.3 ml of SB juice corresponds to 500–600 ml of it in humans. It is real to drink this volume of juice and it can protect against genotoxic action of highly effective but at the same time very biologically aggressive drug CP. This is in agreement with data obtained by a group of German investigators who showed that fresh fruits juice consumption modulates antioxidative status, immune status and DNA damage in humans [17].

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ПРИЕМ СОКА ОБЛЕПИХИ СНИЖАЕТ ГЕНОТОКСИЧЕСКИЙ ЭФФЕКТ ЦИСПЛАТИНА *IN VIVO*

Цель: **исследовать влияние сока облепихи (SB) на частоту появления микроядер (MN) в клетках костного мозга и патологические изменения сперматозоидов, возникающие при действии цисплатина (CP, цис-дихлордиаминоплатина-II).** ***Материалы и методы:*** **эксперименты проведены на мышах-самцах Swiss albino. Животных поили соком SB (0,3 мл), приготовленным *ex tempore,* в течение 5 или 10 дней. Через 3 ч после последнего его приема мыши получали CP в/б в дозе 1,2 мг/кг или 2,4 мг/кг. Уровень MN исследовали в полихроматофильных эритроцитах костного мозга через 24 ч после инъекции CP. Изменение морфологии головок сперматозоидов изучали по данным микроскопии.** ***Результаты*****: отмечено значительное уменьшение числа MN в клетках костного мозга (при дозе CP 1,2 мг/кг — на 36,5% и 47,9% соответственно, при дозе 2,4 мг/кг — на 19,0%; $p > 0,05$), а также значительное снижение выраженности повреждающего действия CP в низкой (но не высокой) дозе в отношении головок сперматозоидов. Антигенотоксический эффект сока составил 45,0 и 16,6% соответственно.** ***Выводы:*** **прием SB значительно снижает генотоксическое действие CP в дозе 1,2 мг/кг на соматические (костный мозг) и половые (сперматозоиды) клетки мышей. В более высоких дозах эти эффекты CP статистической значимости не имеют.**

Ключевые слова**: цисплатин, сок облепихи, антигенотоксический эффект, микроядерный тест, патология спермы, мыши.**