Effect of carboplatin and *Nigella sativa* oil on human breast cancer cells *in vitro* and Ehrlich ascites tumor bearing mice *in vivo*

(Received: 15. 11. 2010; Accepted: 15 .02 .2011)

El-Gohary N. [*], Safwat G. [*,], Ibrahim M. [***], Diab A. [*,****] and Hussein, M. H. [*****]**

[*]Faculty of Biotechnology, October University for Modern Science and Arts (MSA), October, Egypt

[**]Horticulture Research Institute, Agricultural Research Center, Giza, Egypt

[***]Molecular Drug Evaluation, National Organization for Drug Control and Research (NODCAR), Ministry of Health, Giza, Egypt.

[****]Agricultural Genetic Engineering Research Institute (AGERI), Giza, Egypt.

[*****]Department of Genetics, Faculty of Agriculture, Cairo University, Giza, Egypt.

ABSTRACT

Carboplatin is a synthetic antineoplastic agent used for cancer treatment and is considered to be the analogous of cisplatin. Nigella sativa oil is a herbaceous plant, it has been used for thousands of years for culinary and medical purposes. This study aimed to evaluate the effect of carboplatin and Nigella sativa oil alone and in combination together on human breast cancer cells (MCF-7) in vitro and Ehrlich ascites tumor bearing female mice (in vivo). The in vitro experiment on MCF-7 cells illustrated that IC_{50} of carboplatin was 11.8 µg/ml, also IC_{50} of Nigella sativa oil was 39 µg/ml on MCF-7 cells. In addition, IC_{50} of the combination between carboplatin and Nigella sativa oil was found to be 3.78 and 40 µg/ml, respectively. The in vivo experiment illustrated that carboplatin (10mg/kg) increased the enzyme activity of aspartate amino transferase (GOT) and aniline amino transferase (GPT)by 56.52% and 51.14%, respectively as compared to both healthy control (non-tumor transplanted mice) and negative control. Also, the activity of GOT and GPT was increased by 14.75% and 19.84%, respectively as compared to healthy control under the effect of Nigella sativa oil (12ml/kg). While the activity of GOT and GPT was decreased as compared to negative control. The combination of carboplatin and Nigella sativa oil appeared to increase the enzyme activity of GOT and GPT by 62.41 and 49.39%, respectively as compared to both healthy control and negative control. Also, carboplatin induced DNA damage of liver tissue was performed by agarose gel electrophoresis and comet assay, while Nigella sativa oil showed intact DNA without any damage. The combination of carboplatin and Nigella sativa oil appeared to decrease the DNA damage as compared to carboplatin alone.

Key words: *Carboplatin, Nigella sativa oil, cytotoxicity, breast cancer cells (MCF-7), Ehrlich ascites tumor.*

INTRODUCTION

Carcinoma is a type of cancer that represents 80-90% of cases and originates in epithelial tissue, which includes the skin, the covering, lining of the organs and internal passage ways (Murphy *et al.*, 1997). Ehrlich Ascites Tumor (EAT) is a type of tumors originates from the carcinomas tumors. Carboplatin (cis-diammine (1,1-cyclo-butanedicarboxylato)-platinum (II), CBDCA) is an anti-neoplastic drug presenting an activity profile similar to cisplatin but a reduced toxicity and especially a reduced nephrotoxicity, which is the limiting factor to cisplatin therapy. Carboplatin is also submitted to cellular responses leading to a resistance. For example, carboplatin presents some cross-resistance with cisplatin in different cell lines and GSH can be associated with these phenomena (Jansen *et al.*, 2002). Carboplatin a second generation platinum-containing anti-cancer drug is currently used clinically against lung, ovarian, head, and neck cancers (Fujiwara *et al.*, 2003). Carboplatin is more water-soluble and produces fewer adverse reactions than its analog cisplatin, but its DNA-damaging activity is equivalent to cisplatin at similar toxic doses (Alberts, 1995). Carboplatin induced bone marrow suppression with diminution of leukocytes, neutrophils, lymphocytes and erythrocytes with subsequent immuno-compromised related infection may be the explanation for high mortality in carboplatin treated mice (Feng *et al.*, 2008). Although carboplatin related cardiomyopathy has seldom been reported in clinical conditions, nevertheless, its frequent use in combination with doxorubicin and cyclophosphamide that causes cardiomyopathy (Zver *et al.*, 2007). *Nigella sativa* seeds (Black seed) have been used traditionally in Middle Eastern folk medicine as a natural remedy for various diseases for over 2000 years (Phillips, 1992). It, commonly known as black seed, belongs to the botanical family of *Ranunculaceae*. Recently, the plant has been subjected to a range of pharmacological investigations justifying its broad traditional therapeutic value. Also, it was reported to inhibit eicosanoid generation in rat peritoneal leukocytes and ox brain membrane lipid peroxidation and ox brain membrane lipid peroxidation (Houghton *et al.*, 1995). *Nigella sativa* oil is an effective free radical scavenger showing antioxidant activity and protecting against the damage caused by free radicals. Therefore, the oil is useful in diseases in which free radicals are involved, e.g. anoxia and ischemia of brain and heart as well as arteriosclerosis, rheumatism and cancer (Houghton *et al.*, 1995 and Al-Ghamdi, 2001).

Aboul-Ela (2002) reported that *in vivo* experiments show that treatment of mice with *Nigella sativa* or thymoquinone induced insignificant cytogenetic effect on the chromosomal aberration of bone marrow cells comparing to control values. Exposure of MCF-7 breast cancer cells to aqueous and alcohol extracts alone or in the presence of descending potency for H_2O_2 completely inactivated growth of these cells (Swamy and Tan, 2000), suggesting that *N. sativa* alone or in combination with an oxidative stress is effective anti-cancer agent.

The active principle fatty acids derived from *N. sativa*, completely inhibited the growth of Ehrlich ascites carcinoma and Dalton's lymphoma ascites cells (Salomi *et al.*, 1992).

This research aimed to evaluate the cytotoxicity of carpoblatin and *N. sativa* fixed oil and their combination on breast carcinoma cells MCF-7 (*in vitro*), and on Ehrlich ascites tumor bearing female mice (*in vivo*).

MATERIALS AND METHODS

Chemicals

Carboplatin (cis-diammine (1,1-cyclo-butanedicarboxylato)- platinum (II) (manufactured in Korea by KUP packed by EUP), *N. sativa* oil was extracted by El-Captain company (CAP PHARM), Dimethylsulphoxide (DMSO) (Sigma), RPMI-1640 medium (Sigma) and Trypan blue (Sigma) were used.

Animals and tumor maintenance

Inbred female albino mice (*Mus musculus*), weighing 25-30g, 10-12 weeks old were used. Animals were obtained from the animal house of National Research Center, Giza, Egypt. They were kept under environmental and nutritional conditions for 2 weeks. The tumor was maintained in female Swiss albino mice by weekly interapertoneal (i.p) transplantation of 2.5 X 10^6 cells in the National Cancer Institute (NCI), Cairo, Egypt. Tumor cells were taken from transplanted animals after 7 days of transplantation and resuspended by appropriate volume of saline, then 2 X10^6 cells (approximately 0.2 ml) of this suspension were injected (i.p) in each female mouse.

Experimental design

This study was carried out on 50 female albino mice, each one was injected (i.p) with 2.5 X 10^6 (0.2 ml) of Ehrlich ascites carcinoma cells (EACCs) except the first group which represented as healthy female mice (non-tumor transplanted). After 6 days of tumor transplantation; animals were divided into 5 groups, each group contained 10 animals:

Group (1): the healthy female mice (non-tumor transplanted) were injected (i.p) with distilled water once, it represented the negative control.

Group (2): the mice were injected (i.p) with distilled water once, it represented the positive control.

Group (3): the mice were injected (i.p) with a single dose of carpoblatin "10mg/kg" (which is 1/5 of the maximum tolerable dosage, Feng *et al.*, 2008).

Group (4): the mice were orally administrated with *Nigella sativa* oil "12ml/kg" for 6 days (Ilhan *et al.*, 2005)

Group (5): the mice were orally administrated with *Nigella sativa* oil "12ml/kg" for 6 days and a single dose of carpoblatin "10mg/kg".

After 24 hours of the administration of carpoblatin, blood was collected to separate serum for determination the activity of GOT and GPT. Liver tissues were isolated to determine DNA fragmentation by single cell gel electrophoresis (Comet assay) and running on agarose gel electrophoresis.

Sulphorhodamine-B (SRB) assay of cytotoxic activity

This method was carried out according to that of Skehan *et al.* (1990). The sensitivity of the human tumor cell lines to thymoquinone was determined by the SRB assay. SRB is a bright pink aminoxanthrene dye with two sulphonic groups. It is a protein stain that binds to the amino groups of intracellular proteins under mildly acidic conditions to provide a sensitive index of cellular protein content. Cells were used when 90 % confluence was reached in T25 flasks. Adherent cell lines were harvested with 0.025 % trypsin. Viability was determined by trypan blue exclusion using the inverted microscope (Olympus 1x70, Tokyo, Japan). Cells were seeded in 96-well microtiter plates at a concentration of $5x10^4$-10^5 cells/ well in a fresh medium and left to attach to the plates for 24 hrs. After 24 hrs, cells were incubated with the appropriate concentration ranges of drugs, completed to total of 200 µl volume/well using fresh medium and incubation was continued for 24, 48 and 72 hrs. Control cells were treated with vehicle alone. For each drug concentration, 4 wells

were used. Following 24, 48 and 72 hrs treatment, the cells were fixed with 50 µl cold 50 % trichloroacetic acid for 1 hr at 4°C. Wells were washed 5 times with distilled water and stained for 30 min at room temperature with 50 µl 0.4 % SRB dissolved in 1 % acetic acid. The wells were then washed 4 times with 1 % acetic acid. The plates were air-dried and the dye was solubilized with 100 µl/well of 10 mM tris base (pH 10.5) for 5 min on a shaker (Orbital shaker OS 20, Boeco, Germany) at 1600 rpm. The optical density (O.D.) of each well was measured spectropho-tometrically at 564 nm with an ELIZA microplate reader (Meter tech. Σ 960, U.S.A.). The mean background absorbances was automatically subtracted and mean values of each drug concentration was calculated as Survival fraction = O.D. (treated cells)/ O.D. (control cells).

Determination of aspartate aminotransferase (GOT)

Aspartate aminotransferase was determined in serum according to Murray (1984).

Determination of aniline aminotransferase (GPT)

Aniline aminotransferase (GPT) was determined in serum according to the method of Murray (1984).

Determination of alkaline phosphatase (ALP)

Alkaline Phosphatase (ALP) was determined in serum according to the method of Marsh *et al.* (1959).

Determination of DNA fragmentation on agarose gel electrophoresis

Genomic DNA was isolated from liver tissue according to the technique of Duke and Cohen (1986).

Determination of DNA fragmentation by single cell gel electrophoresis (Comet assay)

DNA fragmentation by single cell gel electrophoresis of liver tissue was determined according to the method of Singh, *et al.* (1988). Evaluation of DNA damage was evaluated by a Komet 5 image analysis software developed by Kinetic Imaging, Ltd. (Liverpool, UK) linked to a CCD camera to assess the quantitative and qualitative extent of DNA damage in the cells by measuring the length of DNA migration and the percentage of migrated DNA. Finally, the program calculates tail moment. Generally, 50 to 100 randomly selected cells are analyzed per sample.

Statistical analysis

Data were subjected to statistical analysis according to Fisher and Yates (1957) and Snedecor and Cochran (1967).

RESULTS AND DISCUSSION

1. Cytotoxicity induced by carboplatin, *N. sativa* oil and their combination on human breast cancer cells MCF-7 (*in vitro*)

The *in vitro* experiment on MCF-7 cells illustrated that carboplatin with multiple doses 5, 12.5, 25 and 50 µg/ml decreased the surviving fraction of MCF-7 cells, so IC_{50} of carboplatin was 11.8 µg/ml as shown in Figure (1a). Also, *Nigella sativa* oil with multiple doses of 50, 100, 150 and 200 µg/ml decreased the surviving fraction of MCF-7 cells, so IC_{50} of *Nigella sativa* oil was 39 µg/ml as shown in Figure (1b). IC50 of the combination between carboplatin and *Nigella sativa* oil was 3.78 µg/ml and 40 ug/ml, respectively as shown in Figure (1c), also all ICSO's of carboplatin, *Nigella sativa* and their combination oil were collected in Figure (1d).

In vitro and *in vivo* studies indicate that both the oil and the active ingredients of *N. sativa* seeds possess anti-tumor effects. By investigating the effect of the volatile oil of *N.*

sativa seeds on different human cancer cell lines, the oil expressed marked cytotoxic effects against a panel of human cancer cell lines (Islam *et al.*, 2004). Exposure of MCF-7 breast cancer cells to aqueous and alcohol extracts alone or in the presence of descending potency for H2O2 completely inactivated growth of these cells (Swamy and Tan, 2000), suggesting that *N. sativa* alone or in combination with oxidative stress is effective anti-cancer agent. Studies attempted to define the anti-tumor mechanisms of the whole *N. sativa* oil show that *N. sativa* extracts induced, in a concentration-dependent manner, inhibition of the metastasis- induced factors, including type 4 collagenase, metalloproteinase, and serineproteinase inhibitors

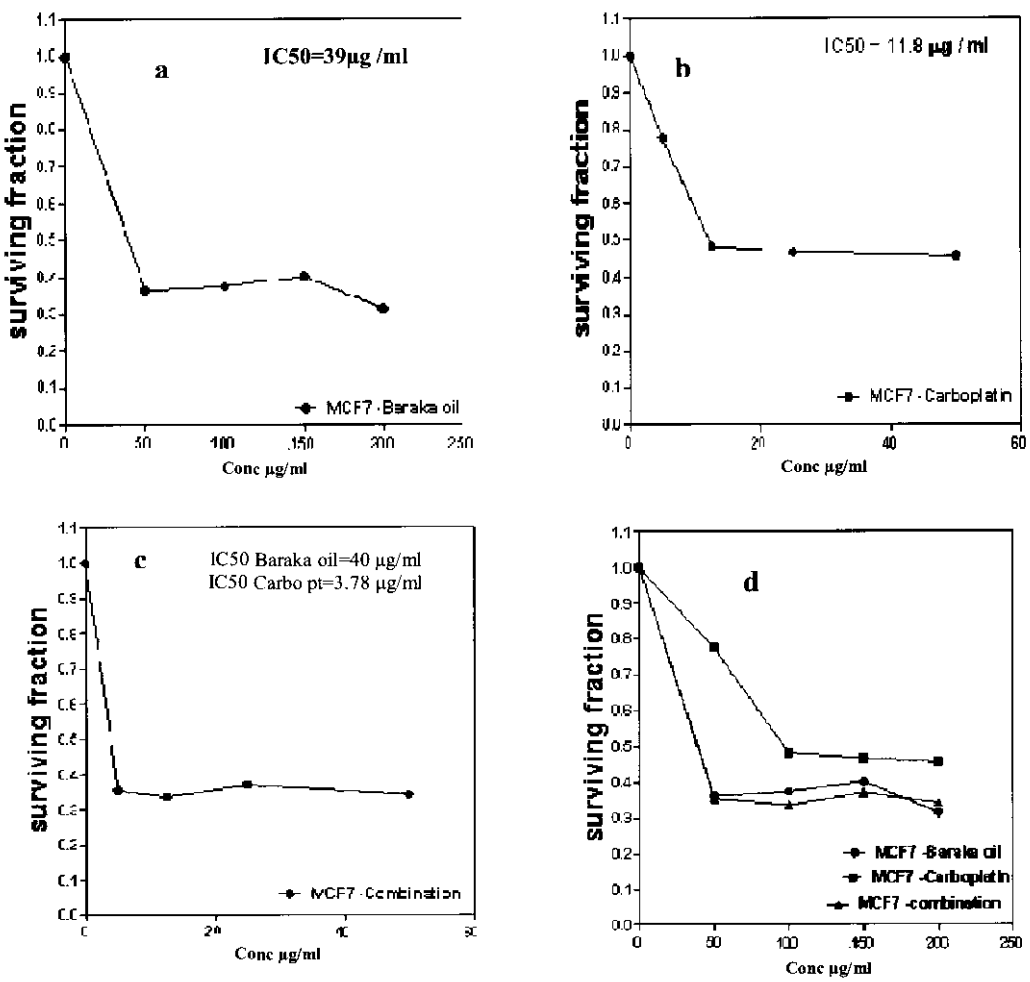

Fig. (1): Cytotoxicity induced by carboplatin alone (a), N. sativa oil alone (b) and their combination (c and d) on human breast cancer cells MCF-7.

(Medenica *et al.*, 1997), angiogenic protein-fibroblastic growth factor (Swamy and Tan, 2000), tissue-type plasminogen activator, urokinasetype plasminogen activator, and plasminogen activator inhibitor type 1 (Awad, 2005). Because tumor cells to ensue their

metastasis produce these factors, it can be suggested that the anti-tumor effects of *N. sativa* oil might be mediated through anti-angiogenic effects through inhibition of local tumor invasion and metastasis *in vivo*.

2. Effect of carboplatin (10mg/kg), *N. sativa* oil (12ml/kg) and their combination on GOT and GPT activities in serum of Ehrlich ascites tumor bearing female mice *in vivo*

Data in Table (1) showed that the enzyme activity of GOT and GPT was increased by 24.15% and 29.84%, respectively in negative control as compared to healthy control. On the other hand, the treatment of carboplatin (10mg/kg) revealed that the activity of GOT and GPT was increased by 56.52% and 51.14%, respectively as compared to both healthy control and negative control, *N. sativa* oil (12ml/kg) showed that the enzyme activity of GOT and GPT was increased by 14.75% and 19.84%, respectively as compared to healthy control, while the enzyme activity was decreased as compared to negative control (Table 1). The effect of combination of carboplatin and *N. sativa* oil on GOT and GPT activities appeared to be increased by 62.41% and 49.39%, respectively as compared to healthy control and negative control (Table1).

Table (1): Effect of carboplatin (10mg/kg), N. sativa oil (12ml/kg) and their combination on GOT and GPT activities in serum of Ehrlich ascites tumor bearing female mice.

	Parameters		
Treatments	**GOT activity µ/l**	**GPT activity µ /l**	**ALP activity (I µ /L)**
Healthy Control	48.48±2.70	36.29±5.66	117.00±8.26
Negative control % change	60.19±0.32 24.15%**	47.12±5.49 29.84%*	Not detected
Carboplatin % change	75.88±5.73 56.52%**	54.85±6.20 51.14%**	Not detected
N. sativa oil % change	55.63±5.90 14.75%†	43.49±1.50 19.84%*	Not detected
Carb.+ *N. sativa* oil % change	62.41±5.87 28.73%*	49.39±0.72 36.1%**	Not detected

* and ** indicate significance at 0.05 and 0.01 probability levels, respectively

Alkaline phosphatase (ALP) activity in serum was measured, there was no observed activity. On the other hand, ALP activity in healthy mice was appeared as 117 IU/L. The active principle fatty acids derived from *N. sativa*, completely inhibited the growth of Ehrlich ascites carcinoma and Dalton's lymphoma ascites cells (Salomi *et al.*, 1992). Moreover, oral feeding with *N. sativa* extract suppressed hepatic tumor in rat induced by diethylnitrosamine or by partial hepatectomy (Iddamaldeniya *et al.*, 2003). Our present study revealed that GOT and GPT were increased in the negative control as compared to healthy group, also there was no ALP activity in all groups except the healthy group, this may be due to the effect of tumor transplantation in female mice. Furthermore, *N. sativa* oil suppressed colon carcinogenesis induced by methylnitrosourea (Mabrouk *et al.*, 2002), or by 1,2- dimethylhydrazine (Salim and Fukushima, 2003). Mahmoud *et al.*, (2002) reported that the liver functions were improved by a decrease of the elevated serum levels of ALT, GGT, ALP and by a

normalization of albumin under the effect of *N. sativa* oil.

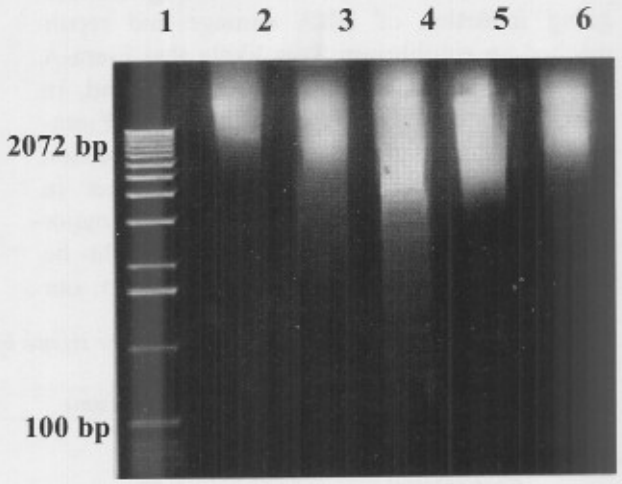

Fig. (2): Genomic DNA of liver tissue on agarose gel electrophoresis (2%), (1) Marker (100 bp), (2) healthy control, (3) neg. control, (4) caboplatin (10mg), (5) N. sativa oil (12 ml/kg), (6) combination of carboplatin and N. sativa oil.

3. Effect of carboplatin (10mg/kg), *N. sativa* oil (12ml/kg) and their combination on genomic DNA on agarose gel electrophoresis

Fig. (2) illustrated the different lanes profiling of the genomic DNA on agarose gel (2%). As revealed from the figure of genomic DNA of healthy control and *N. sativa* oil groups showed no observed fragmentation, while the other bands of different group's revealed damage and smear bands. This is due to the cytotoxicity of CDDP and the protective effect of *N. sativa* oil on genomic DNA. Carboplatin (CBP) is a DNA-damaging agent that causes S-phase blockade of dividing cells. Its DNA-damaging effect may be mediated through the formation of CBP-DNA adducts (Alonso *et al.*, 2006); *N. sativa* oil is an effective free radical scavenger showing antioxidant activity and protecting against the damage caused by free radicals.

Therefore the N. *sativa* oil is useful in diseases in which free radicals are involved, e.g. anoxia and ischemia of brain and heart as well as arteriosclerosis, rheumatism and cancer (Al-Ghamdi, 2001). Platinum-resistant cells may have several chromosomal abnormalities (Abdel-Latif *et al.*, 2005). Telomere length, telomerase activity, and telomerase mRNA expression were reduced in cisplatin-resistant OC cell lines (Kiyozuka *et al.*, 2000). Cytochrome P-450 has the role in producing free radicals, which may then react with molecular oxygen to form superoxide anion radicals. The radicals thus formed may give rise to highly reactive species such as hydroxyl radicals, singlet oxygen and hydrogen peroxide (Pratibha *et al.*, 2006). Cisplatin induced hepatotoxicity was enhanced by elevated expression of Cyp2E1 and may involve increasing production of ROS and oxidative stress (Lu and Cederbaum, 2006).

4. Effect of carboplatin (10mg/kg), *N. sativa* oil (12ml/kg) and their combination on quantitation of DNA incision by the alkaline single cell gel electrophoresis (comet assay)

Data in Table (2) and Figure (3) illustrated that the tail moment appeared to be high under the effect of carboplatin as compared to each group, while the tail moment appeared to be low under the effect of *N. sativa* oil alone. On the hand, the combination between carboplatin and *N. sativa* oil appeared to decrease the tail moment as compared to carboplatin alone. Takagi *et al.*, (2004) reportid that the incorporation of thymidine and the tail moment after treatment with carboplatin confirmed that quiescent lymphocytes were proficient in repairing DNA damage initiated by carboplatin up to 150 mM. When carboplatin was administered at a dose range of 75–450 mM, the peak concentration ranged from 40 to 230 mM. Takagi *et al.*, (2004) suggested that lymphocytes were able to accomplish DNA repair during 24 hr continuous exposure to carboplatin, DNA incision reached a plateau after 3–6 hr

incubation with carboplatin, indicating that on-going induction of DNA damage and repair reached an equilibrium. It is likely that F-ara-A was incorporated into the repair patch and, in turn, inhibited DNA repair. Therefore, F-ara-AMP incorporation and DNA incision accumulated in a time-dependent manner in continuously treated-cells that induced major cellular damage, but these lesions might be insufficient in pulse-treated cells. From our present study, carboplatin as anticancer drug causes many deleterious effects not only on tumor cells, but also on intact organs. On the other hand, the combination with *Nigella sativa* oil decreases the dangerous side effects of carboplatin and enhances carboplatin activity against tumor cells. We recommend that any chemotherapeutic agent should be taken in combination with antioxidant agent.

Table (2): Induction of DNA incision of liver tissue by carboplatin (10mg/kg), N. sativa oil (12ml/kg) and their combination.

Parameter	Tail length (um)	DNA%	Tail moment
Healthy Control	1.585	1.501	02.38
Negative control	5.254	4.521	32.75
Carboplatin	8.281	7.216	59.76
***N. sativa* oil**	1.657	1.494	02.48
Carb.+ *N. sativa* oil	4.392	3.953	17.36

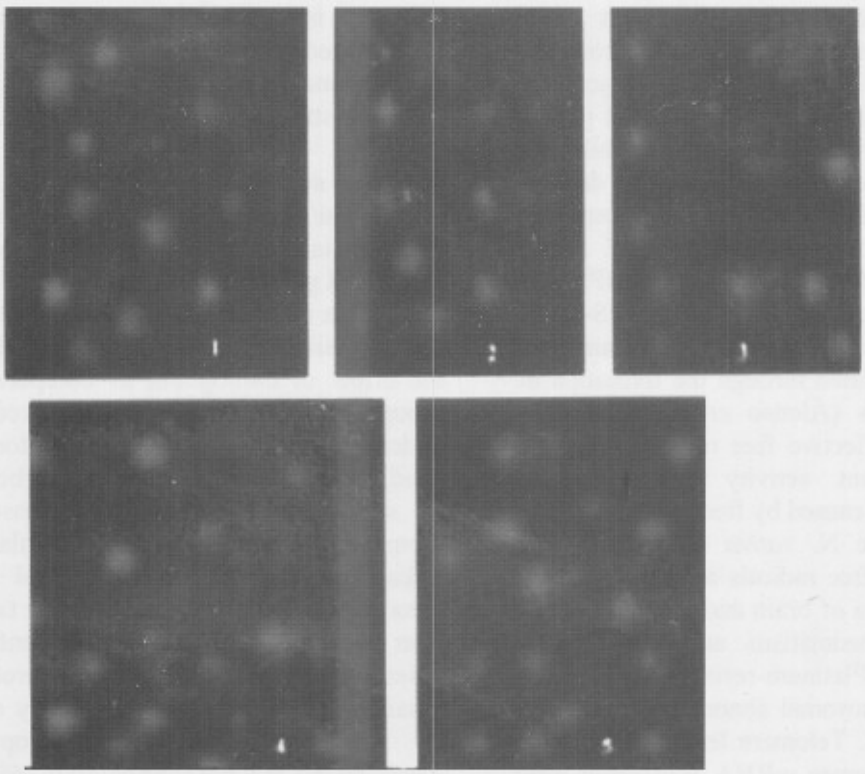

Fig. (3): Induction of DNA incision by carboplatin, (1) healthy control, (2) neg. control, (3) carboplatin (10mg), (4) N. sativa oil (12 ml/kg), (5) combination of carboplatin and N. sativa oil.

REFERENCES

Abdel-Latif, M. M.; Raouf, A. A.; Sabra, K.; Kelleher, D. and Reynolds, J. V. (2005). Vitamin. C enhances chemosensitization of esophageal cancer cells *in vitro*. J. Chemother., 17:539-49.

Aboul-Ela, E. I. (2002). Cytogenetic studies on *Nigella sativa* seeds extract and thymoquinone on mouse cells infected with schistosomiasis using karyotyping. Mutation Research, 516: 11-17.

Alberts, D. S. (1995). Carboplatin versus cisplatin in ovarian cancer. Semin. Oncol., 22:88-90.

Al-Ghamdi, M .S. (2001). The anti-inflammatory, analgesic and anti-pyretic activity of *Nigella sativa*. Journal of Ethnopharmacology., 76:45-48.

Alonso, A.; Almendral, M. J.; Curto, Y.; Criado, J. J.; Rodríguez, E. and Manzano, J. L. (2006). Determination of the DNA-binding characteristics of ethidium bromide, proflavine, and cisplatin by flow injection analysis: usefulness in studies on antitumor drugs. Anal. Biochem., 355: 157-164.

Awad, E. M. (2005). *In vitro* decreases of the fibrinolytic potential of cultured human fibrosarcoma cell line, HT1080, by *Nigella sativa* oil. Phytomedicine, 12:100-7.

Duke, R. C., and Cohen, J. J. (1986). Endogenous endonuclease-induced DNA fragmentation: an early event in cell-mediated cytolisis. Proc. Natl. Acad. Sci. U.S.A., 80: 6361.

Feng, G.; Wang, D. Z.; Chen, H. Q.; Hu, J. and He, J. (2008). A new method to induce multi-drug resistance to carboplatin in a mouse model of human tongue squamous cell carcinoma. Int. J. Oral Maxillofac. Surg., 37: 1141-1147.

Fisher, A. R. and Yates, F. (1957). In: The Statistics for Biology; Agriculture and Medical Research. 5th ed.; Olea europear and Boyd Publications; Edinburgh; London. Food Chem. Toxicol.; 11:85-94. Food Chem., 49:7-30.

Fujiwara. K.; Sakuragi, N. and Suzuki, S. (2003). First-line intra-peritoneal carboplatin-based chemotherapy for 165 patients with epithelial ovarian carcinoma: results of long term follow up. Gynecol. Oncol., 90:637-643.

Houghton, P. J.; Zarka, R.; de la Heras, B. and Hoult, J. R. S. (1995). Fixed oil of *Nigella sativa* and derived thymoquinone inhibit eicosanoid generation in leukocytes and membrane lipid peroxidation. Planta Medica, 61:33-36.

Iddamaldeniya, S. S.; Wickramasinghe, N., Thabrew, I.; Ratnatunge, N. and Thammitiyagodage, M. G. (2003). Protection against diethylnitrosoamine - induced hepatocarcinogenesis by an indigenous medicine comprised of *Nigella sativa*, Hemidesmus indicus and Smilax glabra: a preliminary study. J. Carcinog., 2:6.

Ilhan, A.; Gurel, A.; Armutcu, F.; Kamisli, S. and Iraz, M. (2005). Antiepileptogenic and antioxidant effects of *Nigella sativa* oil against pentylenetetrazol-induced kindling in mice. Neuropharmacology, 49: 456-464.

Islam, S. N.; Begum, P.; Ahsan, T.; Huque, S. and Ahsan M. (2004). Immunosuppressive and cytotoxic properties of Nigella sativa. Phytother. Res.,18:395-8.

Jansen, B. A.; Brouwer, J. and Reedijk, J. (2002). Glutathione induces cellular resistance against cationic dinuclear platinum anticancer drugs. J. Inorg. Biochem., 89:197-202.

Kiyozuka, Y.; Yamamoto, D. and Yang, J. (2000). Correlation of chemosensitivity to anticancer drugs and telomere length, telomerase activity and telomerase RNA

expression in human ovarian cancer cells. Anticancer Res., 20:203-12.

Lu, Y. and Cederbaum, A. I. (2006). Cisplatin-induced hepatotoxicity is enhanced by elevated expression of cytochrome P450 2E1. Toxicol. Sci., 89:515-523.

Mabrouk, G. M.; Moselhy, S. S.; Zohny, S. F.; Ali, E. M.; Helal, T. E. and Amin, A. A. (2002). Inhibition of methylnitrosourea (MNU) induced oxidative stress and carcinogenesis by orally administered bee honey and *Nigella* grains in Sprague Dawely rats. J. Exp. Clin. Cancer Res., 21:341-6.

Mahmoud, M. R.; El-Abhar, H. S. and Saleh, S. (2002). The effect of *Nigella sativa* oil against the liver damage induced by *Schistosoma mansoni* infection in mice. Journal of Ethnopharmacology, 79: 1-11

Marsh, W. H.; Finger hut, B. and Krish, E. (1959). Clin. Chem., 5:119.

Medenica, R.; HJanssens, J.; Tarasenko, A.; Lazovic, G., Corbitt, W. and Powell, D. (1997). Antiangiogenic activity of *Nigella sativa* plant extract in cancer therapy. Proc. Annu. Meet. Am. Assoc. Cancer Res, 38:A1377.

Murphy, G. P.; Morris, L. B. and Lange, D. (1997). Informed Decisions: The Complete Book of Cancer Diagnosis; Treatment and Recovery. Viking Penguin, N.Y.

Murray, R. (1984). Alanine aminotransferase. Keplan, A. *et al.*, Clin. Chem. The C. V. Mosby Co. St Louis. Toronto. Princeton 1088-1090.

Phillips, J. D. (1992). Medicinal plants. Biologist, 39: 187-191.

Pratibha, R.; Sameer, R.; Padmanabh, V. R.; Dayanand, A. B. and Chitra, Y. D.(2006). Enzymatic studies of cisplatin induced oxidative stress in hepatic tissue of rats. European Journal of Pharmacology, 532:290-293.

Salim, E. I. and Fukushima, S. (2003). Chemopreventive potential of volatile oil from black cumin (*Nigella sativa* L.) seeds against rat colon carcinogenesis. Nutr. Cancer, 45:195- 202.

Salomi, N. J.; Nair, S. C.; Jayawardhanan, K. K.; Varghese, C. D. and Panikkar, K. R. (1992). Antitumour principles from *Nigella sativa* seeds. Cancer Lett., 63:41-46.

Singh, N. P.; McCoy, M. T.; Tice, R. R. and Schneider, E. L. (1988). A simple technique for quantitation of low levels of DNA damage in individual cells. Exp. Cell Res., 175 184-191.

Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S. and Boyd, M. R. (1990). New colorimetric cytotoxicity assay for anticancer-drug screening. J. Natl. Cancer Inst., 82 (13):1107-1112.

Snedecor, G.W. and Cochran, G.C. (1967). In, Statistical Methods. Iowa State University Press, Iowa, U.S.A.

Swamy, S. M. and Tan, B. K. (2000). Cytotoxic and immunopotentiating effects of ethanolic extract of *Nigella sativa* L. seeds. J. Ethnopharmacol., 70:1- 7.

Takagi, K.; Kawai Y.; Yamauchi, T. and Ueda, T. (2004). Inhibition of repair of carboplatin-induced DNA damage by 9-b-D-arabinofuranosyl-2-fluoroadenine in quiescent human lymphocytes. Biochemical Pharmacology, 68:1757-1766.

Zver, S.; Zadnik, V.; Bunc, M.; Rogel, P.; Cernelc, P. and Kozelj, M. (2007). Cardiac toxicity of high-dose cyclophosphamide in patients with multiple myeloma undergoing autologous hematopoietic stem cell transplantation. Int. J. Hematol .,85:408-414.

الملخص العربي

تأثير عقار الكربوبلاتين وزيت حبة البركة على الخلايا البشرية السرطانية (MCF-7) وإناث الفئران الحاملة لخلايا Ehrlich ascites tumor

نورهان الجوهرى*، جيهان صفوت,*** ، ماجد إبراهيم ***، أيمن دياب ****,*، منى هاشم*****

*كلية البيوتكنولوجى-جامعة أكتوبر للعلوم الحديثة والأداب-أكتوبر-مصر

**معهد بحوث البساتين-مركز البحوث الزراعية-الجيزة-مصر

***شعبة التقييم الدوائى الجزيئى-الهيئة القومية للرقابة والبحوث الدوائية-وزارة الصحة- الجيزة-مصر

****معهد بحوث الهندسة الوراثية الزراعية-الجيزة-مصر

*****قسم الوراثة- كلية الزراعة- جامعة القاهرة-الجيزة-مصر

يعتبر الكربوبلاتين من الأدوية المضادة للسرطان المصنعة لعلاج السرطان. ويعتبر زيت حبة البركة من المنتجات الطبيعية الذى له تأثيرات صحية جيدة. لذلك كان الهدف من هذه الدراسة هو تقييم تأثير كل من الكربوبلاتين وزيت حبة البركة على حدة وكذلك الخليط بينهما على الخلايا البشرية السرطانية (MCF-7) وإناث الفئران الحاملة لخلايا Ehrlich ascites tumor حيث أوضحت النتائج ان التركيزات المختلفة للكربوبلاتين وهى 5، 12.5، 25، 50ميكروجرام/مللى قللت معدل surviving fraction وكان التركيز المثبط (IC_{50}) للكربوبلاتين 11.8 ميكروجرام/مللى. أما زيت حبة البركة فكانت تركيزاته هى 50، 100، 150، 200 ميكروجرام/مللى أدت إلى تقليل معدلsurviving fraction وكان التركيز المثبط لزيت حبة البركة 39 ميكروجرام/مللى، لكن كان التركيز المثبط للخليط بين الكربوبلاتين وزيت حبة البركة 3.78 ، 40 ميكروجرام/مللى على التوالى. أما عن تأثير الكربوبلاتين وزيت حبة البركة كل على حدة والخليط بينهم على الفئران الحاملة لخلايا Ehrlich ascites tumor فقد أوضحت النتائج بأن الكربوبلاتين بتركيز 10 ميلليجرام/كجم أدى إلى إرتفاع إنزيمات أنشطة الكبد (GPT, GOT) إلى 56.52%، 51.14% على التوالى فى السيرم مقارنة بالفئران السليمة الغير حاملة لخلايا Ehrlich ascites tumor والمجموعة الضابطة الحاملة لخلايا Ehrlich ascites tumor اما زيت حبة البركة بتركيز 12 مللي/كجم أدى إلى إنخفاض إنزيمات أنشطة الكبد (GPT, GOT) إلى 14.75%، 19.84% على التوالى بالمجموعة الضابطة الحاملة لخلايا Ehrlich ascites tumor أما الخليط بين الكربوبلاتين وزيت حبة البركة أدى إلى إرتفاع إنزيمات أنشطة الكبد (GPT, GOT) إلى 62.41%، 49.39% على التوالى مقارنة بالفئران السليمة الغير حاملة لخلايا Ehrlich ascites tumor والمجموعة الضابطة الحاملة لخلايا Ehrlich ascites tumor أيضا ادى تركيز الكربوبلاتين الى حدوث تكسير فى Genomic DNA من خلال الفصل على الأجاروز و comet assay الخاص بخلايا الكبد لكن أظهرزيت حبة البركة عدم حدوث تكسير فى Genomic DNA الخاص بخلايا الكبد أما الخليط بين الكربوبلاتين وزيت حبة البركة فقد قلل تكسير فى Genomic DNA الحادث بسبب الكربوبلاتين مقارنة بالفئران السليمة الغير حاملة لخلايا Ehrlich ascites tumor .