Evaluation of the protective activity of beta-carotene *and l-carnitine on doxorubicin -induced genotoxicity in rats*

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ABSTRACT

Doxorubicin is a potent antitumor agent used worldwide against many forms of human cancers. It has been demonstrated to have the potential for initiating genetic events in non-tumor cells in human and in animal systems. Due to the importance of doxorubicin in chemotherapy for the treatment of many types of cancer, it is important to reduce its toxicity to the normal cells. This goal can be achieved by concurrent administration of free radical scavenging agents, such as antioxidants. Beta-carotene and L-carnitine antioxidants were used to evaluate their ability to reduce the induction of genetic damage (MNPCEs) by the anticancer drug (doxorubicin) in bone marrow cells of male and female rats. Results indicated that the treatment with beta-carotene and L-carnitine concurrently with doxorubicin induced reduction in the frequencies of MNPCEs. No significant difference was recorded between the frequency of MNPCEs in male and in female rats within each group. Treatment of rats with beta carotene did not induce any significant variation in the incidence of MNPCEs as compared to control values, while, L-carnitine induces significant increase in the frequency of MNPCEs when compared to control.Therefore, it is concluded that Beta- carotene and L-carnitine may act as positive modulators of cytotoxic anticancer agents.

Key words: *Doxorubicin - beta-carotene - L-carnitine- MNPCEs -bone marrow cells - rat.*

INTRODUCTION

Antitumor agents are common therapy against many of human cancers. However, as with many agents that have mammalian cell toxicity as a target, physiological side effects can occur, and genotoxic effect rise to secondary tumors (Beretta, 1991). The anthracycline antibiotic adriamycin (doxorubicin) is one of the most effective chemotherapeutic agents against a wide variety of cancers. The tumors that respond better to adriamycin are breast and esophageal carcinomas, asteosarcoma Kaposi's Sarcoma, soft-tissue sarcomas, hodgkin's and non-hodkin's lymphoma. There are other cancers that, despite being less responsive to adriamycin, are still treated with this compound because of its benefitial effects, such as gastric liver, bile-duct, pancreatic and endometrial carcinomas (Quiles *et al.*, 2002).

Doxorubicin induces mutations and chromosome aberrations in normal and tumor cells. The capacity of doxorubicin to inhibit DNA synthesis has been proposed as a mode of action of this drug (Gewirtz, 1999).

Doxorubicin intercalates with DNA and partially uncoils the double-strand helix. Doxorubicin has a high affinity for cell nuclei, as much as 60% of the total intracellular amount of doxorubicin found in the nucleus. Once binding to DNA occurs, several consequences may issue. Doxorubicin binds to DNA polymerase and inhibits nucleic acid synthesis. In addition, anthracyclines are known to stabilize the otherwise cleavable complex between DNA and homodimeric topoisomerase II enzyme subunits, resulting in the formation of protein-linked DNA double strand breaks (Evert *et al.*, 2001).

In addition, cellular enzymes are capable of converting doxorubicin into free radical metabolites (Benckekroun *et al,* 1992). It is becoming clear that doxorubicin cytotoxicity may be mediated by free radicals derived from it (Luo *et al.*, 1997).

Due to the great importance of doxorubicin in chemotherapy for the treatment of many types of cancer, it is important to reduce its toxicity to normal cells, a goal that can be achieved by concurrent administration of free radical scavenging agents, such as antioxidants (Amaramokrane *et al.*, 1996) .

Beta-carotene is an excellent antioxidant and free radical scavenger. A large number of epidemiological studies suggested that the antioxidant nutrients, especially beta-carotene, has a protective effect against genetic damage and the development of cancer induced by carcinogenic chemicals (Salvadori *et al.*, 1992).

Also, in recent years, dietary supplements such as L-carnitine have been reported to influence the development and amelioration of numerous disease states. The biochemically active amino acid L-carnitine influences fatty acid dependent energy use and also prevents oxygen free radical-induced cellular damage. This antioxidant activity may explain many of L-carnitine beneficial effects that do not appear to be directly associated with enhanced fatty acid beta-oxidation (Maher, 2001).

The micronucleus test is a reliable *in vivo* test for the evaluation of clastogenic effect of mutagens. Micronuclei arise from acentric chromosome fragments or chromosomes which are not incorporated into daughter nuclei during mitosis (Schmid, 1976).

This assay was employed to evaluate the ability of beta-carotene and L-carnitine antioxidants to reduce the genetic damage (micronucleated polychromatic erythrocytes) induced by the anticancer drug (doxorubicin) in bone marrow cells of male and female rats.

MATERIALS AND METHODS

Drugs

Doxorubicin hydrochloride(Adriblastina) was supplied by Pharmacia and Upjohn S.P.A. Millan.Italy.B-carotene and L-carnitine were supplied by Arab Co. for Pharmaceuticals and Medical plants MEPACO, Egypt.

Animal groups

Seventy-two adult albino rats of both sexes, weighing approximately 130-150 gm were used in this experiment. All animals received a standard diet and tap water libitum. Animals were divided into nine groups. Each group contained eight animals, half of the animals was males and the other half was females.

Group 1: Control animals were injected I.P. with distilled water.

Group 2: Animals were injected I.P.with 30 mg/Kg/day of beta-carotene for two weeks.

Group 3: Animals were injected I.P. with 200 mg/kg/day of L-carnitine for two weeks.

Group 4: Animals were injected I.P. with single acute dose (15 mg/kg bwt) of doxorubicin three days before capitation .

Group 5 and 6: Animals were injected with the same doses of beta-carotene and L-carnitine for two weeks, then single acute dose of doxorubicin at day 3 before capitation.

Group 7: Animals were injected I.P with the therapeutic dose of doxorubicin (5 mg/kg bwt) two doses weekly for two weeks.

Groups 8 and 9: Animals were injected with the same doses of beta-carotene and L-carnitine plus the therapeutic dose of doxorubicin for two weeks.

Twenty-four hours after the last dose, all animals were sacrificed and the bone marrow of femur bones was collected.

Micronucleus analysis

Slides were prepared according to Salamone *et al.* (1980). The bone marrow of the femur was flushed with fetal bovine serum into tubes. Smears were fixed with methanol and stained with Giemsa. The slides were coded and micronucleated polychromatic erythrocytes frequencies among 2000 polychromatic erythrocytes were estimated for each individual using a 100x magnification (oil immersion).

Statistical analysis:

Student's paired (t) test was used to detect statistical significance among the different groups (Byrkit, 1980).

RESULTS AND DISCUSSIN

The mean frequencies of micronucleated polychromatic erythrocytes in the different studied groups and the standard deviations are presented in Table (1) and Figure (1). The results showed that animals treated with both acute and therapeutic doses of doxorubicin exhibited a high frequency (232.60 ± 35.81 and 134.00 ± 14.43,respectively) of micronucleated polychromatic erythrocytes in bone marrow cells as compared with control (15.00 ± 2.74).

Meanwhile, the treatment with beta-carotene and L-carnitine concurrently with doxorubicin showed a very significant decrease in the frequency of micronucleated polychromatic erythrocytes (68.60 ± 9.76, 40.60 ± 9.15) and (95.40 ± 24.88,57.00 ± 7.17) respectively when compared with doxorubicin groups (232.60 ± 35.81 and 134.00 ± 14.43).

Treatment of rats with beta carotene did not induce any significant variation in the incidence of micronucleated polychromatic erythrocytes (23.40 ± 6.35) as compared to control values (15.00 ± 2.74), while, L-Carnitine induced significant increase in the frequency of micronucleated polychromatic erythrocytes (25.60 ± 3.51) when compared to control (15.00 ± 2.74).

No significant difference was recorded between the frequency of MNPCEs in male and in female rats within each group (Table 2).

Micronuclei are cytoplasmic chromatin masses with the appearance of small nuclei that arise from chromosome fragments of intact whole chromosome lagging behind at the anaphase stage of cell division and they can be easily recognized in the cytoplasm of immature polychromatic erythrocytes. It is a reliable *in vivo* test for evaluating the clastogenic effect of mutagens (Czyzewska and Mazur , 1995).

Doxorubicin is a potent antitumor agent used worldwide against many forms of human cancers. It has been demonstrated to have the potential for initiating genetic events in non-tumor cells in human and in animal systems (James *et al.*, 1998).

The results showed that doxorubicin increases the incidence of micronucleated polychromatic erythrocytes in rat bone marrow cells. These results are in agreement with other reports concerning with doxorubicin toxicity. Anderson *et al.* (1997) demonstrated that doxorubicin has genotoxic and clastogenic effects. Villani *et al.* (1998) reported that

doxorubicin induced genetic damage in human lymphocytes. Antunes and Takahashi (1998) and Duffaud *et al.,* 1998), reported that doxorubicin is genotoxic,it intercelates into DNA molecule generating free radicals and giving positive results with micronucleus and chromosome aberration testes.

In relation to combined therapy, although antitumor action of doxorubicin may be mediated by a wide number of mechanisms, free radical production is among the main causes of its toxicity. This fact could be used in a trial to reduce the toxic effects of doxorubicin without interfering with its antitumor properties. The most immediate approach has been the combination of the drug delivery together with an antioxidant in order to reduce oxidative stress (Singal *et al.,* 2000).

Beta-carotene has been considered as an effective antioxidant that enhances the repair processes of injured DNA (Cunnigham *et al.,* 1987). Also, Konpacka *et al.* (1998) reported that beta carotene as an antioxidant may play a role in trapping peroxy free radicals and its ability to act as an antioxidant is due to the stabilization of organic peroxide free radicals within its conjugated alkyl structure. El-Habit *et al.* (2000) indicated that beta-carotene exerts its genoprotective effects independent of the nature of genotoxins, whether physical as in the case of ionizing radiation or chemical as in the case of carcinogens, mutagens or clastogens.

In the present study, the treatment with beta-carotene plus doxorubicin significantly reduced the clastogenic effect of doxorubicin, this was shown by the significant decrease in the frequency of micronucleated polychromatic erythrocytes. Similar results were reported by Durnev *et al.* (1997); Formenko *et al.* (1997); Sarkar *et al.* (1997); *Kai-Xian et al.* (1998); Konpacka *et al.* (1998); El-Habit *et al.* (2000) and Glei *et al.* (2002), where they indicated that pretreatment with B-carotene can exert protective effect against genetic damage induced *in vivo* by exposure to various genotoxins. It can enter the cell and protect against strand breaks not against oxidized DNA bases.

L-carnitine has been found to be a metabolic antioxidant. As such, it may attenuate some of the adverse consequences of oxygen free radical overproduction as observed in a number of disease states (Maher, 2001).

The results of the present study showed that the concurrent treatment of L-Carnitine and doxorubicin induced significant decrease in the frequency of MNPCEs. This result is in agreement with Atroshi *et al.* (1999). They demonstrated that L-carnitine treatment decreased DNA damage in the liver and spleen of rats treated with fumonisin B1.Sayed-Ahmed *et al.* (2001) reported that L-carnitine has a powerful antioxidant defense mechanism against doxorubicin-induced lipid peroxidation of cardiac membranes. Also, Dayanandan *et al.* (2001) mentioned that L-Carnitine treatment caused significant reduction in the tissue lipid peroxidations, and it also shows marked improvement in the antioxidant status. By this way carnitine maintains the normal function of the cells.

L-propionyl-carnitine showed a dose dependent free radical scavenging activity. Infact, it was able to scavenge superoxide anion, to inhibit the lipoperoxidation of linoleic acid, and to protect DNA from cleavage (Vanella *et al.,* 2000). Yatim and *Sachan(2001)*concluded that carnitine diverts aflatoxin B(1) epoxide away from DNA by promoting binding to proteins. The modulation of AFB(1) binding to proteins and DNA by carnitine alters the carcinogenic and hepatotoxic potential of AFB(1).Neuman *et al.* (2002) reported that carnitine has antioxidant properties that protect sperm membranes of white Leghorn roosters against toxic reactive

oxygen species, and also carnitine functions to reduce the availability of lipid for peroxidation. From the results of this study, it is concluded that each of Beta-carotene and L-carnitine may act as positive modulators of cytotoxic anticancer agents.

Table (1): Frequency of polychromatic erythrocytes (PCEs) with micronuclei in rat bone marrow cells in the different studied groups.

Groups of animals	No.of counted PCEs/animal		Total MNPCEs	PCEs with 1 MN	PCEs with 2 MN	PCEs with 3 MN	PCEs with 4 MN
Control	2000	M±SD	15.00±2.74	12.00±2.12	3.20±0.83	0.00 0.00	0.00 0.00
Beta-carotene	2000	M±SD	23.40±6.35	19.60±5.77	3.80±1.30	0.00 0.00	0.00 0.00
L-carnitine	2000	M±SD	25.60±3.51*	21.40±2.20**	4.20±1.78	0.00 0.00	0.00 .0.00
Acute dose of doxorubicin	2000	M±SD	232.60±35.81**	129.40±20.95**	64.20±10.31**	23.60 3.91**	15.40 2.40**
Beta-carotene + acute dose of doxorubicin	2000	M±SD	68.60±9.76**	27.80±3.96**	25.60±5.85**	11.20 2.16**	4.00 1.58**
L-carnitine + acute dose of doxorubicin	2000	M±SD	95.40±24.88**	51.80±5.02**	26.40±3.13**	12.80 0.83**	4.40 0.54**
Therapeutic dose of doxorubicin	2000	M±SD	134.00±14.43**	73.80±7.98**	37.80±4.20**	13.60 1.51**	8.80 0.83**
Beta-carotene + therapeutic dose of doxorubicin	2000	M±SD	40.60±9.15**	22.50±5.02**	11.50±2.51**	4.60 0.83**	2.00 0.70**
L-carnitine + therapeutic dose of doxorubicin	2000	M±SD	57.00±7.17**	31.40±3.43**	16.40±2.00**	6.80 1.30**	2.40 1.14**

* Significant differences at (P< 0.05). MN =micronucleus ** Significant differences at (P< 0.01).
M ± SD = mean ± standard deviation

Table (2): Comparison between frequencies of micronucleated polychromatic erythrocytes in rat bone marrow cells of male and female rats.

Animal groups	Male M± S.D.	Female M±S.D.	t -value	P
Control	15.000 ± 1.224	16.200 ± 1.529	0.967	0.271
B-Carotene	23.400 ± 6.348	23.800 ± 5.974	0.667	0.004
L-Cartinine	25.600 ± 3.507	26.200 ± 4.658	0.244	0.856
Acute dose of doxorubicin	234.400 ± 7.474	238.400± 7.938	3.508	0.011
B-Carotene + acute doxorubicin	68.600 ± 9.762	73.000 ± 7.778	1.866	0.073
L-Cartinine + acute doxorubicin	95.400 ± 24.885	106.600± 8.783	0.892	0.752
Therapuetic dose of doxorubicin	134.000±14.439	139.000±14.258	0.758	0.579
B-Carotene + therapeutic dose doxorubicin	40.000 ± 9.154	41.000±10.222	0.104	0.273
L-Cartinine + therapeutic dose doxorubicin	56.800 ± 6.978	58.800 ± 7.563	2.236	0.008

M = Mean S D = Standard Deviation P=Propability

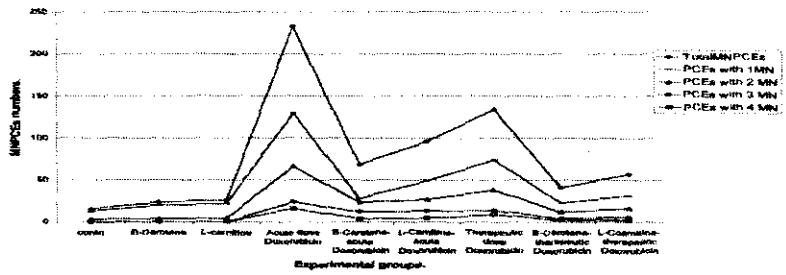

Fig .(1): Frequencies of polychromatic erythrocytes (PCEs) with micronuclei in rat bone marrow cells in the different experimental groups.

REFERENCES

Amaramokrane, Y.A., Lebucher, M.P., Balansard, G., Dumenil, G. and Botta B. (1996). Protective effects of alpha-hederin, chlorophillin and ascorbic acid towards the induction of micronuclei by doxorubicin in cultured human lymphocytes. Mutagenesis, II, 161-167.

Anderson, D., Yu, T.W. and Browne, M.A. (1997). The use of the same image analysis system to detect genetic damage in human lymphocytes treated with doxorubicin in the comet and fluorescence *in situ*hybridization (Fish) assay. Mutat. Res., 390 (1-2): 69-77.

Antunes, L.M. and Takahashi, C.S. (1998). Effects of high doses of vitamin C and E against doxorubicin induced chromosome damage in Wistar rat bone marrow cells. Mutat. Res., 419 (1-3): 137-43.

Atroshi, F., Rizzo, A., Biese, I., Veijalainen, P., Saloniemi, H., Sankari, S. and Andersson, K. (1999). Fumonisin B1-induced DNA damage in rat liver and spleen: effects of pretreatment with Coenzyme Q10, L-Carnitine, alpha-tocopherol and Selenium. Pharmacol. Res., 40 (6): 459 67.

Benckekroun, M.N., Sinha, B.K. and Robert, J. (1992): Doxorubicin induced oxygen free radical formation in sensitive and doxorubicin-resistant variants of rat glioblastoma cell lines. FEBS lett., 322: 295-298.

Beretta, G. (1991). Cancer statement Medical Guide 10th ed. Formitalia Carlo Erba-Erabamont, Milan.

Byrkit, D.R. (1980). Elements of Statistics 3rd ed., Van Nostrand, New York, PP. 275-358.

Cunnigham, M. L., Peak, J.G. and Peak, M.J. (1987). Single-strand DNA breaks in rodent and human cells produced by superoxide anion or its reduction products. Mutat. Res., 184: 217-222.

Czyzewska, A. and Mazur, L. (1995). Suppressing effect of WR 2721 on micronuclei induced by cyclophosphamide in mice. Teratog Carcinog. Mutagen, 15 (3): 109-114.

Dayanandan, A., Kumar, P. and Panneer, S.C. (2001). Protective role of L-carnitine on liver and heart lipid peroxidation in atherosclerotic rats. J. Nutr. Biochem., 12 (5): 254-257.

Duffaud,F., Orsiere, T., Baciuchka-Palmaro, M., Digue, L., Favre, R. and

Botta, A. (1998). Doxorubicin and cisplatin genotoxicity: search for a real indication using the micronucleus test. Ann. Biol. Clin. (Paris), 56(2):183-7.

Durnev, A.D., Tiarina, L.S., Guseva, N.V., Oreshehenko, A.V. and Seredenin, S.B. (1997). Mutagen- modifying effects of beta carotene *in vivo*. Genetika, 33 (5) : 717-20.

El-Habit, O.H.M., Saada, H.N., Azab, K.h.Sh., Abdel Rahman, M. and El Malah, D.F. (2000). The modifying effect of beta-carotene on gamma radiation-induced elevation of oxidative reactions and genotoxicity in male rats. Mutat. Res., 466: 179 186.

Evert, L. De Beer, Antonio, E.B. And Emile, E.V. (2001). Doxorubicin and mechanical performance of cardiac trabeculae after acute and chronic treatment: a review, European Journal of Pharmacology, 415 (1): 1-11.

Fomenko, L.A., Bezlepkina, T.A., Anoshkin, A.N. and Gaziev, A.1. (1997). A vitamin-antioxidant diet decreases the level of chromosomal damages and the frequency of gene mutations in irradiated mice. IZV. Akad. Nauk. Ser. Biol. (4): 419-24.

Gewirtz, D.A. (1999). A critical evaluation of the mechanisms of action proposed for the antitumor effects of the anthracycline antibiotics adriamycin and daunorubicin. Biochem. Pharmacol. , 57:727-741.

Glei, M., Liegibel, U.M., Ebert, M.N., Bahm, V., Pool, Z.B.L. (2002). Beta-carotene reduces Bleomycin-induced genetic damage in human lymphocytes. Toxicology and applied pharmacology, 179(2): 65-73.

James, M.G., Saum, R., Julie, Z., Glenda, J.G. and Lynnette, R.F. (1998). Effect of selected antimutagens on the genotoxicity of antitumor agents. Mutat. Res., 402:289-298.

Kai-Xian, X., Jian-Zhong, W., Guo-Jian, M., Sheng, y. and Hai-Lan, Q. (1998) . Comparative studies on genotoxicity and antigenotoxicity of natural and synthetic Beta-Carotene stereoisomers. Mutat. Res., 418:73-78.

Konpacka, M., Widel, M. and Rzeszowska-Wolny, J. (1998). Modifying effect of vitamin C, E and beta-carotene against gamma-ray induced DNA damage in mouse cells. Mutat. Res., 417: 85-94.

Luo, X., Evrovsky, Y., Cole, D., Trines, J., Benson, L.N. and Lehotay, D.C. (1997). Doxorubicin induced acute changes in cytotoxic aldehydes, antioxidant status and cardiac function in the rat. Biochem. Biophys. Acta., 1360: 45-62.

Maher, T.J. (2001): L-carnitine: Continuing Education Module, Natural Healing Track pp: 1-8.

Neuman, S.I., Lin, T.L. and Heste, P.Y. (2002). The effect of dietary carnitine on semen traits of white leghorn roosters. Poult. Sci., 81 (4): 495-503.

Quiles, J.L., Huertas, J.R., Battino, M., Mataix, J. and Ramirez-Tortosa, M.C. (2002). Antioxidant nutrients and adriamycin toxicity. Toxicology, 180 (1): 79-95.

Salamone, M.F., Heddle, J.A., Stuart, E. and Katz, A. (1980). Towards and improved micronucleus test: Studies on 3 model agents, mitomycin C, cyclophosphamide and dimethyl benznthracene. Mutat. Res. , 74: 347-356.

Salvadori, D.M.S., Ribeiro, L.R., Oliveira, M.D.M. and Becak, W. (1992). The protective effect of beta-carotene on genotoxicity induced by cyclophosphamide. Mutat. Res. , 256: 237-44.

Sarkar, A., Basak, R., Bishayee, A., Basak, J. and Chatlerjee, M. (1997). Beta-carotene inhibits rat liver chromosomal aberrations and DNA chain break after a single injection of diethylnitrosamine. Br. J. Cancer., 76 (7): 855-61.

Sayed-Ahmed, M.M., Salman, T.M., Gaballah, H.E., Abou El Naga, S.A., Nicolai, R. and Calvani, M. (2001). Propionyl-L-carnitine as protector against adriamycin-induced Cardiomyopathy. Pharmacol. Res., 43(6): 513-20.

Schmid, W. (1976). The micronucleus test for cytogenetic analysis, In Chemical Mutagenes Principles and Methods for their Detection. Vol.4, pp.31-52.

Singal, P.K., Li, T., Kumar, D., Danelisen, I. and Iliskovic, N. (2000). Adriamycin-induced heart failure: mechanism and modulation. Mol.Cell.Biochem. 207, 77-85.

Vanella, A., Russo, A., Acquaviva, R., Campisi, A., Di Giacomo, C., Sorrenti, V. and Barcellona, M.L. (2000). L-propionyl-carnitine as superoxide scavenger, antioxidant and DNA cleavage protector. Cell Biol. Toxicol. , 16 (2): 99-104.

Villani, P., Orsiere, T., Duffaud, F., Digue, L., Bouvenot, G. and Botta, A. (1998). Genotoxic and clastogenic effects of doxorubicin. Therapie, 53 (4): 391 5.

Yatim, A.M. and Sachan, D.S. (2001). Carnitine alters binding of aflatoxin to DNA and proteins in rat hepatocytes and cell free systems. J. Nutr., 131 (7): 1903-8.

الملخص العربي

تقييم التأثير الوقائى للبيتاكاروتين و ل- كارنيتين على السمية الوراثية لعقار الدوكسوريبيسين

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عقار الدوكسوريبيسين المضاد للسرطان من الأدوية الفعالة فى علاج العديد من الأورام السرطانية فى الانسان. وقد تبين أن له تأثير وراثى ضارعلى خلايا الانسان غير السرطانية . ونتيجة لأهمية هذا العقار حيث أنه يستخدم كعلاج كيماوى فى العديد من الأورام السرطانية كان من الضرورى ايجاد وسيلة لمقاومة التأثير الوراثى الضارلهذا العقار على الخلايا السليمة . وبالدراسة وجد أنه قد يمكن تحقيق هذا الهدف باستخدام بعض الأنواع من مضادات الأكسدة. ففى هذه الدراسة تم اختيار نوعين من مضادات الأكسدة الطبيعية وهما بيتا- كاروتين و ل - كارنيتين لاختبار مدى قدرتهما على تقليل التأثير الوراثى الضار لعقار الدوكسوريبيسين فى خلايا نخاع الفئران البيضاء الكبيرة . ولقد أثبتت النتائج أن معالجة الفئران بكل من مادتى البيتا- كاروتين و ل - كارنيتين مع عقار الدوكسوريبيسين نتج عنه نقص معنوي فى أعداد الأنوية الصغيرة فى كرات الدم الحمراء متعددة الصبغات بنخاع العظام . ولم يتم تسجيل أى تغيرات ذات دلالة احصائية فى أعداد الأنوية الصغيرة فى كل من الذكور والاناث فى المجموعة الواحدة.

كما أوضحت النتائج أن المعالجة بالبيتا كاروتين لم تحدث أى زيادة معنوية فى اعداد الأنوية الصغيرة بالمقارنة مع المجموعة الضابطة . بينما أحدث ل- كارنيتين زيادة معنوية فى أعداد الأنوية الصغيرة عنها فى المجموعة الضابطة .

ومن نتائج هذا البحث يمكن استنتاج أن كلا من البيتا- كاروتين و ل- كارنيتين كان له تأثير ايجابى فى مقاومة التأثير الوراثى الضار لعقار الدوكسوريبيسين.