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RESEARCH ARTICLE

ROLE OF *SPIRULINA PLATENSIS* ON MITOMYCIN C- INDUCED GENOTOXICITY IN EHRLICH ASCITES CARCINOMA BEARING ALBINO MICE: SINGLE-CELL GEL ELECTROPHORESIS (COMET ASSAY) AND SEMIQUANTITATIVE RT-PCR ANALYSIS

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ABSTRACT

In cancer therapy, Mitomycin C (MMC) is a commonly used drug to fight several human malignancies; it is a powerful anti-bacterial and anti-tumor fungal antibiotic. However, during clinical use several side effects may occur. The current study was designed to investigate the potential effect of pre- and post- addition of *Spirulina platensis* (Sp) to the diet on mitomycin C (MMC) induced genotoxic and molecular alterations. Sixty Ehrlich Ascites Carcinoma Cell (EAC) bearing female albino mice (i.p injection of $2.5x10^6$ EAC cell/ mouse) were classified into six groups which received different treatment of SP powder 1% in diet and MMC (i.p. 1mg/kg BW). After 7 days from MMC injection, specimens were collected for evaluation of DNA damage using comet assay and transcriptional estimation of mutant apoptotic P53 and glutathione peroxidase mRNA expression level by semiquantitative (RT-PCR). Results showed that MMC induced a genotoxic effect in mice bone-marrow and liver cells, SP significantly reduced the level of DNA damage and augmentation of expression levels of studied genes mRNA. In conclusion, our results confirm the antigenotoxic potential of SP and provide strong evidence to support administration of SP during MMC-therapy.

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INTRODUCTION

Mitomycin C is a prototypical alkylating anti-tumor antibiotic isolated from *Streptomyces caespitosus*. Although current use of MMC is limited due to its toxicity in several normal tissues (Turkez *et al*., 2012), this agent continues to be a key element in several clinical trials due to its intrinsic efficacy against many solid tumors including colon, breast, lung, head and neck and preferential activity in hypoxic tumor cells. It is metabolically activated to induce DNA adducts formation and DNA cross-linking that leads to cytotoxicity and cell death. MMC is a direct-acting clastogen requiring only intracellular reductive activation to initiate its potent DNA cross-linking action (Kumar *et al*., 1997). At present, *Spirulina platensis* (SP) is the most commonly consumed and commercialized blue-green algae for more than 10 years as a nutraceutical and potential source of pharmaceutical targets, because it contains high quality protein and its nutritional and various medicinal properties such as natural biochelated vitamins, minerals, essential fatty acids, phycocyanin and β-carotene (Sharma *et al*., 2007 and Lordan *et al*., 2011). SP is well known for its antioxidant and anti-cancerous properties as well as its ability to amend the carcinogen damaged DNA (Romay *et al*., 2003). Several studies have looked at it's therapeutic properties in animals. It has been shown to reduce oxidative stress and hypercholesterolaemia in rabbits fed a high-cholesterol diet (Kim *et al*., 2010). Moreover, pre-treatment with a Spirulina-enhanced diet protected rats from cyclophosphamide-induced nephrourotoxicity via its antioxidant and anti-apoptotic properties (Sinanoglu *et al*., 2012). Chronic treatment with Spirulina prevented ischaemia/reperfusion-induced apoptosis (Wang *et al*., 2005). The genotoxic effects of MMC have been proven in chromosome aberration tests and in micronucleus assays in mouse spermatocytes, bone-marrow cells and in *Drosophila melanogaster* (Niikawa *et al*., 2007 and Li *et al*., 2009). In this context, strategies to protect against MMC-induced genotoxicity are of clinical interest and cyto-protective agents are essential to provide this protection. Therefore the aim of the present work was to investigate the protective effect of SP against MMC-induced genotoxicity in bone-marrow cells and liver of female albino mice under different conditions of treatment. For this purpose, the DNA-damage level was measured with the comet assay and transcriptional estimation of mutant apoptotic P53 mRNA and glutathione peroxidase (GPx) mRNA expression level by semiquantitative RT-PCR.

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MATERIALS AND METHODS

Tested compounds and chemicals

Mitomycin C vials (MMC; $C_{15}H_{18}N_4O_5$) Kyowa. Co. Ltd (Tokyo-Japan), contains Mitomycin C hydrochloride 10 mg potency, it was purchased from Eman- Elazab pharmacy, Zagazig City, Sharkia Province, Egypt. *Spirulina platensis* (Sp) is bright, blue-green powder with characteristic odor (pH: 4.5-6.5), it was stored in fresh, dry, pest-free and hygienic store rooms to prevent deterioration of *Spirulina* pigments. It was purchased from EL-Hellowa for Biological Products. The parent line of Ehrlich Ascites Carcinoma cells (EAC) was initially supplied by the National Cancer Institute, Cairo University, Egypt.

Experimental Design

Sixty female albino mice weighed 30-40 g were kept in metal cages under hygienic conditions, provided with food and water *ad-libitum* through the experiment. The animals were observed along the experimental period. Mice were i.p injected with 0.2 ml of ascetic

Table 1. Experimental groups, line of feeding, induction of cancer, and treatment

Treatment / Groups	Pre treatment with 1% Sp powder in food for 3 weeks before tumor induction (SP1) (Linjawi, 2011)	Tumor induction by i.p injection of $2.5x10^6$ EAC cell/ mouse	MMC i.p injection of 1 mg/kg BW in 3 rd day of tumor induction and for 7 consecutive days (Pawar *et al*., 2009)	Post treatment with 1% Sp powder in food after MMC injection and for 7 days (SP2)
Control	-	+	-	-
Sp1	+	+	-	-
Sp1+Sp2	+	+	-	+
MMC	-	+	+	-
SP1+MMC	+	+	+	-
Sp1+MMC+Sp2	+	+	+	+

Table 2. Oligonucleotide primers and PCR condition used for Semi-quantitative RT-PCR analysis of GAPDH, GPx and P53 genes

Gene	Sequences (5'-3')	Product size/pb	Refrences
GAPDH	Forward: AGT ATG ATG ACA TCA AGA AGG Reverse: ATG GTA TTC AAG AGA GTA GGG	421	Peters *et al*., 2002
GPx	Forward: GCC CTC CCA CTG CAG AAC TCC Reverse: GCT GCC TGC CGC CTC ATG	193	Jayara *et al*., 2006
P53	Forward: GGG ACA GCC AAG TCT GTT ATG Reverse: GGA GTC TTC CAG TGT GAT GAT	350	Chew *et al*., 2003

fluid containing 2.5×10^6 EAC viable cells (the viability was checked by the trypan blue exclusion test) (Zeinab, 2009) and divided into six groups of ten animals each as shown in Table 1. At the end of the experimental period (7days post MMC injection), animals of each group were sacrificed. Both femurs were removed and the content was directly flushed out with a 24-gauge needle into a micro-centrifuge tube. The cell suspension was prepared in PBS containing 20 mM EDTA and 1% DMSO and kept frozen at -20°C in phosphate buffer saline until single-cell gel electrophoresis assay analysis. Liver tissues were snap-frozen in liquid nitrogen and kept at -80°C until gene expression analysis of GPx mRNA and P53 mRNA.

Single-cell gel electrophoresis (SCGE, Comet assay)

From the thawed femoral bone marrow suspension, 5 µl of sample (containing approximately $2–5\times10^4$ cells/ml) were added to 95 µl of 0.5% low-melting agar (LMA) (in PBS) to prepare the final cell–agarose suspension. The comet assay was performed as described by Singh *et al*. (1988). From the final cell-agarose suspension, 80 µl was spread over the microscope slide, which was pre-coated with 1% normal-melting agar (NMA). The cells were then lysed in a buffer containing 2.5 M NaCl, 100 mM EDTA, 10 mM Tris (pH 10.0), with freshly prepared 1% Triton X-100 and 10% DMSO for 24 h at 4 ◦C. After lysis, slides were rinsed three times in de-ionized water to remove salt and detergent. Slides were placed in a horizontal electrophoresis unit and DNA was allowed to unwind for 20 min in alkaline solution containing 300 mM NaOH and 1 mM EDTA, pH > 13. Electrophoresis was performed at 0.7 V/cm for 30min (300mA, 24 V). The slides were neutralized with 0.4 M Tris (pH 7.5), stained with Acridine Orange (20 µg/ml) before examination with a fluorescence microscope (Olympus BX51 TRF, USA). To prevent secondary DNA damage, preparative steps were conducted in darkness. A total of 50 randomly selected images were subjected to image analysis with Comet Assay Project Software (CAPS). Mean values of tail moment, tail length and tail DNA intensity (%) were separately analyzed for statistical significance.

Expression levels of Glutathione peroxidase (GPx) mRNA and Mutant apoptotic gene P53 mRNA in the liver

To compare expression levels of genes mRNAs between control and treated groups, a semi-quantitative RT-PCR protocol was used according to (Mallet *et al*., 1995). Expression was normalized by glyceraldehyde 3-phosphate dehydrogenase (GAPDH) mRNA gene expression, which was used as an internal housekeeping control.

Tissue preparation and RNA extraction

Total RNA was extracted from 30 mg Liver tissue using RNeasy Mini Kit (Qiagen, Heidelberg, Germany), according to the manufacturer's instructions. Quality of RNA was checked by electrophoresis on 2% agarose gel. Concentrations and purities of the isolated RNA were assessed by spectrophotometer. Only high purity samples (OD 260/280 >1.8) were further processed.

Reverse transcription reactions

Aliquots of 1 µg RNA were reverse transcribed into cDNA using RobusT™ I RT-PCR Kit (Finnzymes, Oy, Findland) according to the manufacturer's instructions. The PCR thermal cycler (2720 Thermocycler Applied Biosystems) was prepared as following; at 48ºC for 30 minutes for c DNA synthesis, followed by a 2 minutes at 94°C to inactivate the AMV RT enzyme and denaturation of the cDNA-RNA hybrid, then stored at 4°C.

PCR reactions

The cDNA was used to amplify the genes of interest. The list of primers sequence has been shown in Table (2). The amplification reaction was carried out in 25 µl final volume containing 1.5µl primer (100 pmol/µl), 12.5µl 2X Tag mastermix, 5µl template cDNA (50 ng/µl) and 6µl ddH_2O. Amplification of cDNA was performed in Thermal Cycler (Master Cycler Gradient, Eppendorf, Germany) with the following cycling program; 30 cycles: 94˚C, 30 s ; 55˚C, 30 s; 72˚C, 45 s, final extension: 72˚C, 5 min for both GAPDH and GPx while P53 was: 35 cycles: 94˚C, 45 s; 54˚C, 45 s; 72◦C, 1 min, final extension: 72◦C, 10 min. The 20 µl PCR products were loaded in 1.5% agarose gel and captured through gel documentation system. The expression levels of the gene bands intensity on gel were analyzed using Image J soft ware (version 1.24).

Statistical analysis

Data are presented as means ± standard error of the mean. All statistical analyses were performed using computer software (SPSS/PC+; 2001) for comparisons between means of control and treated groups, P value < 0.05 was considered significant. The statistical method was one way ANOVA test.

RESULTS

In the present study, three mice died from control group and two mice died from MMC treated group at 6th day from MMC injection, other

mice showed an increase of abdomen size with signs of emaciation and alopecia especially in MMC treated groups. After decapitation there are large amount of ascetic fluid in abdomen due to EAC inoculation.

Single cell gel electrophoresis (Comet assay)

Results of the visual scoring of total DNA damage are illustrated in Fig. 1, there are a significant increase ($p < 0.05$) of the total DNA damage in bone-marrow cells of mice treated with MMC compared with the control group. Treatment with Sp (1%) in diet for 3 weeks pre- and post- i.p injection of MMC was significantly reduced comet tail length, % of DNA damage and tail moment compared with MMC treated group (Table 3).

Table 3. Oxidative DNA damage in the bone marrow of EAC bearing female mice in different groups (means ± S.E)

Groups	Tail length (µm)	DNA tail (%)	Tail moment (units)
Control	4.33±0.07^{c}	2.97±0.03^{cd}	13.90± 0.41^{c}
SP1	4.01±0.01^{d}	3.01±0.01^{cd}	14.19±0.61^{c}
SP1 +SP2	2.41±0.17^{f}	2.66±0.02^{d}	7.05 ±0.98^{d}
MMC	5.25± 0.03^{a}	6.15±0.58^{a}	30.49±0.87^{a}
SP1+MMC	4.74±0.11^{b}	4.44±.042^{b}	18.92±0.53^{b}
SP1+MMC+SP2	3.35±0.05^{e}	3.46±0.18^{c}	17.31±0.86^{b}

Means within column carrying different superscripts are significant at (P <0.05).

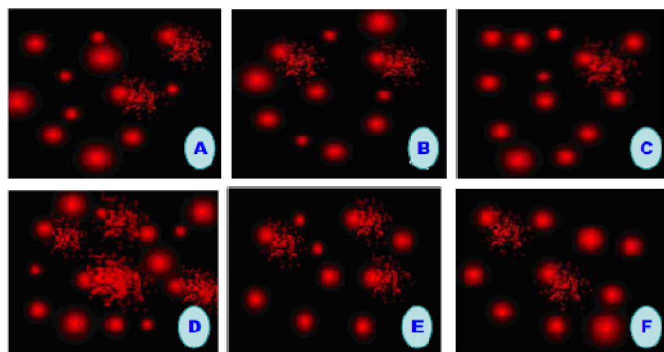

Fig. 1. Representative Comet images of bone marrow cells of EAC bearing female mice in different groups; A. control, B. Sp1, C. Sp1+Sp2, D. MMC, E. Sp1+MMC, F. Sp1+MMC+Sp2.

Expression levels of Glutathione peroxidase (GPx) mRNA and Mutant apoptotic gene P53 mRNA in the liver

The expression of p53 mRNA in the liver of MMC treated group was up regulated in comparison to control and Sp treated groups while the expression level was gradually down regulated in Sp treated groups either pre- or post- MMC treatment in comparison with MMC treated group.

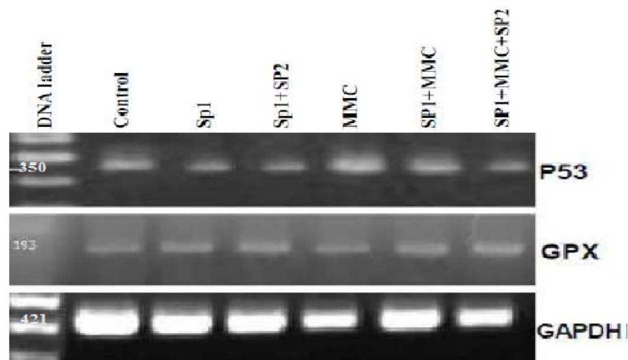

Fig. 2. Gel electrophoresis images showing genetic alteration in P53, GPx mRNA gene expression in the liver of EAC bearing female mice in different groups.

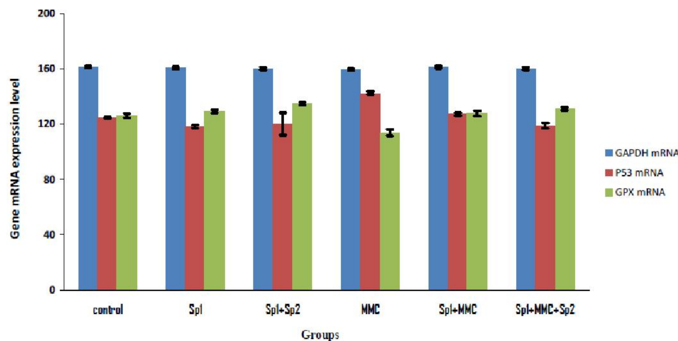

Fig. 3. Quantitative analysis of GPx, P53 and GAPDH mRNA in the different groups

Concerning with GPx mRNA expression in liver, the results revealed that it was down regulated in MMC treated group in comparison to control and Sp groups while its expression was gradually up regulated in Sp treated groups pre- and post- MMC treatment in comparison with MMC treated group.

DISCUSSION

Genotoxicity refers to the capability of chemotherapeutic agents to damage DNA and /or cellular components regulating the fidelity of the genome. The main problem posed anticancer drugs is that they target not only the tumor, but also other cells, thus causing the same damage to both abnormal and normal cells (Granados *et al*., 2010). The Genotoxicity is believed to be an important mechanism in the development of MMC toxicity, so the protection of normal tissues against MMC genotoxicity is of clinical interest. In the present study we evaluated the oxidative DNA damage level in bone marrow cells to elucidate the possible anti-genotoxic mechanism of Sp against MMC induced toxicity. The oxidative DNA damage of MMC was evaluated by means of the comet assay, which is widely used in genotoxicity testing *in vitro* and also becoming an important tool for evaluating the genotoxic potential and mutagenicity of many chemicals and natural compounds *in vivo* where as it play important roles in the determination of DNA damage level (Nan *et al*., 2013). The present study revealed that MMC treated mice significantly showed an increase of DNA damage through increased tail length, tail moment and tail DNA % in bone marrow cells comparing to control group. This result became in harmony with (Linjawi, 2011) who recorded that single i.p injection of MMC (2mg/kg b.wt) induced DNA damage in male rats and attributed this damage to the high concentration of nitric oxide (NO) generated by MMC either directly or through secondary molecules, by nitrose- active deamination, DNA strand breakage and DNA modifications, also this result in accordance with Pfuhler and Wolf (1996) who found that MMC led to an increase in DNA migration at concentrations of 100–800µM in human whole blood cultures, this owed to the ability of MMC to form H_2O_2 and hydroxyl radicals after reductive bioactivation induced DNA damage with high sensitivity.

The pre- and post- treatment with Sp in the current study leads to a significant reduction of DNA damage caused by MMC as this results were in agreement with (Kaji *et al*., 2002) who reported that the unique polysaccharides of SP enhance cell nucleus enzyme activity and potentiate the process of DNA repair and (Bhat and Madyastha, 2000) who found that phycocyanin and phycocyanobilin contents of Sp were also have strong anti- cyclooxygenase-2, antioxidant activity to scavenger peroxidinitrite and reduce OONO-induced oxidative damage to DNA, also the present result were in parallel to (Qishen *et al*., 1988) who reported that the presence of the polysaccharide content of SP enhanced significantly both the repair activity of damaged DNA excision and the unscheduled DNA synthesis. Also these results were in accordance with Gad *et al*. (2010) and Hassan *et al*. (2012) who demonstrated that supplementation of Sp reduced the oxidative stress induced by aflatoxicosis which indicated by decreased lipid peroxidation level, increased glutathione content and

up-regulation of GPx mRNA gene expression which accompanied with down-regulation the gene expression of apoptotic genes that is in turn succeeded to inhibit DNA damage and decreased the percentage of DNA fragmentation. In the present study, there was up regulation of p53 gene expression in MMC treated group in comparison with control group which is in harmony with (Linjawi, 2011) who reported that the expression profile of p53 gene was significantly higher in hepatic tissues of male rats exposed to MMC at (0.5 and 2mg/ kg b.wt) and (Pirnia *et al*., 2002) who explained that MMC as DNA damaging agent is a very strong activator of p53 function compared to other anticancer drugs, this may be attributed to reactive nitrogen species induced DNA damage can lead to p53 accumulation which mediated apoptosis as high concentrations of nitric oxide products generated by MMC can cause DNA damage, either directly or through secondary molecules, by nitroso-active deamination, DNA strand breakage, and DNA modifications, whereas Abbas *et al*. (2002) reported that MMC induced DNA damaging in the form of DNA cross links as well as a variety of DNA mono adducts is known to induce p53. Many studies proved that p53 has regulatory responses to a variety of cellular stresses, including DNA damage, nucleotide depletion, chemotherapeutic drugs and oxidative stress, genotoxic damages, oncogene activation and hypoxia (Grawish, 2008).

P53 mRNA in Sp treated mice was significantly down regulated in the liver of Sp treated groups either pre- or post-treatment of MMC and this agreement with (Linjawi, 2011) who reported that Sp significantly reduced MMC induced rat hepatic tissue genetic alterations whereas, the Sp was able significantly to down regulate Bcl-2, P53, and P21 expression in the hepatic cells at the dose of 1% of the rat diet and also the results were in agreement with (Ismail *et al*., 2009) who found that the treatment with Sp inhibited the incidence of liver carcinogensis and expression level of P53 mRNA in liver of doxorubicin treated rats. Oxidative stress was considered to be one of the mechanisms implicated in MMC-induced toxicity (Turkez *et al*., 2012). MMC oxygen-dependent toxicity occurs via cyclic one electron reduction of the MMC molecule, followed by oxidation with molecular oxygen, producing the active MMC molecule and the superoxide radical. Excess generation of reactive oxygen species can attack biomolecules, such as DNA, lipids, and thiols in proteins and glutathione leading to inactivation of enzymes, genotoxic damage, cell dysfunction, and cell death (Klaunig *et al*., 2011). The present study revealed that GPx mRNA in the liver of MMC treated mice was down regulated comparing with the control and Sp treated groups. This result became in accordance with (Lee *et al*., 2004) who reported that MMC induced oxidative damage against small cell lung cancer cells through the enzymatic reduction leading to depletion or oxidation of GSH by oxidative attack of free radicals. Hassan *et al*. (2012) suggested that the direct oxidative stress evidenced by increased lipid peroxidation and decreased GSH content an indirect pathway expressed by the down-regulation of phospholipids hydroperoxide and glutathione peroxidase (GPx) gene expression in liver of aflatoxicosis in mice.

Addition of Sp 1% in diet of EAC bearing female mice leading to up regulation of GPx mRNA in liver in comparison with MMC treated group. Previous studies were recorded that Sp inhibited lipid peroxidation induced by several xenobiotics and scavenging of free radicals which cause oxidative stress (Khan *et al*., 2005 and Ou *et al*., 2010). This effects may attributed to its antioxidants contents such as phycocyanin, β-carotene, γ-linolenic acid, phenolic compounds and selenium-containing phycocyanin which has been shown to have strong superoxide and hydrogen peroxide radical-scavenging activities and enhancing the activities of superoxide dismutase and GPx (Li *et al*., 2009). Tochopherol other constituent of Sp act as chain breaking antioxidant and as chemical scavengers of O_2 radicals, as it able to repair oxidizing radical directly and preventing the chain propagation step during lipid peroxidation (Karpinski *et al*., 1999). In conclusion, from the findings we suggest that SP may play a role in reducing genotoxicity induced by anti-neoplastic drugs during cancer chemotherapy treatment with MMC through augmentation of the DNA damage and expression of P53 and GPx mRNA expression. It is recommended to supplement SP in the diet befor and after a minimum of 3 weeks to exert its beneficial effects. Additional researches are needed to study the possible additional desired effects of SP.

REFERENCES

Abbas, T., Olivier, M., Lopez, J., Houser, S., Xiao, G., Kumar, G.S., Tomasz, M., and Bargonetti, J. 2002. Differential activation of p53 by the various adducts of mitomycin C .J Biol Chem, 227:40513- 40519.

Bhat, V.B. and Madyastha, K.M. 2000. C-phycocyanin: a potent peroxyl radical scavenger *in vivo* and *in vitro*. Biochem. Biophys. Res. Commun, (275): 20–25.

Chew, B.P., Brown, C.M., Park, J.S. and Mixter, P.F. 2003. Dietary lutein inhibits mouse mammary tumor growth by regulating angiogenesis and apoptosis. Anticancer Res, 23 (4): 3333–3339.

Gad, A.S., Khadrawy, Y.A., El-Nekeety, A.A., Mohamed, S.R., Hassan, N.S. and Abdel-Wahhab, M.A. 2010. Antioxidant activity and hepatoprotective effects of whey protein and *Spirulina* in rats. Nutrition, 27(5):582-589.

Granados, P.S., Quiles, J.L., Ramirez, T. and Scnchez, R.P. 2010. New advances in molecular mechanisms and the prevention of Adriamycin toxicity by antioxaidant nutrients. Food and chemical Toxicology, (48): 1425-1438.

Grawish, M.E. 2008. Effects of *Spirulina platensis* extract on Syrian hamster cheek pouch mucosa painted with 7, 12 dimethylbenz[a] anthracene. Oral Oncol, (44): 956–62.

Hassan, A.M., Abdel-Aziem, S.H. and Abdel-Wahhab, M.A. 2012. Modulation of DNA damage and alteration of gene expression during aflatoxicosis via dietary supplementation of spirulina and whey protein concentrates. Ecotoxicology and Environmental Safety, 79: 294-300.

Ismail, M.F., Ali, D.A., Fernando, A., Abdraboh, M.E., Gaur, R.L., Ibrahim W.M., Raj M.H. and Ouhtit,A. 2009. Chemoprevention of rat liver toxicity and carcinogenesis by Spirulina. Int J Biol Sci., 2(4): 377-87.

Jayara, R., Anand, T. and Rao, P.V. 2006. Activity and gene expression profile of certain antioxidant enzymes to microcystin-LR induced oxidative stress in mice. Toxicology, 220:136–146.

Kaji, T., Fujiwara, Y. and Inomata, Y. 2002. Repair of wounded monolayers of cultured bovine aortic endothelial cells is inhibited by calcium spirulina, a novel sulfated polysaccharide isolated from *Spirulina platensis*. Life Sci., 70:1841-1848.

Karpinski, S., Reynolds, H.B., Karpinksa, G., Wingsle, G. and Mullineaux, P. 1999. Systemic signaling and acclimation in response to excess excitation energy in Arabidopsis. Sci., 284: 654-657.

Khan, M., Shobha, J.C., Mohan, I.K., Naidu, M.U., Sundaram, C., Singh, S., Kuppusamy P. and Kutala,B.K. 2005. Effect of *Spirulina* against doxorubicin-reduced cardiotoxicity. Phytother. Res., 19 (12): 1030–1037.

Kim, M.Y., Cheong, S.H., Lee, J.H., Kim, M.J., Sok, D.E., Kim, M.R. 2010. Spirulina improves antioxidant status by reducing oxidative stress in rabbits fed a high-cholesterol diet. J. Med. Food, 13: 420–426.

Klaunig, J.E., Wang, Z., Pu, X., Zhou,S. 2011. Oxidative stress and oxidative damage in chemical carcinogenesis. Toxicol. App. Pharmacol., (254): 86–99.

Kumar, G.S., Lipman, R., Cummings, J. and Thomaz, M. 1997. Mitomycin CDNA adducts generated by DT-diaphorase. Revised mechanism of enzymatic reductive activation of mitomycin C. Biochemistry, 36: 14128–14136.

Lee, C.S., Park, S.Y., Ko H.H., and Han, E.S. 2004. Effect of change in cellular GSH levels on mitochondrial damage and cell viability loss due to mitomycin C in small cell lung cancer cells. Biochem. Pharmacol, 68: 1857–1867.

Li, F., Xua, J., Zhoua, L., Zhao, J. , Sheng, G. and Sund, Q. 2009. Inhibition of mitomycin C-induced chromosomal aberrations by micrometer powder of selenium enriched green tea in mice spermatocytes. Mutat. Res., 675:11–16.

Linjawi, S.A. 2011. Protective Effect of *Spirulina* Against Mitomycin C-Induced Genotoxic Damage in male Rats. Journal of American Science, 7(1): 922-931.

Lordan, S., Ross R.P., Stanton, C. 2011. Marine bioactives as functional food ingredients: potential to reduce the incidence of chronic diseases. Mar. Drugs, 9: 1056–1100.

Mallet, F., Oriol, G., Mary, C., Verrier, B. and Mandrand, B. 1995. Continuous RT-PCR using AMV-RT and Taq DNA polymerase: characterization and comparison to uncoupled procedures. Biotechniques, (18):678-687.

Nan, P., Xiao X., Yan D.U., Jian C. and Zhong, C. 2013. Genotoxic effects of 8-hydroxylquinoline in loach (*Misgurnus anguillicaudatus*) assessed by the micronucleus test, comet assay and RAPD analysis. Environmental toxicology and pharmacology, (35): 434–443.

Niikawa, M., Shin S. and Nagase, H. 2007. Suppressive effect of post- or pre-treatment of aspirin metabolite on mitomycin C-induced genotoxicity using the somatic mutation and recombination test in *Drosophila melanogaster*. Biomed. Pharmacother, 61: 113–119.

Ou Y., Shan, Z., Lin, L., Qizhou, J. and Xuegan, Y. 2010. Protective effect of C-phycocyanin against carbon tetrachloride-induced hepatocyte damage *in vitro* and *in vivo*. Chemico-Biological Interactions, (185): 94–100.

Pawar, A.S., Vikram, A., Tribathi, D.N., Badmanabhat, S., Ramarao,P. and Jena, G. 2009. Modulation of mitomycin C induced genotoxicity by acetyl- and thiol-analogues of salicylic acid. *In vivo,* (23): 303-308.

Peters, S., Bauermeister, O., Simon, K., Branke, J.P. and Wagner, T. 2002. Quantitative polymerase chain reaction-based assay with fluorogenic Y-chromosome specific probes to measure bone marrow chimerism in mice. J Immunol Methods, 260:109–116.

Pfuhler, S. and Wolf, H.U. 1996. Detection of DNA-crosslinking agents with the alkaline comet assay. Environ. Mol. Mutagen., 27:196–201.

Pirnia, F., Schneider, E., Betticher, D.C. and Borner, M.M. 2002. Mitomycin C induces apoptosis and caspase-8 and -9 processing through a caspase-3 and Fas-independent pathway, Cell Death Differ. 9 (9): 905-914.

Qishen, P., Guo, B.J. and Ruan, J.H. 1988. Enhancement of endonuclease activity and repair DNA synthesis by polysaccharide of Spirulina. Chinese Genetics Journal, 15 (5): 374-381.

Romay, C., Gonza´lez R., Ledo´n, N., Remı´rez, D. and Rimbau,V. 2003. C phycocyanin: abiliprotein with antioxidant, anti inflammatory and neuroprotective effects. Curr Protein Pept Sci, 4:207–16.

Sharma, M.K., Sharma, A., Kumar, A. and Kumar, M. 2007. Evaluation of protective efficacy of *Spirulina* against mercury induced nephrotoxicity in Swiss albino mice. Food and Chemical Toxicology, (45): 879–887.

Sinanoglu, O., Yener, A.N., Ekici, S., Midi, A., Aksungar, F.B. 2012. The protective effects of *spirulina* in cyclophosphamide induced nephro- toxicity and urotoxicity in rats. Urology, 80 (6), 1392.e1–e6.

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.

Turkez, H., Aydin E. and Aslan, A. 2012. DNA damage and oxidative stress of mitomycin C in human lymphocytes. Cytotechnology, 64 (6): 679-86.

Wang, Y., Chang, C., Chou, J., Chen, H.L., Deng, X., Harvey, B.K., Caded, J.L. and Bick ford, P.C. 2005. Dietary supplementation with blueberries, spinach, or Spirulina reduces ischemic brain damage. Exp. Neurol., 193: 75–84.

Zeinab, E.H. 2009. Ginger extracts antimutagens as cancer chemopreventive agent against Ehrlich ascites carcinoma. Academic Journal of Cancer Research, 2(2):61:67.

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